Synthesis and Characterization of Starch-based Cationic Flocculants for Harvesting Microalgae

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Dedication

This dissertation is dedicated to my dear grandpa. You have lightened my thirst for knowledge, that’s why I can pursue so much in these 25 years. Hope you live in peace in heaven.

Abstract

Harvest of tiny microalgae cells is a technically and economically challenging step in algal biomass production and utilization. Many techniques have been developed and tested, and some of them are used with limited success. However none of these techniques has broad commercial applications, especially in the area of algae based fuels production. The purpose of this thesis project was to develop a novel flocculation process which would be able to concentrate algal biomass, allowing efficient separation and collection of algal biomass from culture broth. The core of the research was to develop a procedure to synthesize starch-based cationic flocculants which would be renewable, biodegradable, and non-toxic with harvest performance comparable with or better than commercial flocculants. The procedure involves cationization reaction between starch and glycidyltrimethylammonium chloride (GTAC), where the hydroxyl groups on starch are substituted by quaternary ammonium cations with the help of alkaline catalyst. In this project, experiments were designed and carried out to study the effects of key reaction variables, namely temperature, time, GTAC dosage, water content, and catalyst dosage on the degree of substitution (DS), an indication of how well GTAC was utilized in the reaction, and reaction efficiency (RE). The DS and RE generally increased and then decreased with increasing temperature, time, water content, and catalyst dosage. An increase in GTAC dosage increased DS but decrease RE. Pretreatments of starch using acid, alkaline, and microwave did not significantly affect the DS and RE. The resultant cationic starch flocculants and cationic polyacrylamide (CPAM), a commercial flocculant, were tested in harvesting experiments involving live algae grown in fresh water or animal manure. The harvest efficiency was affected by DS of the cationic starch, flocculant dosage, pH, and flocculation time. The cationic starch based flocculant performed better than CPAM in all conditions. Pilot scale cationic starch production and harvest tests were conducted. The results agreed well with those obtained from the lab scale experiments. Based on these results, an optimized cationic starch synthesis procedure was proposed. The novel procedure has great potential for commercial production of renewable, biodegrable, and non-toxic starch based flocculants for cost effective and eco-friendly harvest of algal biomass.

Table of Contents

List of Tables

List of Figures

Background

There is increasing attention to microalgae which are a potential feedstock for production of renewable energy and products to substitute for the fossil fuel (Chisti 2007). The production of algal biomass can be coupled with flue gas CO_2 mitigation (Sawayama, Minowa et al. 1999), wastewater treatment (Wang, Min et al. 2010), and recovery of valuable resources in the form of the co-products such as biofuels, animal feeds (He, Hollwich et al. 2002), fertilizers, foods and high value chemicals and nutrient (Barclay, Meager et al. 1994) (Becker 2007). Developments in microalgae cultivation, harvesting, and downstream processing are expected to further enhance the cost effectiveness of biofuel and other products from microalgae (Li, Du et al. 2008). Hence, every step in the algae to fuel approach is considered to be important to the commercialization and sustainability of an algae-based fuel technology. The present study was focused on the development of cost effective harvesting techniques.

Harvesting Methods of Microalgae

Microalgae harvesting is thought to be one of the most difficult steps in microalgae cultivation and utilization, due to extremely small size (around 5-40µm) and low concentration (<1-3%) of algae cells in the culture broth. A number of harvesting techniques have been tested. They include sedimentation, filtration, flotation, flocculation, and electric method, as well as combinations of the above. Some of these traditional techniques are simple and easy to operate. For example, gravity sedimentation and simple filtration are good choice due to the low cost and ease of manipulation.

Flocculation is a process in which dispersed particles are aggregated together to form large particles for settling, and is considered one of the most convenient method.

Flotation, chemical flocculation, centrifugation, some combined processes, and new methods are being used or developed nowadays. Selection of a method must consider the shape, size, motility of algae cells and target products (Shelef, Sukenik et al. 1984; Vlaški, Van Breemen et al. 1997). Therefore it is very difficult to find one technique that fits all situations. Following is a brief discussion of the common harvesting techniques.

Sedimentation

Sedimentation is considered as a low cost simple technique because no chemicals and extra force other than gravity is used. This technique is limited to large cell strains which are capable of self-aggregating through a process called auto-flocculation (Demirbas 2010), forming ample size particles that settle down without any additives. However, in most cases, sedimentation is not usually used for algae cells harvesting but for algae suspension enrichment in industry (Mohn 1980). For small sized algae, gravity sedimentation must be combined with chemical flocculation to be useful for algae harvest (Shelef, Sukenik et al. 1984).

Filtration

Membrane is the key component in filtration method, and in some systems, extra force is added (pump in most case) in addition to gravity (Jaouen, Vandanjon et al. 1999) which could accelerate the water removal. Previous research on microbial collection also mentioned “drum filter” (Ludwig and Lochmann 2000) and “disc filter” (Pessoa and Vitolo 1998). The key issues with filtration are that the membrane is easily fouled by algal cells (Wilde, Benemann et al. 1991) and the residual water in the biomass is often high. Magnetic filtration was tested for algal mass removal in many lakes near Gainesville, Florida, and for some algae species/strains, the removal efficiency could reach 94% (Bitton, Fox et al. 1975). The tangential flow filtration was developed to remove microalgae of mixed strains from very low algae concentration liquid (Petrusevski, Bolier et al. 1995). The membrane with an average pore size of 0.45/μm was found to minimize membrane fouling, and was able to remove 70-89% of algae from the culture broth. However, in most cases, filtration is inefficient (Papazi, Makridis et al. 2010).

