Open access • Journal Article • DOI:10.1007/S10811-009-9488-8

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Published on: 01 Aug 2010 - Journal of Applied Phycology (Springer Netherlands)

Topics: Modified starch, Starch, Parachlorella, Flocculation and Scenedesmus

Flocculation of microalgae using cationic starch

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Abstract

Due to their small size and low concentration in the culture medium, cost-efficient harvesting of microalgae is a major challenge. We evaluated the potential of cationic starch as a flocculant for harvesting microalgae using jar test experiments. Cationic starch was an efficient flocculant for freshwater (*Parachlorella*, *Scenedesmus*) but not for marine microalgae (*Phaeodactylum*, *Nannochloropsis*). At high cationic starch doses, dispersion restabilisation was observed. The required cationic starch dose to induce flocculation increased linearly with the initial algal biomass concentration. Of the two commercial cationic starch flocculants tested, Greenfloc 120 (used in wastewater treatment) was more efficient than Cargill C*Bond HR 35.849 (used in paper manufacturing). For flocculation of *Parachlorella* using Greenfloc 120, the cationic starch to algal biomass ratio required to flocculate 80% of algal biomass was 0.1. For *Scenedesmus*, a lower dose was required (ratio: 0.03). Flocculation of *Parachlorella* using Greenfloc 120 was independent of pH in the pH range of 5 to 10. Measurements of the maximum quantum yield of PSII suggest that Greenfloc 120 cationic starch was not toxic to *Parachlorella*. Cationic starch may be used as an efficient, non-toxic, cost-effective and widely available flocculant for harvesting microalgal biomass.

Keywords: modified starch, quaternary ammonium, microalgal harvesting, flocculation

Introduction

Compared to traditional crops, microalgae have a high areal productivity, a relatively high oil and protein content and do not depend on arable land and freshwater. Therefore, microalgae receive much interest as a potential source of biofuels (Chisti 2007; Sialve et al. 2009) and/or bulk protein (Becker 2007). Microalgae can grow on nitrogen and phosphorus from wastewater (Olguin 2003) and CO_2 from flue gas (Keffer and Kleinheinz 2002). Therefore, they can be used to polish nutrients from wastewaters and capture CO_2 from flue gas.

Microalgae are currently being produced on a limited scale by several small companies around the world. The main purpose is high-value products such as natural pigments, health food products, live feed for fish larvae or a source of poly-unsaturated fatty acids (Raja et al. 2008; Spolaore et al. 2006). The cost of production for these applications is high. For applications such as biofuel production, bulk food or feed production, wastewater treatment, CO_2 capture or even a combination of these, the cost of production has to be reduced by at least an order of magnitude.

The concentration of microalgal biomass in cultures is typically only about 0.5 to 5 g l^{-1}, or 0.05 to 0.5 %. Moreover, microalgae are small (5-20 μm) and have a density comparable to that of water. As a result, harvesting microalgae from their medium is a major challenge (Grima et al. 2003; Gudin and Therpenier 1986). The high cost of harvesting is an important reason why previous attempts to produce microalgae at large scales for low-value applications such as biofuels or bulk feed/food have failed. Most existing commercial systems use centrifugation for harvesting microalgae, but this is an energy-intensive process (Heasman et al. 2000). Relatively large microalgae such as *Arthrospira* can be harvested using gravity filtration (Becker 1994). Smaller microalgae can theoretically be harvested using ultrafiltration, but extracellular organic matter generally results in rapid fouling of membranes (Rossi et al. 2004; Rossignol et al. 1999). Microalgae can also be harvested using standing ultrasound waves, but due to the necessity of cooling, the energy cost of large scale harvesting systems is high. (Bosma et al. 2003).

One of the other possibilities for harvesting microalgae is by means of flocculation. Inorganic flocculants such as alum and iron chloride are efficient but are required in high doses and result in contamination of the biomass with aluminum or iron (Becker 1994). Biodegradable organic flocculants do not contaminate the algal biomass and are often required in lower doses (Singh et al. 2000). Biodegradable organic flocculants are based on biopolymers like chitin, guar gum, alginic acid or starch. Of these, chitosan has been shown to be an effective flocculant for microalgae (Divakaran and Pillai 2002). It has no apparent toxic effects on fish feeding on the harvested algae (Knuckey et al. 2006). It is, however, a high value product with a market value of about $ 10 kg^{-1} (Becker 1994; Kumar 2000).

