

Harvesting of microalgae by bio-flocculation

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Abstract The high-energy input for harvesting biomass makes current commercial microalgal biodiesel production economically unfeasible. A novel harvesting method is presented as a cost and energy efficient alternative: the bio-flocculation by using one flocculating microalga to concentrate the non-flocculating microalga of interest. Three flocculating microalgae, tested for harvesting of microalgae from different habitats, improved the sedimentation rate of the accompanying microalga and increased the recovery of biomass. The advantages of this method are that no addition of chemical flocculants is required and that similar cultivation conditions can be used for the flocculating microalgae as for the microalgae of interest that accumulate lipids. This method is as easy and effective as chemical flocculation which is applied at industrial scale, however in contrast it is sustainable and cost-effective as no costs are involved for pre-treatment of the biomass for oil extraction and for pre-treatment of the medium before it can be re-used.

Keywords Harvesting · Microalgae · Bio-flocculation

Introduction

Oil-accumulating microalgae are a promising feedstock for biodiesel production (Benemann et al. 1977; Lee et al. 2009). Commercial microalgal biodiesel production is not economically feasible yet, mainly due to the high-energy

inputs required for water pumping, mixing and for harvesting the microalgal biomass combined with large investment costs (Schenk et al. 2008).

Harvesting in commercial microalgae production plants is generally done by centrifugation. Different studies showed a contribution of the costs for harvesting to more than 30% of the total cost in case of algal production in open ponds (Zittelli et al. 2006). These high costs can only be justified in case of microalgal production for high value products. For low-value bulk products, both the investment as well as the operational costs should drastically decrease to make commercial production feasible (Wijffels and Barbosa 2010).

To minimize the energy consumption of harvesting microalgae, an integrated approach is needed (Benemann 1997). Evaluation of several harvesting methods showed that flocculation combined with flotation or sedimentation and subsequent further dewatering by centrifugation or filtration is the most promising cost and energy efficient alternative (Schenk et al. 2008). During flocculation, the dispersed microalgal cells aggregate and form larger particles with higher sedimentation rate.

Flocculation can be induced in different ways. Induced chemical flocculation using Zn^{2+} , Al^{3+} , Fe^{3+} or other chemical flocculants has been studied extensively (McGarry 1970; Lee et al. 1998; Papazi et al. 2010) and some of them are applied at industrial scale, especially in wastewater treatment plants (De la Nouë et al. 1992). Although this is an easy and effective method, this is not an appropriate method for cheap and sustainable harvesting of microalgae in large-scale microalgae production plants because excess cationic flocculant needs to be removed from the medium before it can be re-used and this leads to extra operational costs (Schenk et al. 2008). Flocculation can also be induced by changing the culture conditions by

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applying extreme pH, nutrient depletion, temperature changes and changes of the level of dissolved O₂. For pre-harvesting of microalgae at large-scale these flocculation methods are not preferred. Most of the latter methods cannot be applied for controlled flocculation and they may induce undesired changes in cell composition (Benemann and Oswald 1996). All of them again require treatment of the medium to be re-used (Schenk et al. 2008). The third method that has been proposed for induced flocculation of microalgae is biologically induced flocculation with bacteria as has been applied successfully in wastewater treatment (Lee et al. 2009). Bio-flocculation of microalgae with bacteria, however, demands additional substrate as well as an extra energy source for bacterial growth and this will evoke undesirable bacterial contamination of the algal production plant. Recently, the naturally flocculating diatom *Skeletonema* was used to form flocs of *Nannochloropsis* (Schenk et al. 2008). As diatoms have a silica-based cell wall, they require different medium composition than most of microalgal strains used for biodiesel production which leads to additional cultivation costs.

In this paper, bio-flocculation of a non-flocculating microalga with another autoflocculating microalga has been evaluated as a promising alternative effective method for harvesting of microalgae. The presented bio-flocculation method enables the harvesting of microalgae without addition of chemical flocculants and allows for re-use of the cultivation medium without any additional treatment. Another advantage of this method in comparison with other applied flocculating microorganisms (bacteria, diatoms) is that it does not require different cultivation conditions and therefore avoids additional costs and prevents undesired contaminations. Furthermore the lipid content of the strains used as the flocculating and non-flocculating microalgae in this study is on average more than 25% of the dry weight biomass (Table 1). The presence of the flocculating microalgae in the final biomass concentrate does thus not interfere with further downstream processing of the lipids