For the large-scale and high-density algae cultivation, cell packing is another serious problem (Dodson and Thomas 1964). Stirring or shaking the suspension was proven to be helpful for reducing cells accumulation in the pores of the membrane (Morris and Yentsch 1972). However, stirring a large amount of liquid will consume a lot of energy, and it is therefore not cost effective, and time consuming too. That is why filtration can only be used to harvest large cell species/strains in small quantities. As to small sized algae in large scale, the algae cells can be chemically agglomerated to form larger sized flocs (Petrusevski, Van Breemen et al. 1996), to reduce cell packing and membrane fouling.

AlgaeVenture, a private company in Ohio, developed a harvesting and dewatering system which utilizes a special fabric membrane with strong capillary water absorption capacity to remove water from algae paste while it travels on the moving belt (Fig. 1). The company claimed that the system is very energy and cost effective.

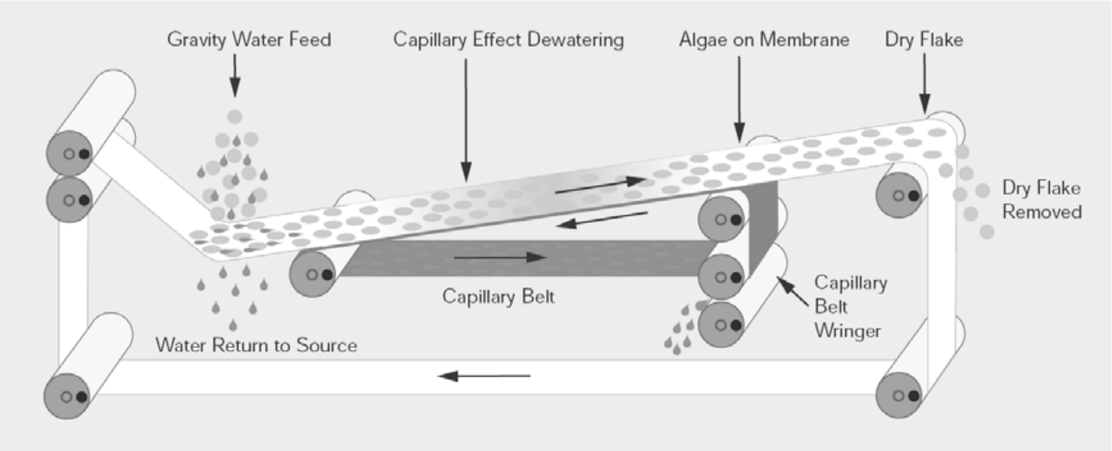


Fig. 1 AlgaeVenture microalgae harvesting and dewatering system

Flotation

Flotation was developed for clarifying drinking water (Edzwald 1993). In this method, suspended solid particles adhere to tiny bubbles created in the water through air injection, and float to the surface and form a particle-air-water foam which can be easily removed and collected. This principle should be applicable to algae removal.

Early research found that air flotation is a very effective way of concentrating or removing microalgae. In Fig. 2, the middle column is the original culture broth, the left one is harvested algae, and the right column is clarified water. They also reported that lowering the pH of the culture broth helped stabilize the foam, resulted in an improved algae recovery through froth flotation. However, the acidic condition could erode the algae growth (Levin, Clendenning et al. 1962). Acid condition may also corrode facilities and release inclusions into culture broth which might have some negative effect on algae growth and later applications. Flotation alone is still considered inefficient because of low removal rate and high residual water content of the harvested algal biomass. There is also serious water loss through evaporation during aeration (Levin, Clendenning et al. 1962).

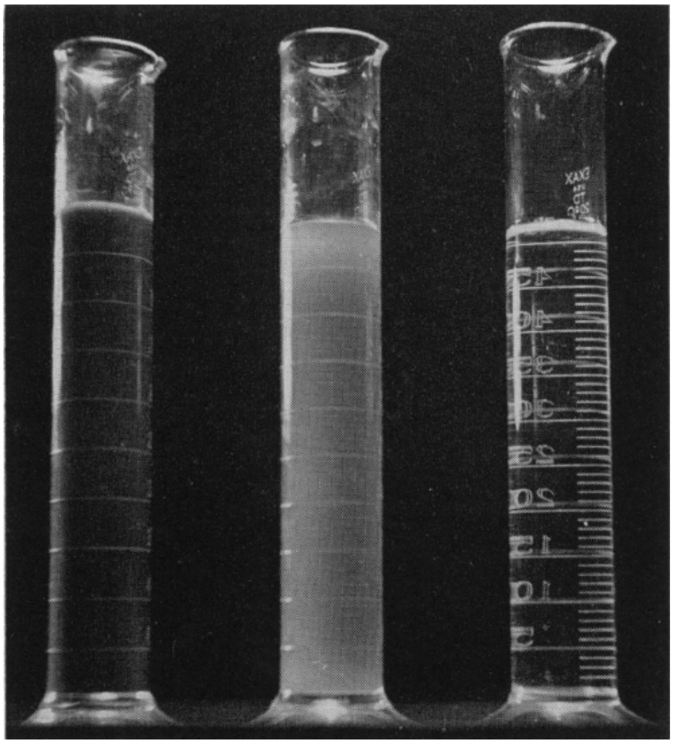


Fig. 2 Comparison of harvested, original, and clarified liquid of froth flotation (from left to right) (Levin, Clendenning et al. 1962)