Starch consists of a mixture of amylose and amylopectin and is one of the most abundant natural polymers. Chemically modified starches have properties very different from the parent starch and have many applications in industrial processes (Prakash et al. 2007). Cationic starch is prepared by addition of quaternary ammonium groups to the glucose hydroxyl groups and is an effective flocculant (Pal et al. 2005). Because of its low cost (about $ 1-2 kg^{-1}), cationic starch is increasingly being used as an alternative for inorganic and synthetic organic flocculants in liquid solid separation processes, more specifically in wastewater treatment and papermill industries. As polymer flocculants are often specific (Bratby 2006), flocculants that are effective for clay dispersions or cellulose are not necessarily applicable to algal cells. The goal of this study was to evaluate the potential of cationic starch for flocculation of microalgae.

Materials & methods

Materials

Four microalgal species were obtained from culture collections: *Parachlorella kessleri* (SAG 27.87), *Scenedesmus obliquus* (CCAP 276j3A), *Phaeodactylum tricornutum* (CCAP 1055/1) and *Nannochloropsis salina* (SAG 40.85). The microalgae were cultured in Wright's cryptophytes (WC) medium which was prepared from pure salts and deionised water. The concentration of the medium was increased 5 times to allow the microalgae to attain a biomass concentration comparable to commercial culture systems (up to 0.5 g dry weight l^{-1}). For the marine species, synthetic sea salt (Ultramarine Synthetica, Waterlife Research, U.K.) was added at a concentration of 30 g l^{-1}. The medium was adjusted to pH 8 and autoclaved. An inoculum was added under a sterile hood at a 1/10 ratio. The microalgae were cultured in 5 parallel 2 l bottles incubated in a temperature controlled room (20°C). The bottles were irradiated with daylight fluorescent tubes (light intensity: 100 µEinst m-2 s^{-1}) and were bubbled with sterile-filtered air at a rate of approximately 200 ml min^{-1} to create turbulence and avoid CO_2 limitation. Flocculation experiments were carried out when algae were in exponential growth phase. The algal biomass concentration in the reactors at that moment varied between 0.15 and 0.5 g dry weight l^{-1}. Algal biomass was estimated from optical density measurements at 550 nm using a spectrophotometer (Hach Lange DR 2800). Optical density was calibrated against dry weight measured gravimetrically on pre-weighed GF/F glass fiber filters ($R^2 > 0.98$).

Two commercial cationic starches were used in the experiments. Greenfloc 120 (Hydra 2002 Research, Development and Consult, Hungary) is a cationic starch with a degree of substitution of 0.15 that is mainly used in wastewater treatment. It was supplied as a concentrated solution in water (16%) that was ready for use. Cargill C*bond HR 35.849 (Cargill Deutschland, Germany) is a cationic starch with a degree of substitution of 0.11 that is used in the paper manufacturing industry. It was supplied as a dry product that was dissolved in water and heated to 80°C for 20 min before use.

Flocculation experiments

Flocculation of microalgae after addition of cationic starch was evaluated using jar tests (Cohen 1957; Hudson and Wagner 1981). The algal suspensions were divided over replicate 100 ml beakers. The initial algal biomass concentration in the beakers was estimated from the optical density at 550 nm. Cationic starch was added at a specific dose under intensive stirring (1000 rpm) using a magnetic stirrer. After 5 min, the stirring speed was reduced to 250 rpm. Stirring was stopped 30 min after addition of the cationic starch. After another 30 min, the optical density of the supernatant was measured at half the height of the clarified layer. The percentage of algal biomass removed was estimated from the ratio of the initial over the final optical density. To evaluate the influence of pH on flocculation, pH was adjusted using 0.5 N HCl or 0.5 N NaOH. Results were statistically evaluated using One Way ANOVA and a Tukey's Test (Sigmaplot 11, Systat Software, Inc.). The potential toxicity of cationic starch on the microalgae was evaluated using measurements of the maximum quantum yield of photosynthetic efficiency of photosystem II, measured using an AquaPen-C fluorometer (Photon Systems Instruments, Czech Republic). This parameter is a sensitive indicator of stress experienced by microalgae and is often used for evaluating toxicity of substances towards microalgae (Cid et al. 1995). The quantum yield of photosynthetic efficiency of photosystem II was measured 3 hours after addition of cationic starch and after 20 min of dark adaptation of the microalgae. Statistical analysis was performed using One Way ANOVA (Sigmaplot 11, Systat Software, Inc.).