Table 1 Maximum and minimum reported lipid contents for the three flocculating microalgal strains used in this study and for the two non-flocculating microalgae

Strain	Habitat	Lipid content (% DW)
Flocculating microalga		
<i>A. falcatus</i>	Freshwater	28–37
<i>S. obliquus</i>	Freshwater	21–42
<i>T. suecica</i>	Marine	18–26
Non-flocculating microalga		
<i>C. vulgaris</i>	Freshwater	25–42
<i>N. oleoabundans</i>	Marine	36–42

The data are adapted from Griffiths and Harrison (2008)

into biodiesel. Unfortunately, the overall lower lipid productivity of these flocculating microalgae makes them, as such, less attractive for biodiesel production than the faster growing non-flocculating microalgae (Griffiths and Harrison 2008).

The bio-flocculation method will be compared with the chemically induced flocculation, in terms of recovery efficiency and time needed for sedimentation.

Materials and methods

Chlorella vulgaris (211-11b) and *Scenedesmus obliquus* (276-3a) were obtained from University of Göttingen, Germany (SAG), *Neochloris oleoabundans* (1,185) from University of Texas, Austin, USA (UTEX), *Tetraselmis suecica* (66/38) from SAMS, UK (CCAP) and *Ankistrodesmus falcatus* (211) from the Center of Phycology, Třeboň, Czech Republic (CCALA).

Culture conditions The marine medium contained NaCl (27.00 g L⁻¹), MgSO₄·7H₂O (6.60 g L⁻¹), MgCl₂·6H₂O (5.60 g L⁻¹), CaCl₂·2H₂O (1.50 g L⁻¹), KNO₃ (1.45 g L⁻¹), NaHCO₃ (0.04 g L⁻¹), TRIS (hydroxymethyl) amino-methane (3.94 g L⁻¹), EDTA-Na₂ (95 µg L⁻¹), ZnSO₄·7H₂O (11 µg L⁻¹), CoCl₂·6H₂O (5 µg L⁻¹), MnCl₂·4H₂O (90 µg L⁻¹), Na₂MoO₄·2H₂O (30 µg L⁻¹) and CuSO₄·5H₂O (5 µg L⁻¹) dissolved in demineralized water. For the freshwater medium KNO₃ (3 g L⁻¹), NaH₂PO₄·2H₂O (0.26 g L⁻¹), KH₂PO₄ (0.74 g L⁻¹), HEPES (2.38 g L⁻¹), H₃BO₃ (61.80 µg L⁻¹), EDTA-Fe(III)-Na, (0.11 g L⁻¹), EDTA-Na₂ (37 mg L⁻¹), ZnSO₄·7H₂O (3.20 mg L⁻¹), MnCl₂·4H₂O (13 mg L⁻¹) and CuSO₄·5H₂O (1.83 mg L⁻¹) were added to demineralized water. The pH of the solution was set at 6.8 using 4 M HCl. One hundred milliliters of this medium was dispensed into 300-mL Erlenmeyer flasks, sealed with cotton and an aluminum cap and autoclaved for 20 min at 121°C. After cooling the marine medium, K₂HPO₄ (100 mg L⁻¹), KH₂PO₄ (2 mg L⁻¹), EDTA-Fe(III)-Na (1.36 mg L⁻¹), vitamin B₁₂ (1 µg L⁻¹), D-biotin (1 µg L⁻¹), and thiamine-HCl (200 µg L⁻¹) were added using a 0.2 µm non-pyrogenic sterile filter (Sartorius Stedim Biotech, FR). For the freshwater medium MgSO₄·7H₂O (0.4 g L⁻¹), CaCl₂·2H₂O (13 mg L⁻¹), vitamin B₁₂ (1 µg L⁻¹), D-biotin (1 µg L⁻¹), and thiamine-HCl (200 µg L⁻¹) were added after cooling. The microalgae were grown in a light and climate controlled shaking incubator at 100 rpm and 25°C with a 2% CO₂ enriched airflow (3 L·min⁻¹), illuminated using fluorescent light (50 µmol·photons m⁻² s⁻¹) with a 16 h/8 h light/dark cycle.