Harvesting efficiency can be improved by combining flotation with other methods such as flocculation (Sandbank and Shelef 1987) and filtration, or by adding some other chemicals like coagulants (Chen, Liu et al. 1998) and ozone (Betzer, Argaman et al. 1980). Coagulant and surfactants were used in flotation process to greatly facilitate generation of fine air bubbles, effectively reduce the equipment size and energy consumption while improving harvest rate (Wiley, Brenneman et al. 2009). Sim et al.

designed an algae harvesting system which consists of flocculation, dissolved air flotation and continuous filtration, addition of chitosan. The harvesting efficiency could achieve 95%, and the harvesting cost was proven to be much cheaper than centrifugation.(Sim, Goh et al. 1988). Ozone is commonly used in treatment of drinking water and was found to also help remove algal cells and produce relatively low water content algae froth (8.5% solid content) because ozone can react with algae cell wall to result in a hydrophobic suspension amenable separation (Betzer, Argaman et al. 1980).

Centrifugation

High-speed centrifugation is a widely used method with reasonable algae recovery rate although it may collapse some algal cells during the process (Moraine, Shelef et al. 1979). Compared with other methods, algal pastes harvested using centrifugation have higher solid contents, usually above 15% (Sim, Goh et al. 1988). It was reported that a cream separator at a centrifuge force of 13000g, which is very high, could recover over 95% of algae cells with relatively low cell damage percentage (0-3%). Lower speed centrifugation 6000g was proven much less efficient (60% recovery) (Heasman, Diemar et al. 2000).

Similarly, centrifugation was used in combination with other processes in both pilot and bench scales. For example, centrifugal force was used to help the filtration to dewater algal mass. For large scale algae production, centrifugation is not practical because of the high costs needed to treat large volumes of culture broth, especially when the suspended algal mass content is low (Sim, Goh et al. 1988). However, centrifugation is an option for further removal of water from algal pastes which were harvested using other methods such as sedimentation, floatation, and flocculation.

Flocculation

Microalgae cells usually carry negative charges on their surface which keep the cells separated due to repulsion (Ives 1959). This property is important to the suspension of the cells in culture media for healthy growth but also can be utilized for harvest. Positively charged electrodes and cations containing polymers will attract algal cells to form flocs which can be easily collected. Both the electrode and polymer based methods have been studied (Sridhar, Namasivayam et al. 1988), (Alfafara, Nakano et al. 2002). It is widely believed that chemical flocculation is more convenient, more practical and faster than electrode and other conventional methods. In chemical flocculation, algae cells are agglomerated together and form larger particles which can be separated from water easily. Many researchers suggested that flocculation is the best way for algae harvesting in large scale.

Algae flocculation may occur if the pH of the culture medium is changed, and the degree of flocculation varies wth algal species and strains. Lime and $Mg(OH)_2$ were used to raise the pH to as high as 10-11 to cause flocculation (Folkman and Wachs 1973). Lowering pH can also be used to flocculate algae. One down side of pH based flocculation is that costly neutralization of the remaining water must be carried out before the water can be reused for cultivation. Furthermore, the extreme pH environment may result in algae cell lysis. In addition to pH adjustment, inorganic and organic flocculants are commonly used in wastewater treatment, and their uses in algae harvest have also been reported (Van Vuuren and Van Duuren 1965), (Tenney, Echelberger Jr et al. 1969). The fact that chemical flocculants are used in the wastewater treatment industry indicates the great potential of using chemical flocculants for algae harvest. The next section is therefore dedicated to the discussion of flocculants.

Flocculants

Inorganic Flocculants

Inorganic flocculants like aluminum and ferric salt as well as their polymeric compounds are being used in many places like sewage treatment and drinking water clarification (Yu, Fang et al. 2001). In theory, the positive metal ions capture the negatively charged algae cells, prompting many researchers to investigate the use of these flocculants for algae harvest (Chow, House et al. 1998) (McGarry 1970). Chow et al reported that ferric chloride keeps algae cells intact so that cell inclusion could be locked in the algae, which is good for subsequent recovery of valuable components from the harvested algae cells and avoiding water pollution (Chow, House et al. 1998). Use of a mixture of aluminum sulfate and polyelectrolyte in wastewater treatment ponds was shown to yield better flocculation than when they were used individually, suggesting that there were some sorts of synergetic effects between the two flocculants (McGarry 1970). The same study also showed that the flocculant concentration, stirring speed of the mix, and flocculation time were very important (McGarry 1970). Poly-aluminum chloride (PAC) was found to be more effective than monomeric aluminum chloride for flocculation (Gao, Chu et al. 2005). However, some reports indicate that the required dosage of inorganic flocculants is too high (Hejzlar, Dolejš et al. 1998), and the metal ions may adversely affect the downstream processing and utilization of the harvested algal biomass (Becker 1994).