Results & discussion

Our results indicate that cationic starch is an efficient flocculant for the freshwater microalgae *Scenedesmus* and *Parachlorella*. Suspensions of unicellular microalgae are stabilized by the negative surface charge of the algal cells. Cationic starch can induce flocculation of negatively charged particles through bridging and/or patch charge neutralisation (Sharma et al. 2006; Bratby 2006). In jar tests using *Parachlorella* and the cationic starch Greenfloc 120, the flocculation efficiency increased strongly over a relatively narrow range of cationic starch concentration (about 10-15 mg l^{-1}) (Fig. 1). At the optimal dose, more than 90% of the biomass was removed by the flocculant. The Greenfloc 120 cationic starch dose required to flocculate 80% of *Parachlorella* biomass was above a certain concentration linearly related to the algal biomass concentration (Fig. 2). A linear relation between flocculant dose and particle concentration is often observed in cationic polyelectrolyte flocculants (Black and Vilaret 1969).

The ratio of cationic starch over *Parachlorella* biomass required to achieve 80% flocculation was approximately 0.1. For *Scenedesmus*, a lower dose of Greenfloc 120 cationic starch was required to induce flocculation. The ratio of cationic starch over algal biomass required to achieve 80% flocculation for *Scenedesmus* was 0.03 or less (Fig. 3). As *Parachlorella* and *Scenedesmus* have a comparable charge density (Henderson et al. 2008), this difference can probably be ascribed to the larger size of *Scenedesmus*. Larger particles often require a lower polymer dose for flocculation than smaller particles (Bratby 2006).

In the experiments with low biomass concentrations of *Parachlorella* as well as in the experiment with *Scenedesmus*, it was clear that overdosing of cationic starch resulted in dispersion restabilization. This phenomenon is commonly observed with polyelectrolyte flocculants, including cationic starch (Fellows and Doherty 2005; Bratskaya et al. 2005; Liu et al. 2009) and is probably the result of steric hindrance and/or electrostatic repulsion.

For the marine microalgae *Nannochloropsis* and *Phaeodactylum*, the ratio of Greenfloc 120 cationic starch over algal biomass required to induce flocculation was around 1 (results not shown). Therefore, it appears that cationic starch is inefficient for flocculating marine microalgae. This is probably due to high NaCl concentrations. In experiments with kaolin dispersions, Bjorklund and Wagberg (1995) observed a decrease in flocculation efficiency of cationic starch at high NaCl concentrations. Like cationic starch, chitosan is also ineffective for flocculating microalgae in seawater (Bilanovic and Shelef 1988; Divakaran and Pillai 2002; Liu et al. 2009; Henderson et al. 2008).

In dense microalgal cultures, pH is often highly variable: it may increase to 10 due to intensive primary production or decrease to 6 during CO_2 addition or as a result of respiration. As pH affects the zeta potential of charged particles, it may interfere with flocculation. Flocculation of *Parachlorella* using Greenfloc 120 cationic starch increased slightly but significantly with pH over a pH range of 5 to 10 (ANOVA, $p < 0.001$). This is in contrast to flocculation of microalgae using chitosan, which is only efficient at a pH below 8 (Divakaran and Pillai 2002; Liu et al. 2009). In cationic starch, the positive charge is due to quaternary ammonium salts, which maintain their positive charge even at relatively high pH. The increase in flocculation efficiency at high pH is probably due to some autoflocculation of *Parachlorella*, which occurs at a pH of 10 or higher (unpublished results).

The Greenfloc 120 cationic starch had no significant (ANOVA, $p = 0.330$) effect on the maximum quantum yield of photosynthetic efficiency of photosystem II in *Parachlorella* (Fig. 5). It therefore appears that cationic starch has no short-term effects on the viability of the algae.

For *Parachlorella*, we compared flocculation by two commercial cationic starch polyelectrolytes, both with a relatively low degree of substitution. Greenfloc 120 is a flocculant designed for wastewater treatment while C*bond HR 35.849 is designed for applications in the paper industry. Using C*bond HR 35.849, a higher dose was required compared to the Greenfloc 120 cationic starch (Fig. 6). The ratio of C*bond HR 35.849 cationic starch over algal biomass required to achieve 80% flocculation was approximately 0.3. Moreover, the flocculation increased more slowly with increasing cationic starch concentration. The lower flocculation efficiency of C*bond HR 35.849 may be

due to the lower degree of substitution (0.11 versus 0.15). It is well known that the flocculation efficiency of polyelectrolytic flocculants in general and cationic starch in particular is related to the degree of substitution (Bratskaya et al. 2005). However, the flocculation efficiency generally increases linearly with the degree of substitution, especially at a low degree of substitution (Krentz et al. 2006). As the degree of substitution of Greenfloc 120 is only 1.4 times that of C*bond HR 35.849 while the optimal dose for flocculation was at least 3 times lower, other factors probably contributed to the difference in flocculation efficiency. Both the location of the substitutions (Shirzad-Semsar et al. 2007), the molecular weight of the polymers (Krentz et al. 2006), the steric configuration (Fellows and Doherty 2005) and the amylose to amylopectin ratio have been shown to influence the flocculation efficiency of cationic starch (Pal et al. 2005).