Turbidity measurements Cell concentration was measured as the optical density at 750 nm (OD₇₅₀) with an Ultraspec

2,000 spectrophotometer (Pharmacia Biotech Ltd. UK) equipped with a temperature controlled carousel cell holder with six positions. Demineralized water served as reference. The microalgal samples were diluted in a 10×10×45 mm polystyrene cuvette (Sarstedt, DE) using filter-sterilized tap water for the freshwater microalgae and with 0.46 mol·L⁻¹ NaCl solution (in demineralized water) for the marine strains (similar ionic strength as the medium applied for the marine strains) to achieve an OD₇₅₀ value below 1.

Sedimentation kinetics Samples of the microalgal suspensions were taken and diluted in a cuvette. After mixing, the suspension was left to settle at 27°C in the dark in a spectrophotometer. The temperature and pH of all samples were measured in the beginning and at the end of the sedimentation period and they were constant respectively at 27°C and pH 7. During the settling period, turbidity of the sample was measured at 750 nm at the same height in the cuvette to determine the recovery. The microalgal recovery (microalgal removal percentage) was calculated with:

$$\text{recovery (\%)} = \frac{\text{OD}_{750}(t_0) - \text{OD}_{750}(t)}{\text{OD}_{750}(t_0)} \cdot 100 \quad (1)$$

where OD₇₅₀(t₀) is the turbidity of sample taken at time zero and OD₇₅₀(t) is the turbidity of the sample taken at time t (Fig. 1). This was done for the suspension of non-flocculating microalga with and without addition of the bio-flocculating microalga. The sedimentation kinetics were measured in cuvettes instead of in conventional jar tests (Vandamme et al. 2010) or recently used cylindrical glass tubes (Papazi et al. 2010). Similar to the conventional tests, the recovery percentage is measured in the top part of the cuvette, where individual cells and formed flocs independently sink.

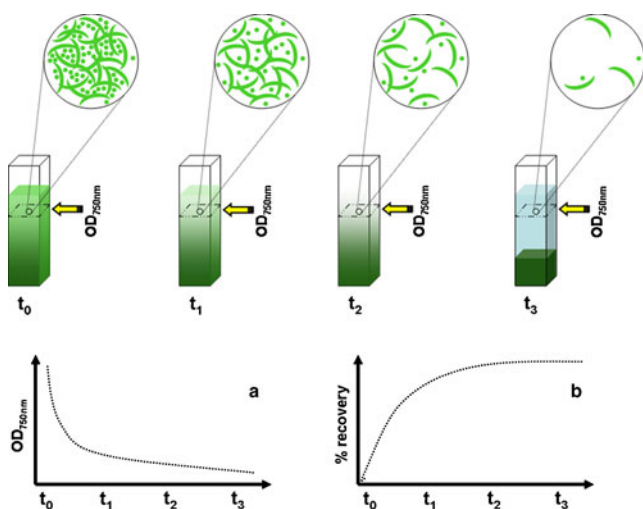


Fig. 1 Recovery of microalgal biomass and sedimentation kinetics calculation. **a** Schematic overview of the microalgal sedimentation test in time. **b** Recovery (%) of the microalgae from the suspension in time

To compare different strains on their ability to be applied as flocculating microalgae, the recovery efficiency is defined as the recovery of the non-flocculating microalga in the presence of the flocculating microalga divided by the recovery of the non-flocculating microalga without flocculating microalga present. The recovery efficiency (adapted from Papazi et al. 2010 and Buelna et al. 1990) was calculated with:

$$\text{recovery efficiency (\%)} = \left[1 - \frac{\frac{\text{OD}_{a750}(t)}{\text{OD}_{a750}(t_0)}}{\frac{\text{OD}_{b750}(t)}{\text{OD}_{b750}(t_0)}} \right] \cdot 100 \quad (2)$$

where OD_{a750}(t₀) and OD_{a750}(t) are the turbidities of samples of non-flocculating microalga with flocculating microalga taken at time zero and at time t, respectively. OD_{b750}(t₀) is the turbidity of sample of non-flocculating microalga taken at time zero and OD_{b750}(t) is the turbidity of the same sample taken at time t.

Three different flocculating microalgae were tested on their ability to improve the recovery efficiency and the rate of harvesting of the non-flocculating microalga. The freshwater microalgae *A. falcatus* and *S. obliquus* were used for harvesting of *C. vulgaris*. The marine microalga *T. suecica* was used to harvest the non-flocculating marine microalga *N. oleoabundans*. For each of the three tested combinations of flocculating and the non-flocculating microalga, four sedimentation experiments were performed: (1) the flocculating microalga, (2) the non-flocculating microalga, (3) the non-flocculating microalga with low concentration of added flocculating microalga and (4) the non-flocculating microalga with high concentration of added flocculating microalga (Table 2). Each of these experiments was performed in duplicate. At the end of sedimentation experiment, samples were taken from the bottom of cuvettes in order to make microscopic pictures of the formed microalgal flocs.