Organic Flocculants

Organic flocculants can be divided to two groups: synthetic polymers such as polyacrylamide and polyethylene imines, and nature macromolecules such as chitosan and modified starch. These flocculants could be cationic, neutral, and anionic. Some studies indicated that only cationic polymers could produce satisfactory microalgae harvesting (Tilton, Murphy et al. 1972).

Industrial organic flocculants

Cationic polyacrylamide (CPAM) is commonly used due to its strong capability of agglomerating algae and low price (Salehizadeh and Shojaosadati 2001). However, though the polymer itself is nontoxic, its monomer is acrylamide, which has been proven to be a peripheral nerve toxin and a cancerogenic agent (Smith, Prues et al. 1996) (Rudén 2004) causing large bowel, kidney and bladder cancers in human (Mucci, Dickman et al. 2003). Commercial CPAM may contain residual monomer acrylamide due to incomplete polymerization. Some monomer acrylamide molecules may be released from CPAM due to degradation and depolymerization to a certain extent in application (Vers 1999) posing a potential risk to public health (Smith, Prues et al. 1996). CPAM will be banned for use in sludge treatment in Germany by the end of 2013 (Krentz, Lohmann et al. 2006).

Biobased flocculants

To address the issues with industrial polymeric flocculants such as CPAM, researchers looked into biobased materials and their derivates, such as chitosan, guar gum and starch (Salehizadeh, Vossoughi et al. 2000). Compared with CPAM, these biodegradable materials are usually toxin free, environmental-friendly, effective at low dosage, and not so susceptible to shearing force in liquid (Karmakar and Singh 1998; Singh, Karmakar et al. 2000).

Chitosan is derived from chitin of crustaceans' exoskeletons. The amino groups on the molecular chain backbone of chitosan are able to coagulate negatively charged suspended matters. It has been tested to harvest both freshwater algae and marine algae. It works very efficiently under appropriate temperature, mixing speed and time, and pH (Lavoie and Noüe 1983). Even in saline condition, chitosan is able to achieve satisfactory flocculation efficiency (Divakaran and Sivasankara Pillai 2002) (Morales, De La Noüe et al. 1985). Due to its natural and completely non-poisonous characteristic (Hirano, Itakura et al. 1990), the algae harvested using chitosan is nontoxic too. However, chitosan cost is considered to be too high for large scale algae harvesting (Ravi Kumar 2000).

Starch is an abundant biopolymer, which can be a cheaper alternative to chitosan. The –OH groups on the backbones of starch molecules can be substituted by positively charged groups such as quaternary ammonium (Pal, Mal et al. 2005), effectively turning starch into a cationic polymer which can attract negatively particles such as algae cells. A good choice of cationization reagents is possible to render cationic starch non-toxic and eco-friendly (Dencs, Nos et al. 2004). N-(3-chloro-2-hydroxypropyl)-N,N,N-trimethylammonium chloride (CHPTAC) and N-(glycidyl)-N,N,N-trimethylammonium chloride (GTAC) are two common etherifying agents (Nichifor, Stanciu et al. 2010). During the reaction, alkaline is catalyst (Fig. 3) (Wei, Cheng et al. 2008).

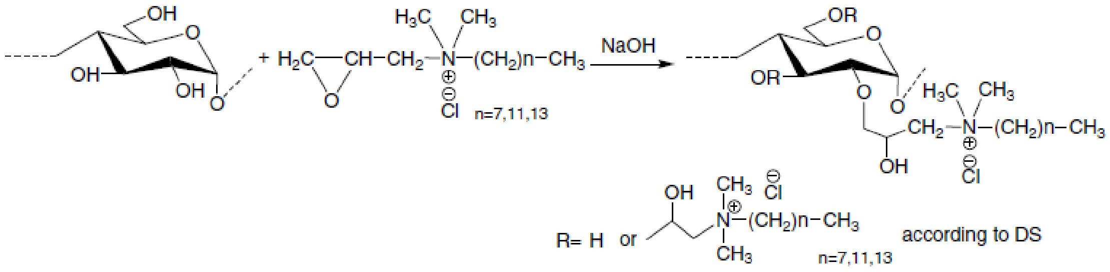


Fig. 3 Process of cationic starch synthesis

Cationic starch is used in municipal and mineral industry wastewater treatment and paper making industry to absorb the negatively charged cellulose fibers in white water (Nachtergaele 1989), and increase the paper strength (Eriksson, Pettersson et al. 2005). Cationic starch has the potential to replace expensive and hazardous inorganic and synthetic organic flocculants in wastewater treatments.

Cationic starch can also be used to collect negatively charged algae cells. Vandamme et al. reported that cationic starch was very effective in fresh water microalgae harvesting (Vandamme, Foubert et al. 2010). Cationic starch works under a wider range of pH value than those inorganic ones (Kato, Gyoten et al. 2004).

A parameter important to the flocculation performance of cationic starch is the degree of substitution (DS), a measure of the extent of cationization reaction and the molecular electro-strength that determines the absorption/flocculation capability. Each anhydroglucose unit has three OH groups, and hence the maximum DS is 3. Molecular weight (MW) is another important parameter that may influence the size of the flocculated particles and settlement velocity. The cationic starch currently available on the market has a relatively low DS and low harvesting rate for microalgae.