Conclusions

Our results show that cationic starch is a potentially useful flocculant for harvesting freshwater microalgae. Compared to inorganic flocculants, cationic starch requires a lower dose. Moreover, it does not contaminate the algal biomass as it is approved for food contact and for use in treatment of drinking water (Krentz et al. 2006). In these aspects, cationic starch is similar to chitosan. Due to the lower number of functional groups, the dose required for cationic starch is higher than that for chitosan. On the other hand, chitosan is more expensive than cationic starch, it is not available in very large volumes and is more difficult to apply due to its pH-dependence.

The cationic starches used in this study were not designed for harvesting algae. The large difference between the two cationic starches tested suggests that there is room for improvement of the efficiency of cationic starches for flocculating algae. The flocculation efficiency might be improved by increasing the degree of substitution. It should be noted, however, that the production cost of cationic starch increases exponentially with the degree of substitution. Other options to improve the flocculation efficiency include modification of the amylose to amylopectin ratio or modification of the polymer chain lengths.

Acknowledgements

The research presented in this paper was financially supported by the Institute for the promotion of Innovation by Science and Technology - Strategic Basic Research (IWT-SBO) project Sunlight and the K.U.Leuven Research Coordination Office -Industrial Research Fund (DOC-IOF) project Algae-Tech. We thank Eline Ryckebosch, Jan Lievens and Donald Samyn for practical support with cultivation of algae.

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Figures

Fig. 1. Effect of cationic starch (Greenfloc 120) dose on the percentage of algal biomass (*Parachlorella*) removed by flocculation for three different initial algal biomass concentrations (a: 0.30 g l^{-1}, b: 0.15 g l^{-1}, c: 0.075 g l^{-1}).

Fig. 2. Relation between the initial algal biomass (*Parachlorella*) concentration and the cationic starch dose (Greenfloc 120) required to achieve 80% flocculation.

Fig. 3. Effect of cationic starch (Greenfloc 120) dose on the percentage of algal biomass (*Scenedesmus*) removed by flocculation. Initial algal biomass was 0.15 g l^{-1}.

Fig. 4. Effect of pH on the flocculation of the alga *Parachlorella* using cationic starch (Greenfloc 120) at an algal biomass concentration of 0.43 g l^{-1} and a cationic starch dose of 70 mg l^{-1}. The white point corresponds to the control in which pH was not adjusted. A, B, C indicate whether pH has a significant influence on flocculation efficiency; means with the same letter are not significantly different ($\alpha = 0.1$).

Fig. 5. Effect of different cationic starch (Greenfloc 120) concentrations on the maximum quantum yield of photosynthetic efficiency of photosystem II in *Parachlorella*.

Fig. 6. Comparison of flocculation of *Parachlorella* using two types of cationic starch (white points: Greenfloc 120 and black points: Cargill C*Bond HR 35.849). Initial algal biomass was 0.3 g l^{-1}.