Morphological analysis At the end of sedimentation experiment, samples were taken from the bottom of cuvettes in order to make microscopic pictures of the formed flocs of the microalgal cells, using a C-3030 zoom 5-megapixel Olympus camera connected to a CK40 Olympus microscope with a SK20-SLP phase contrast filter and a T6 objective (×40 magnification) and a NCWHK 18 L ocular lens (×10 magnification).

Results

Three different autoflocculating microalgae were identified; the freshwater *A. falcatus*, and *S. obliquus* and the marine *Tetraselmis suecica* (Fig. 2d, e, and f, respectively). The freshwater microalgae were used to flocculate the strain *C.*

Table 2 Optical densities ($OD_{750}(t_0)$) of flocculating and non-flocculating microalgae added into the cuvettes for four combinations of three experiments

Combination of flocculating and non-flocculating microalgae	$OD_{750}(t_0)$			
	1*	2*	3*	4*
<i>A. falcatus</i>	0.7	0.7	0.4	0.0
<i>C. vulgaris</i>	0.0	0.9	0.9	0.9
<i>S. obliquus</i>	0.8	0.8	0.4	0.0
<i>C. vulgaris</i>	0.0	0.3	0.3	0.3
<i>T. suecica</i>	1.1	1.1	0.5	0.0
<i>N. oleoabundans</i>	0.0	0.2	0.2	0.2

1* the flocculating microalga, 2* the non-flocculating microalga; 3* the non-flocculating microalga with low concentration of added flocculating microalga; 4* the non-flocculating microalga with high concentration of added flocculating microalga

vulgaris as non-flocculating microalga (Fig. 2a and b), while the marine microalgal strain was used to flocculate *N. oleoabundans* (Fig. 2c). *C. vulgaris* and *N. oleoabundans* show both relatively high growth rates in comparison with the autoflocculating microalgae, but all five microalgae are reported to show relatively high lipid content (Table 1).

Microscopic analysis

Figure 2 shows pictures of the non-flocculating microalgae *N. oleoabundans* (Fig. 2c) and *C. vulgaris* (Fig. 2a and b). The microalgae are present as single cells and no floc formation is observed. In the sediments of all three flocculating microalgae large flocs can be observed (Fig. 2d, e, and f). If the three flocculating microalgae are