In this thesis project, improvements on cationic starch synthesis process were sought to enhance the performance cationic starch in harvesting microalgae.

Hypothesis

Microalgae have negative charges on the surface of cell wall, which can be attracted by positive charged substance. A cationic flocculant could react against a negatively charged suspension. In this project, the cationic starch flocculant will be synthesized by treating granules of starch with a reactive cationic regent which contains a positive charge. Bridging of segments of cationic starch chain attracting negative particles (microalgae) would help them aggregate. Meanwhile cationic starch will counterbalance the negative charge of microalgae particles. So cationic starch could be used to harvest microalgae. Theoretically, the higher the charges strength, the stronger the attraction force between particles. The charge strength of cationic starch is relative to the DS which will have direct influence on microalgae harvesting effiency. So appropriate DS of cationic starch should be obtained to achive better microalgae harvest effect.

During the synthesis process, many conditions may affect the degree of subsititution of cationic starch, and influence significance is different. We need to determine an “optimum” conditions combination of cationic starch synthesis.

Cationic polyacrylamide is considered to be an effective algae harvesting agent, and was applied widely in labs and greenhouses. However, its monomer is toxic so it is hypothetical that cationic starch could replace the cationic polyacrylamide in microalgae harvesting.

Goal and Objectives

The goal of this thesis project was to develop a novel flocculation process which would be able to concentrate algal biomass, allowing efficient separation and collection of algal biomass from culture broth by other techniques. The core of the research was to develop a procedure to synthesize starch-based cationic flocculants which would be renewable, biodegradable, and non-toxic with harvest performance comparable with commercial flocculants. The specific research objectives were:

1. to quantify the effects of key reaction variables on the degree of substation and reaction efficiency during synthesis,
2. to optimize the synthesis procedure,
3. to evaluate the harvest performance of the resultant cationic starch against CPAM, a commercial flocculant,
4. to test the synthesis procedure and flocculation in pilot scale.

Methods and Materials

Lab Scale Cationic Starch Synthesis

In this study, cationic starch was prepared by attaching positively charged groups onto the H of hydroxyl groups of the starch backbone with the help of base catalyst sodium hydroxide. Reaction variables studied included dosage of catalyst, water content, temperature, and polymerization time. Baseline procedure was used in making cationic starch. Five grams of corn starch (National starch, Bridgewater) were placed in a flask, 1 mol/L NaOH solution was prepared by NaOH powder (Fisher, Fair Lawn). A solution containing glycidyltrimethylammonium chloride (GTAC) 80% solution (Sigma, USA), 0.5 ml - 2ml 1 mol/L NaOH solution and 0.5ml -2ml distilled water was added. All the reactants in the flask were well mixed and allowed to react for different times ranging from 2 to 7 hours at temperature at 45-65 ℃ in a water bath. After the reaction completed, an excess amount of 95% alcohol (Sigma) was poured into the flask to stop the reaction and allow the modified starch to settle down. The obtained modified starch slurry was placed on a filter on a vacuum filtration funnel and washed with 70% alcohol twice and 95% alcohol once under vacuum to remove the cationization reagent and NaOH. The cake was dried under 60℃ for 6 hours. The dried modified starch cake was milled to fine powder which was the final starch based flocculant product.

Pilot scale Cationic Starch Synthesis under Optimized Conditions

Corn starch (National starch, Bridgewater), GTAC solid powder (Guofeng, China), NaOH (Fisher, Fair Lawn) and distilled water were mixed homogeneously as the optimized synthesis ratio at larger scale. Firstly, starch powder and solid etherifying agent were mixed and preheated at 90 ℃, then the liquids mixture was added, allowed to react at 90℃ for 160 min.

Pretreatment of Corn Starch Effect on DS and Harvest Rate

The reaction between starch and cationization agents depends very much on properties of functional groups on the starch molecules. Modifications of starch molecules prior to the reaction may affect the reaction and ultimately the substation rate and flocculation performance. In this study, we tested a number of treatments such as acid, alkaline and microwave heating. The total moisture content of the reaction mixtures in all pretreatment experiments was set to 32.7% which was found to be optimal in experiments described in “Lab scale cationic starch synthesis”. .

Acid Pretreatment

Dilute acid treatment could hydrolyze the glycosidic bonds between anhydroglucose units and change the composition ratio of amylose and amylopectin (Hoover 2000; Wang, Truong et al. 2003). This may alter the starch granules structure, viscosity, and texture and expose more reaction sites for cationization and hence increase the chance of substitution.

In this experiment, 0.5 ml 1 mol/L HCl solution was uniformly mixed with 5 grams starch powder for 5 minutes as pretreatment, followed by addition of a mixture of 2.0 ml 1 mol/L NaOH solution, 0.5 ml distilled water and 3 grams GTAC 80% solution and other synthesis steps as described in “*Lab scale cationic starch synthesis*”.

Alkaline Pretreatment

Alkaline functions similarly to acid in partially breaking down polysaccharide chains, which exposes more reaction sites (Gugliemelli, Weaver et al. 1969). NaOH was chosen for alkaline pretreatment because it was used as the catalyst in synthesis process.

