PERSPECTIVE

Chlamydomonas reinhardtii flocculation by metal cations and high pH with different phenotypic traits

Theodore Dixon

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ABSTRACT

The energy and expense required to harvest the algae might be greatly reduced by flocculating the cells first before centrifuging them. However, it is not fully known how variations in phenotypic variables, such as cell surface characteristics, cell size, and motility, affect the effectiveness of metal cations and pH-induced flocculation. Our findings show that the addition of divalent cations like calcium and magnesium (>5 mM) causes both wild-type and cell wall-deficient strains of the green unicellular alga *Chlamydomonas reinhardtii* to flocculate effectively (>90%) at an elevated pH of the medium (pH 11). Under weakly alkaline circumstances (pH 8.5), the trivalent ferric cation (at 10 mM) was found to be crucial for promoting flocculation, with a maximum efficiency exceeding 95% and 85% for wild-type CC1690. A pH of >11 and a calcium concentration of 5 mM could be used to achieve nearly full flocculation, and the medium that was recovered after cell removal could be recycled without altering algal growth rates. Additionally, the effectiveness of flocculation was not significantly affected by the lack of starch in the cell. These discoveries advance our knowledge of flocculation in several *Chlamydomonas* strains and have implications for low-cost techniques for collecting algae with various phenotypic characteristics. It will be possible to develop low-cost methods for harvesting cell biomass with the aid of additional study on the conditions (such as pH and metal ions) required for efficient flocculation of various algal groups with various properties, both at small and large scales

Key Words: *Phenotypic; Chlamydomonas; Flocculation; Centrifuging; Concentration*

INTRODUCTION

In the past ten years, interest in microalgae as a possible feedstock for the large-scale, sustainable production of goods like food, feed, chemicals, minerals, and fuels has grown. The main factors determining the viability of creating sustainable algal biomass production for all potential products will be resolving technological problems and lowering production costs. A significant hurdle for commercial-scale applications is the capacity to affordably extract significant amounts of microalgal biomass from diluted cultures, among other important technical challenges. The generation of high-value biomass or metabolites is the only use for conventional techniques like centrifugation, which are quick and efficient but also expensive and energy-intensive. Membrane filtration, foam fractionation, chemical or biological flocculation, ultrasonic aggregation, magnetic separation, and gravity sedimentation are some of the additional methods that have been employed to harvest microalgae. Each of these techniques has been documented, with some encouraging outcomes under particular production circumstances.

The right harvesting technology must be used in order to produce algal biomass in an environmentally responsible and economically viable manner. When choosing a harvesting technique, it is important to take into account the strain phenotypic, the ionic strength and pH of the culture medium, the recycling of used medium, and the ultimate biomass quality. It has been demonstrated that chemical flocculation with polyvalent metal ions or polymeric flocculants effectively separates microalgal cells from their growing medium. Altering the pH of the medium can also encourage flocculation in some species. However, raising the pH may cause the algal cells as well as magnesium, calcium, phosphate, and carbonate salts to precipitate. The flocculated material can therefore be collected in a much smaller total culture volume and be further concentrated by centrifugation, in contrast to increasing the pH, which neutralises

Editorial Office, Journal of Environmental Microbiology, UK

Correspondence:, Theodore Dixon, Editorial Office, Journal of Environmental Microbiology UK, Email: theodoredixon@gmail.com

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negative charges on the cell surface and promotes flocculation by reducing the dispersal forces among the cells. In a recent study, it was found that the power used to harvest microalgae by coagulation flocculation was significantly lower than the power used to harvest microalgae by conventional centrifugation only 2.1 kWh/kg for Chlorella vulgaris and 0.2 kWh/kg for Phaeodactylum tricornutum compared to 16 kWh/kg when assuming a microalga biomass concentration of 0.5 kg/m^3 in open In conclusion, algal cell flocculation before centrifugation-based harvesting could dramatically lower the energy "cost" of the collecting process.

Although previous research suggests that flocculation is an effective method for pre-harvesting microalgal cells, it is still unclear how universal the process is for various species and strains of a single species that differ phenotypically, such as in terms of cell size, surface charge characteristics, and motility. Additionally, it would be beneficial to examine how production strains lacking or deficient in a cell wall affect cation and pH-triggered flocculation; these strains may be We tested the flocculation of the green unicellular algae *Chlamydomonas reinhardtii* (hereafter *Chlamydomonas)* using a mixture of metal cations in addition to medium of various pHs in order to further develop easy, affordable approaches to facilitate algal cell harvesting by flocculation. In these investigations, various strains with various biological and physical characteristics were investigated to see if certain physical traits affected the degree or effectiveness of flocculation. The cell wall-deficient (cw-) strain cw15 sta6 (BAFJ5, lack flagella), which was derived from strain CC330 by random insertional mutagenesis with the ARG7-containing plasmid cw15 sta6, is unable to synthesise starch as a result of the STA6 gene, as well as two wild-type strains, CC124 and CC1690, that are frequently used in the laboratory, were also examined. In the past two decades, numerous studies have investigated the carbon partitioning in this strain and its potential to synthesise high levels of lipids for the production of sustainable, renewable liquid fuels. This is because the sta6 mutant displays normal growth rates (relative to wild-type cells) in acetate-supplemented medium but does not synthesise starch. We also looked at CC400, a starch-producing cw-strain, and CC4567, a sta6 strain that was genetically rescued for the starchless phenotype by transformation with a wild-type copy of the *STA6* gene sta6::*STA6*, hereby referred to as C6. These later strains aid in separating the effects of cell wall production and starch accumulation on flocculation.