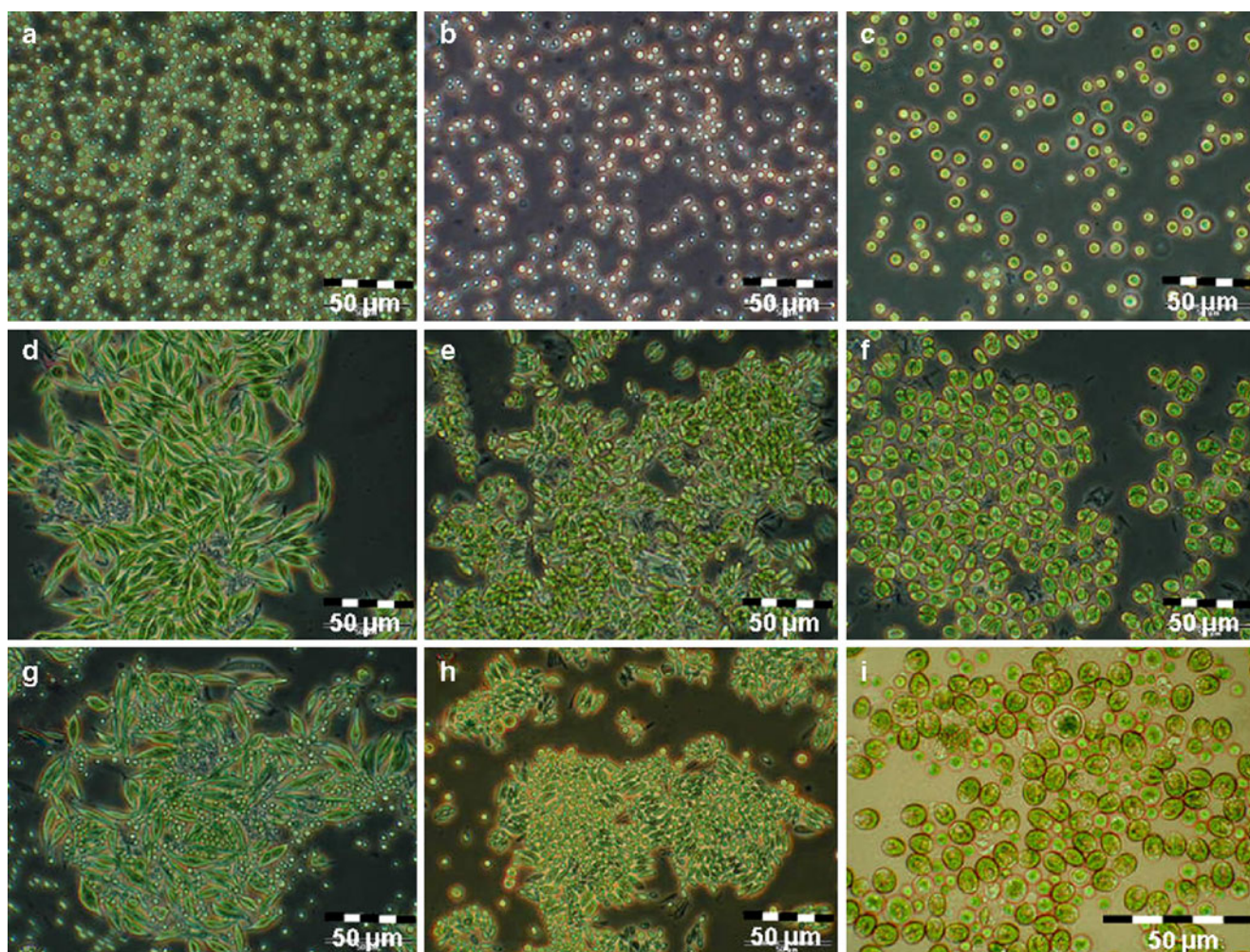


Fig. 2 Microscopic picture of individual and flocculated microalgal cells. The non-flocculating microalgae (a and b *C. vulgaris* and c *N. oleoabundans*), the flocculating microalgae (d *A. falcatus*, e *S. obliquus* and f *T. suecica*) and the flocs of the non-flocculating

microalgae after the addition of accompanying flocculating microalgae (g *C. vulgaris* with *A. falcatus*, h *C. vulgaris* with *S. obliquus* and i *N. oleoabundans* with *T. suecica*). For more details on the morphological analysis and sample preparation see “Materials and methods”

Table 3 Initial sedimentation rate

Combination of flocculating and non-flocculating microalgae	Initial sedimentation rate (% recovery·h ⁻¹)			
	1*	2*	3*	4*
<i>A. falcatus</i> and <i>C. vulgaris</i>	41.1	13.6	10.4	6.8
<i>S. obliquus</i> and <i>C. vulgaris</i>	37.0	20.4	18.7	10.2
<i>T. suecica</i> and <i>N. oleoabundans</i>	46.2	39.9	37.5	18.7

Details for the calculation of these initial sedimentation rates can be found in the main text and “Materials and methods”

1* the flocculating microalga, 2* the non-flocculating microalga, 3* the non-flocculating microalga with low concentration of added flocculating microalga, 4* the non-flocculating microalga with high concentration of added flocculating microalga

added to the non-flocculating microalgae (Fig. 2g, h, and i), the microscopic pictures show that the majority of the non-flocculating microalgae are trapped in flocs formed by the flocculating microalgae and almost no loose cells of non-flocculating microalgae remain in the suspension after the addition of flocculating microalgae. The comparison of the pictures in Fig. 2a, b, and c, respectively, with Fig. 2g, h, and i confirms that the addition of flocculating microalgae from different habitats (marine and freshwater) improves the recovery of various non-flocculating microalgae.

Sedimentation kinetics of various flocculating and non-flocculating microalgae

The sedimentation of the microalgal suspensions was monitored for 8 h and the percentage of microalgal recovery was determined over time. The sedimentation rate of the microalgae in suspension was calculated by linear regression of data in the curves of the recovery percentage in time and use of the slope of the linear regression.