Figure 1
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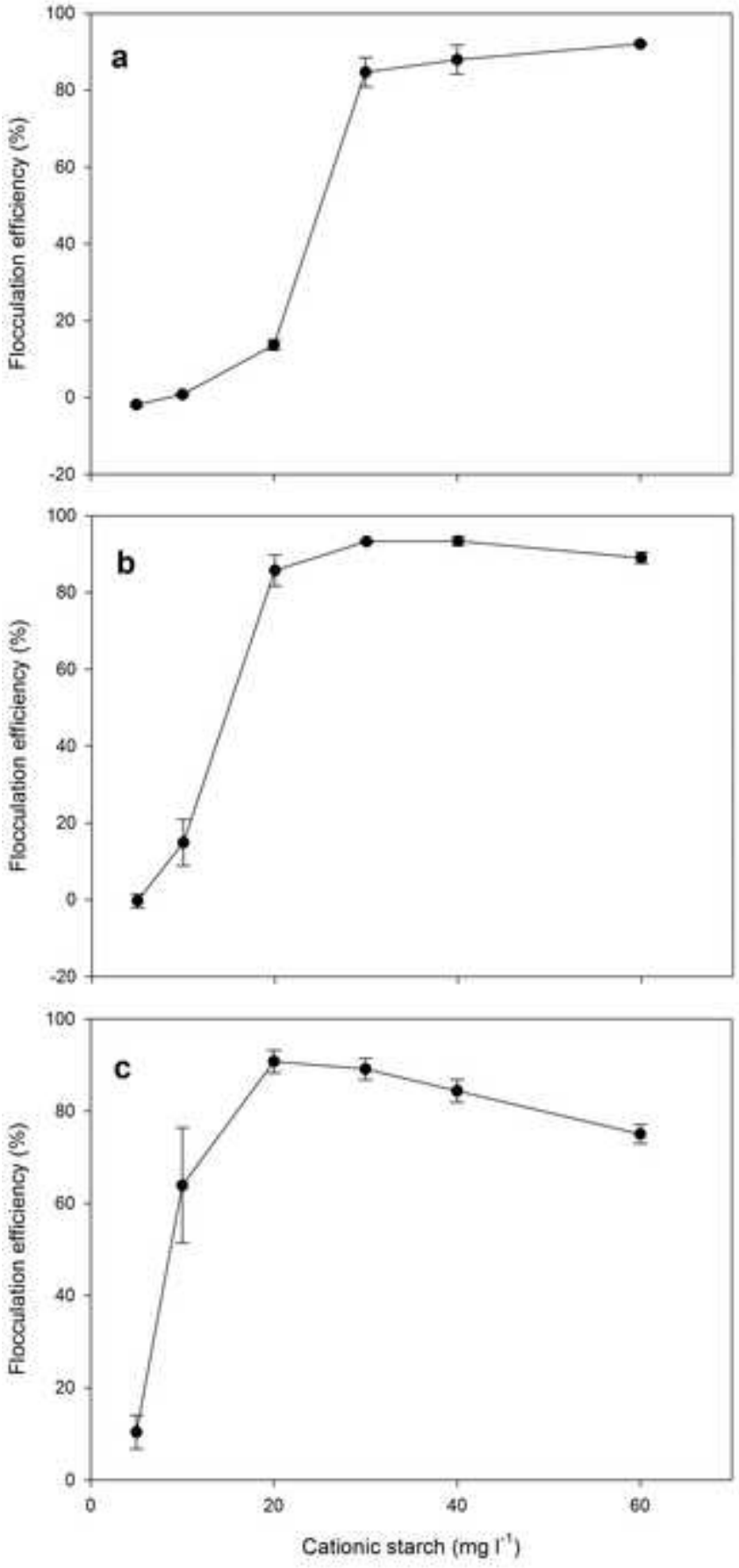

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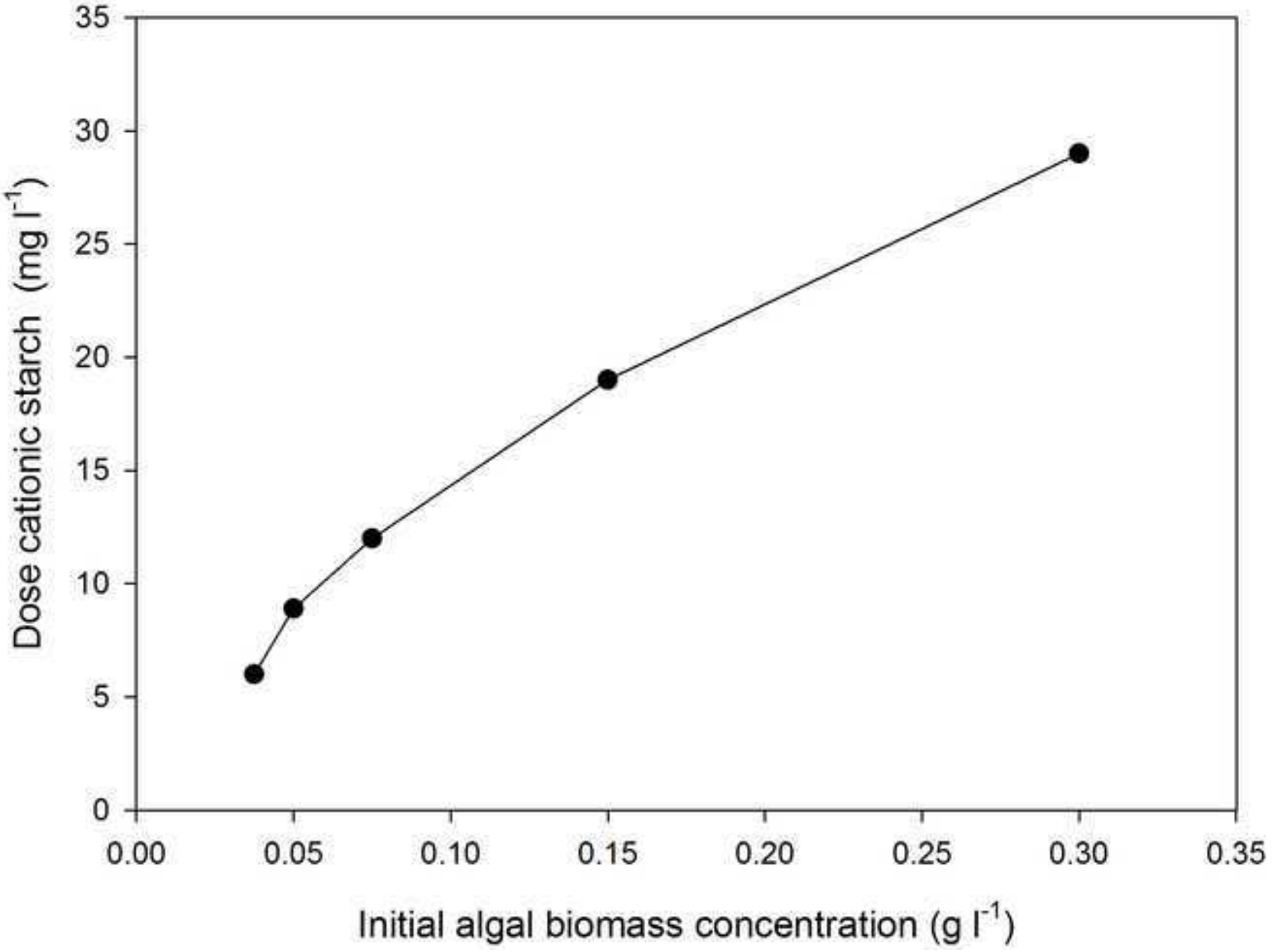