In this experiment, 0.5 ml 1 mol/L NaOH solution was firstly mixed with 5 grams of corn starch powder and allowed to react for 5 minutes, followed by addition of a mixture of 1 ml 1 mol/L NaOH solution, 1.5 ml distilled water and 3 grams of GTAC 80% solution.

Microwave Pretreatment

Singh et al (Singh, Sethi et al. 2003) reported that microwave energy can cause breakage of starch chains. Adebowale and Lawal (Adebowale and Lawal 2003) found that the heat provided by microwave could also help swell the moist starch granules. We speculate that the structural and conformational changes caused by microwave heating may affect the cationization reaction.

In this experiment, 1.5 ml distilled water was added into flask as the microwave absorbent and then mixed with 5 grams of corn starch powder for 5 minutes. The starch slurry was heated in a microwave oven for 10 to 20 seconds at power level 5. The microwave heated corn starch was then subjected to the synthesis steps described in "*Lab scale cationic starch synthesis*".

Measurement of Degree of Substitution

Zero point one gram of cationic starch powder was dissolved in 100ml deionized water (80°C) in a 250ml beaker and stirred with a heated magnetic stirrer (11-498-7SH, Fisher, USA) at speed 3, till the solution became uniform (about 30 minutes). The final concentration of cationic starch solution was 1 gram per liter. The solution was allowed to cool to the room temperature prior to test for the nitrogen content "n" (mg/L) which was tested using total nitrogen kit (HACH Company, Germany) and spectrophotometer (Spectronic Instruments Genesys 5, USA).

The degree of substitution (DS) was calculated using the equation below:

$$DS = \frac{162(g/mol) \times \frac{n(mg/L)}{500(mg/L)}}{100(14g/mol) - 152.5(g/mol) \times \frac{n(mg/L)}{500(mg/L)}}$$

The coefficient 162 g/mol is the molecular weight of the residue of each anhydroglucose unit (AGU)monomer of starch, 14 g/mol is the molecular weight of nitrogen, and 152.5 g/mol is the molecular weight of GTAC group that was connected on to the starch back bone.

The reaction efficiency (RE) was calculated using following equation:

RE= DS / (Mass of GTAC / Mass of AGU) × 100%

Algae Culture

Algal biomass was produced in lab scale for flocculation tests. A locally isolated microalgae strain 10B (*Chlorella*) was cultured on TAP-Medium (Tris-Acetate-Phosphate) (Harris, Stern et al. 1989) contained (all quantities of the ingredients were in g/L for solid and ml/L for liquid): NH_4Cl (Fisher, Fair Lawn), 0.4; $MgSO_4 \cdot 7H_2O$ (Fisher, Fair Lawn), 0.1; $CaCl_2 \cdot 2H_2O$ (Fisher, Fair Lawn), 0.05; K_2HPO_4 (Fisher, Fair Lawn), 0.108; KH_2PO_4 (Fisher, Fair Lawn), 0.056; Tris (hydroxymethyl aminomethane) (Fisher, Fair Lawn), 2.42; glacial acetic acid (Fisher, Fair Lawn), 1; trace elements solution, 1; consisted of the components listed below (grams per liter) Na_2EDTA, 50; $ZnSO_4 \cdot 7H_2O$, 22; $CaCl_2 \cdot 2H_2O$, 0.05; H_3BO_3, 11.4; $MnCl_2 \cdot 4H_2O$, 5.06; $FeSO_4 \cdot 7H_2O$, 4.99; $CoCl_2 \cdot 6H_2O$, 1.61; $CuSO_4 \cdot 5H_2O$, 1.57; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1.10; and KOH 16. Algae seeds were cultured on TAP medium in three 250ml flasks on shaker. To produce algal biomass for the flocculation experiments, 100ml of the seed broth was inoculated on 1L TAP medium in 2L flasks on magnetic stirrers. All cultures were maintained at 20°C with artificial lighting at an illumination intensity of 80 μmol/(㎡/s). The algae density of the culture broth was monitored using a spectrophotometer at 550 nm according to a method by S Ahmed (Ahmed, Gilerson et al. 2004). The culture broth was considered ready for flocculation tests when its algae density reached 1g/L (dry weight), which is close to the normal algae density in industrial photobioreactor cultivation.

Flocculation Experiment

The original algae suspension (concentration over 1g/L) was adjusted using distilled water to 1g/L. The pH was adjusted to different values using 1 mol/L HCl or NaOH solution. The adjusted algae suspension was mixed with cationic starch solution in a 50 ml centrifugal tube at a ratio of 1:12,1:14, 1:16, 1:18, 1:20 (dry mass) respectively, and mixed on vortex (G-560, Fisher, Bohemia) at speed 10 for 10 seconds. After 30 minutes settling, the algae flocs formed a satisfied sedimentation (show a picture here). The sample in the middle part of liquid phase in the tube was taken out for test. The concentration of algae in the sample was measured using the spectrophotometer method described above. The harvest rate was calculated as:

HR (%)= (1-CH (g/L)) / 1 (g/L) x 100%

Here HR is the harvest rate, an indication of flocculation efficiency, and CH is the concentration of algae in supernatant after flocculation.