The initial sedimentation rates of the flocculating microalgae measured over the first 2 h of the test are higher than those of the non-flocculating microalgae (Table 3). Mixing of the flocculating microalga with the non-flocculating microalga increases the initial sedimentation rate considerably. The large flocs formed by flocculating microalgae seem to trap the non-flocculating microalgae (Fig. 2g, h, and i) and sediment faster than individual non-flocculating microalgal cells. Furthermore, an increase in the ratio of the bio-flocculating microalga and the non-flocculating microalga leads to higher sedimentation rates. These observations again confirm that the total recovery as well as the rate of sedimentation of various non-flocculating microalgae improves upon addition of different flocculating microalgae.

Efficiency of various flocculating microalgae

The improvement in the recovery of the non-flocculating microalgae was evaluated for the three flocculating microalgae by calculation of the recovery efficiency percentage. For calculation of the recovery efficiency percentage (Eq. 2 in “Materials and methods”), the average turbidity of duplicate measurements was used. The standard deviation in measured values for sedimentation rate and recovery percentage for all tested samples was less than 3.5%. The recovery efficiency percentage of three flocculating microalgae added at low and high concentration is presented in Fig. 3.

All three flocculating microalgae show higher recovery efficiency when they are applied at higher concentration, although doubling of concentration of the flocculating microalga does not necessarily result in two times higher recovery efficiency of the non-flocculating microalga.

Discussion

The results show that addition of autoflocculating microalgae induce faster sedimentation of non-flocculating microalgae and also increase the harvesting efficiency. Similar positive effects on sedimentation rates and harvesting efficiencies are observed with bio-flocculation of non-flocculating microorganisms with bacteria (Lee et al. 2009). In literature, adsorption of cationic polymers (Lewin 1956; Tilton et al. 1972) excreted by the microorganisms is proposed to explain the mechanism involved in bio-flocculation. Polymer-induced flocculation can be divided in two sub-mechanisms called bridging and patching

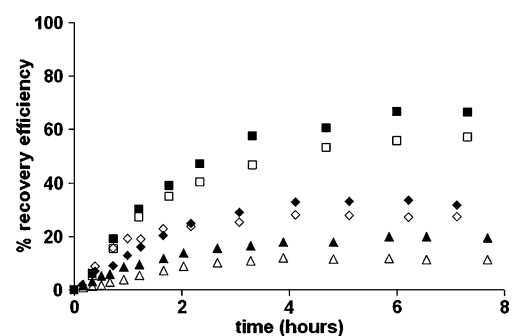


Fig. 3 Recovery efficiency percentage of different flocculating microalgae at two different concentrations. filled square high concentration *T. suecica*, empty square low concentration *T. suecica*, filled diamond high concentration *S. obliquus*, empty diamond low concentration *S. obliquus*, filled upright triangle high concentration *A. falcatus*, empty upright triangle low concentration *A. falcatus*. The standard deviation in measured values for sedimentation rate and recovery percentage for all the tested samples was less than 3.5%. Details for calculation of these recovery efficiency percentages can be found in Materials and methods

(Fig. 4). The positively charged polymers bind partly or completely to microalgal cells. If the polymers bind partly, the unoccupied part of the polymers can bind to other microalgal cells, thereby bridging them and resulting in a network of polymers and microalgal cells. If the polymers bind the microalgal cells completely because they are too short to bind others as well, they adsorb (patch) to the surface and can create positive charges locally. These charges attract other microalgal cells and also result in flocculation of the cells.

Our microscopic observations suggest that bridging is the mechanism behind the floc formation by *A. falcatus* (Fig. 2d) as a large network of microalgal cells is formed. Patching can be the mechanism behind the flocculation of *T. suecica* (Fig. 2f) and *S. obliquus* (Fig. 2e) as they seem to be connected more locally. Based on these observations, our hypothesis is that the extracellular polysaccharides excreted by *A. falcatus* itself bind partly to the surface of *A. falcatus* and positively charged tails of these polysaccharides can bind to the other *A. falcatus* cells. During the formation of the flocs *C. vulgaris* cells are trapped in this large network of *A. falcatus* cells (Fig. 5).