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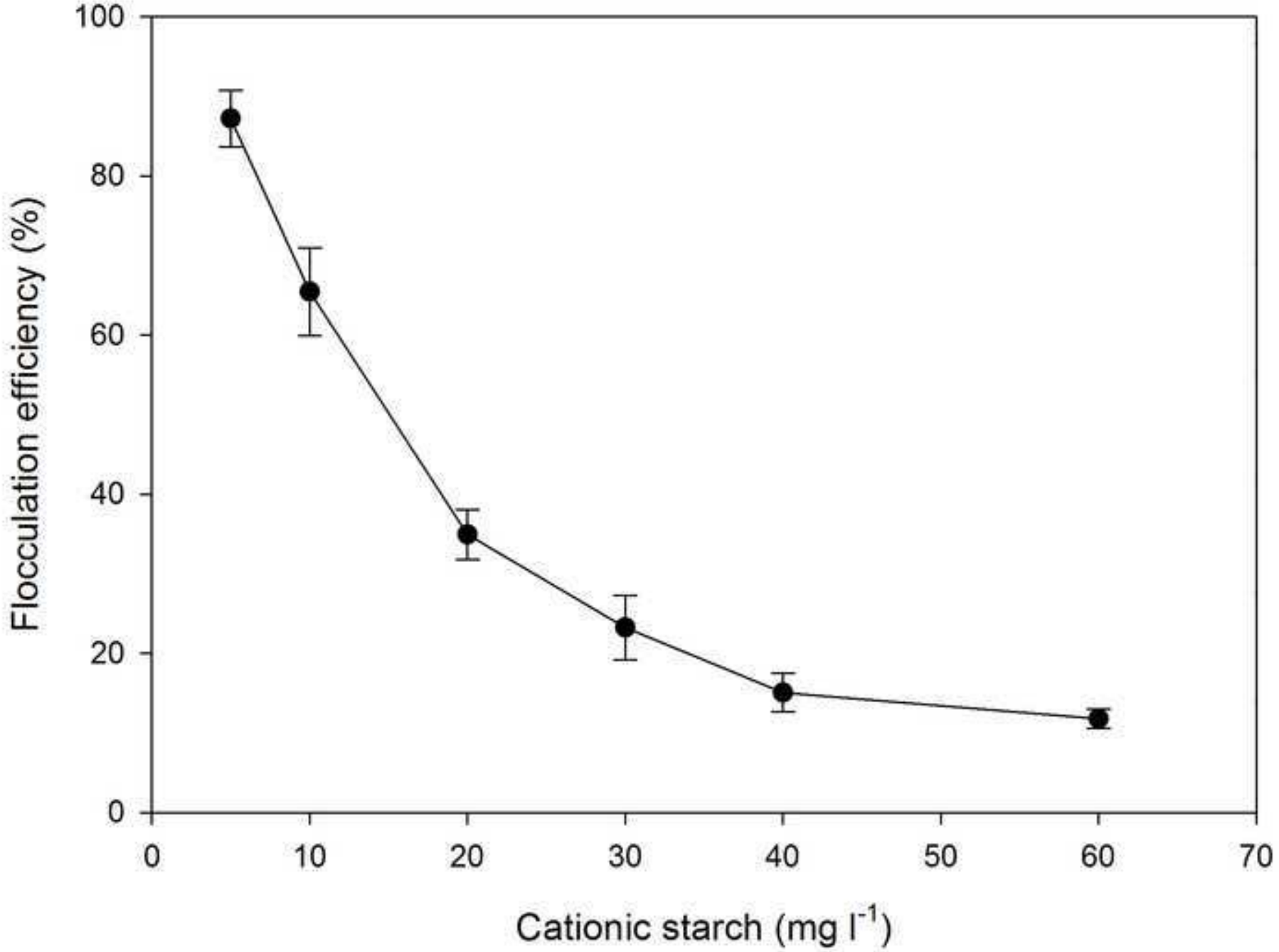