Scanning Electron Spectroscopy

SEM was used to observe delicate morphological features of algae and starch granules. Original corn starch, cationic starch, micro algae and flocculated micro algae were studied using a variable Pressure Scanning Electron M, S3500N, Hitachi, located in the Imaging Center in Department of Biological Science at University of Minnesota.

Results and Discussion

Effects of Process Variables on Degree of Substition and Reaction Efficiency

We first studied the effects of four process variables, namely temperature, reaction time, catalyst dosage, and water content on the reaction rate and degree of substitution (DS). The optimal reaction conditions were determined.

Temperature

High temperature can accelerate the reaction not only by increasing the contacts and collisions between starch and GTAC molecules, but also by enhancing starch gelatinization and swelling. Starch granule’s crystalline structure is so hard and compact that etherifying agents can hardly access the OH groups inside the particles in normal condition. In the presence of water and heat (at 60℃) starch granules swell (Bagley and Christianson 1982), and under higher temperature (between 70 and 82℃) they gelatinize (Lelievre 1974), which breaks down the intermolecular bonds of starch molecules, resulting in mobilized and more reactive starch molecules. However, too high temperature may lead to the positive groups (quaternary ammonium groups) of GTAC decomposition and conversion(Kavaliauskaite, Klimaviciute et al. 2008). Therefore we must find an optimal temperature range.

Figure 2 shows the changes in DS and RE as a function of temperature ranging from 45℃ to 65℃. DS and RE increased with increasing temperature in the range of 45 to 60 C and declined with further increase in temperature. The steep increase in DS and RE observed when temperatures were between 55 and 60℃ may be attributed to the onset of starch swelling (Bagley and Christianson 1982). At temperature above 60℃, thermal side reactions of quaternary ammonium groups resulted in decreased RE and DS. We therefore concluded that 60℃ is the optimal temperature under our reaction conditions.

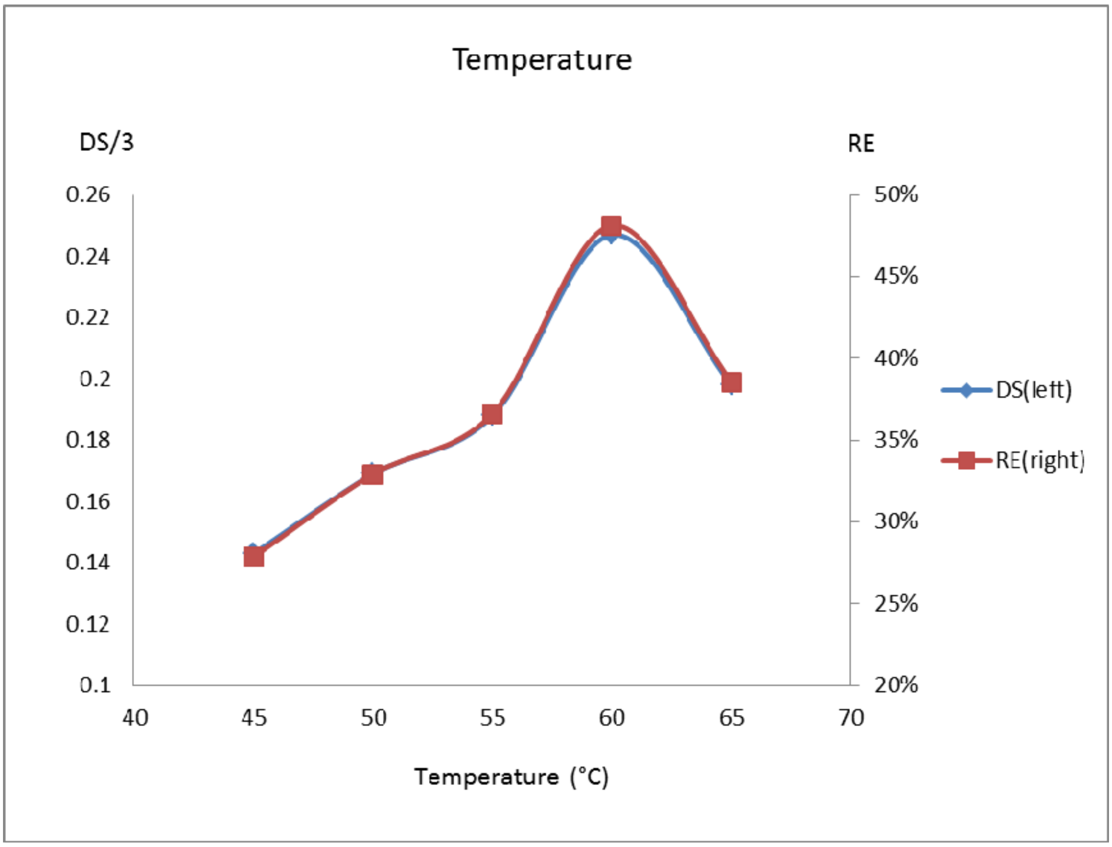


Fig. 4. Change in degree of substitution of cationic starch and reaction efficiency as a function of temperature while water content (50%), catalyst dosage (0.5 ml 1mol/L NaOH solution), reaction time (300 minutes), cationization reagent (GTAC) were kept constant.