The recovery efficiencies and time needed for sedimentation observed here using bio-flocculation are in the same range as the recovery efficiencies found by Papazi et al. (2010) applying chemically induced flocculation for separation of the microalgal biomass. They showed a

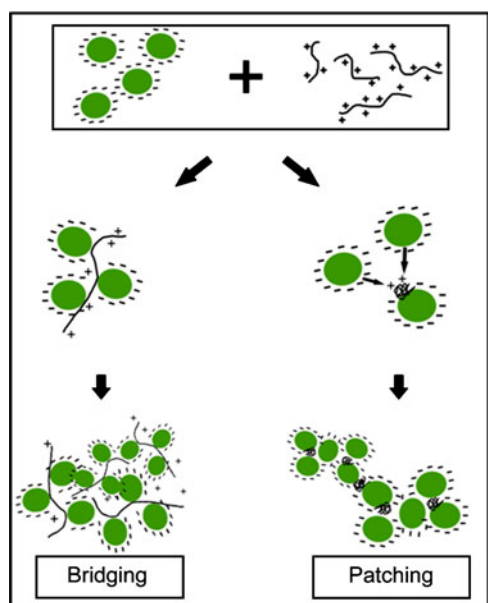


Fig. 4 Schematic view of possible mechanisms involved in polymer-induced flocculation; bridging and patching

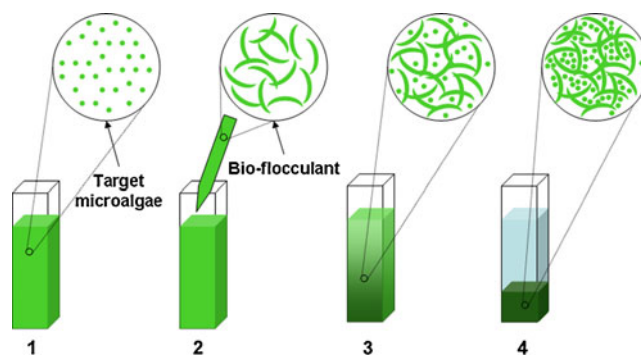


Fig. 5 Schematic view of the proposed mechanism involved in bio-flocculation using *A. falcatus* as the flocculating microalga

recovery efficiency of 60% for harvesting *Chlorella minutissima* by addition of 1 g L^{-1} of $\text{Al}_2(\text{SO}_4)_3$ and ZnCl_2 in, respectively, 1.5 and 6 h. The density of microalgal culture (OD_{750}) used by Papazi et al. (2010) was 2.4 which is comparable with the density of cultures used in this study. Other studies using chemical flocculation reported other concentrations and recovery efficiencies, e.g. Lee et al. (1998), and McGarry (1970) used up to, respectively, 300 and 125 mg L^{-1} of Al^{3+} . However, the microalgal density of the samples used in these studies is not mentioned and the recovery efficiencies are calculated on a different way and therefore cannot be compared with results of the current study.

Future perspectives of sustainable microalgal harvesting

We presented in this study that all three chosen flocculating microalgae improved the recovery efficiency of the accompanying non-flocculating microalga. It can be concluded that the bio-flocculation by using one flocculating microalga for harvesting of another oil-accumulating microalga can be applied as the controlled and reliable pre-concentration step in harvesting of the oil-accumulating microalgae, although large-scale experiments are still needed to prove the feasibility and cost efficiency of this method at industrial scale. Further, it was shown in this study that different flocculating microalgal strains are available for application of bio-flocculation in marine as well as in freshwater environment. Using bio-flocculation followed by sedimentation as the pre-concentration step decreases the recovery time of the non-flocculating microalga. The amount of flocculating microalgae used is still relatively high in comparison with the non-flocculating microalgae (Table 2). A decrease in the amount of

flocculating microalga by half did not show any major effects on the recovery efficiency and time needed for sedimentation of the non-flocculating microalga. This indicates that this method is indeed promising and further optimization of the ratio of the bio-flocculating microalga and the non-flocculating microalga should be done to reveal if large-scale utilization of this technique will indeed result in considerable decrease of harvesting costs and energy.

To summarize, this harvesting method is as easy and effective as chemically induced flocculation which is applied at industrial scale, however in contrast to induced chemical flocculation, this method is sustainable. Although the cultivation of flocculating microalgae requires some extra nutrients and energy, the flocculating microalgae do not require an additional set of nutrients for cultivation in comparison with the microalgae of interest. In the economical analysis of large-scale application of this promising harvesting method the additional costs for a separate cultivation system for cultivation of the flocculating microalga should also be taken into account. In addition, the flocculating microalgae accumulates lipids and no extra operational and investment costs are involved for treatment of the sediment (microalgal biomass) for further downstream processing towards biodiesel or for pre-treatment of the medium before it can be re-used.

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