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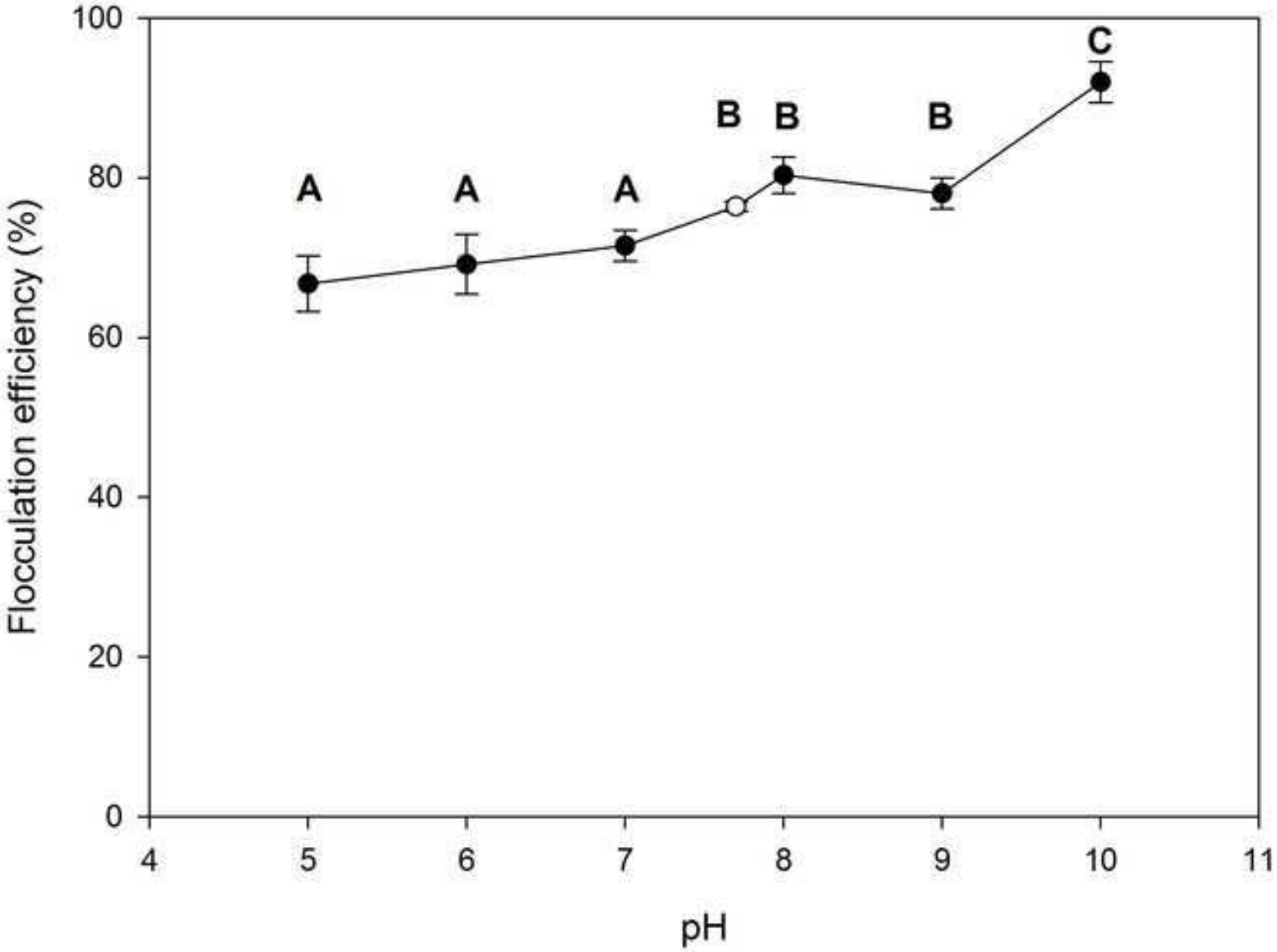

Figure 5

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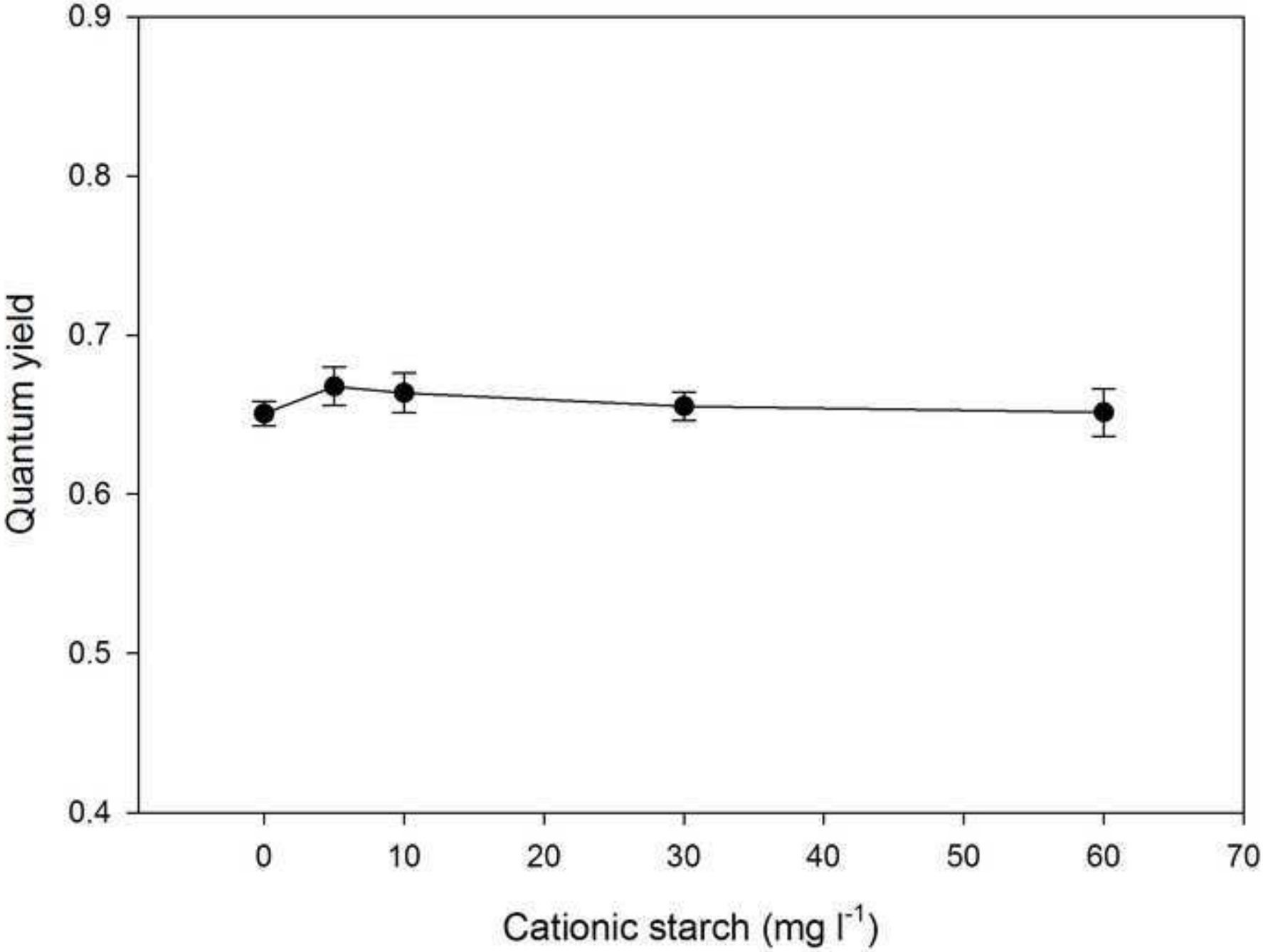

Figure 6
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