Reaction Time

Starch cationization is a lengthy process. It usually requires several hours reaction time to reach a satisfactory utilization rate of the oxidation reagent and in some cases, several days at low temperatures are needed to reach high DS (e.g., close to 1) which may involve reactions between starch and epoxides (Kavaliauskaite, Klimaviciute et al. 2008).

To determine an appropriate reaction time, we studied the changes in RE and DS as a function of reaction time while keeping reaction temperature (60℃), water content (50%), and NaOH dosage (0.0005 mol) constant. Figure 5 shows that both RE and DS increased with increasing time up to 360 min and decreased thereafter. For prolonged reaction time, the reduced DS and RE is due to side reactions involving decomposition of positive charged groups on cationized starch.

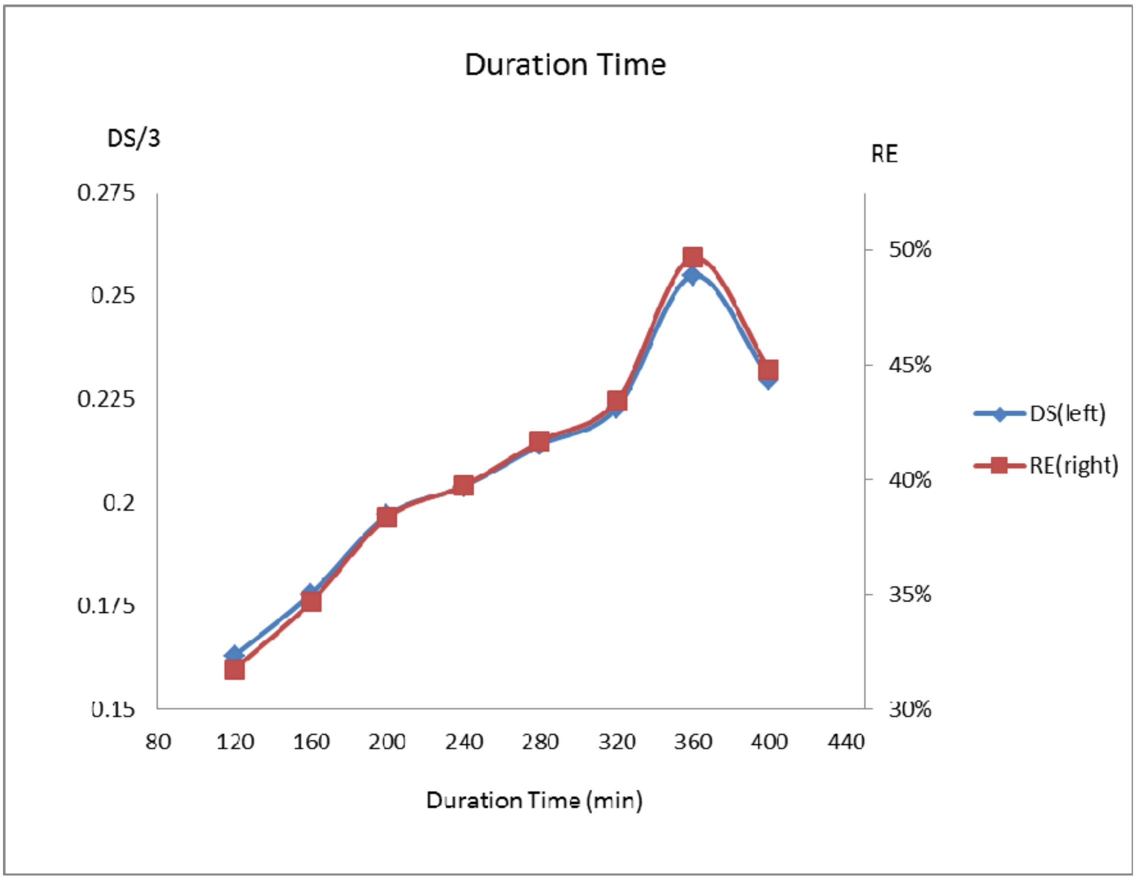


Fig. 5 Change in degree of substitution of cationic starch and reaction efficiency as a function of reaction time while water content (50%), catalyst dosage (0.5 ml 1mol/L

NaOH solution), reaction temperature (60 ºC cationization reagent (GTAC) were kept constant.

Catalyst Dosage

Both DS and RE increased rapidly with increasing catalyst dosage up to 0.001 mol NaOH and decreased gradually when the catalyst dosage was above 0.0015 mol NaOH (Figure 6). It is believed that NaOH provides additional hydroxyl ions in the reaction system so that the chemical reaction equilibrium shifts towards products (cationic starch) (Khalil and Farag 1998) . However, too much NaOH may cause many other side reactions of cationic starch (Carr 1994) due to enhanced hydrolysis of epoxy groups, and produce the N-(2,3-dihydroxy)propyl-N,N,N-trimethylammonium chloride under alkaline condition (Goclik, Stach et al. 2004). De-etherification reaction become more serious under high pH circumstances (Khalil and Farag 1998). These may be the main reasons for the decrease in DS and RE at NaOH concentration above 0.0015 mol. Furthermore, a high NaOH concentration may trigger side reactions between NaOH and GTAC too(Kavaliauskaite, Klimaviciute et al. 2008).

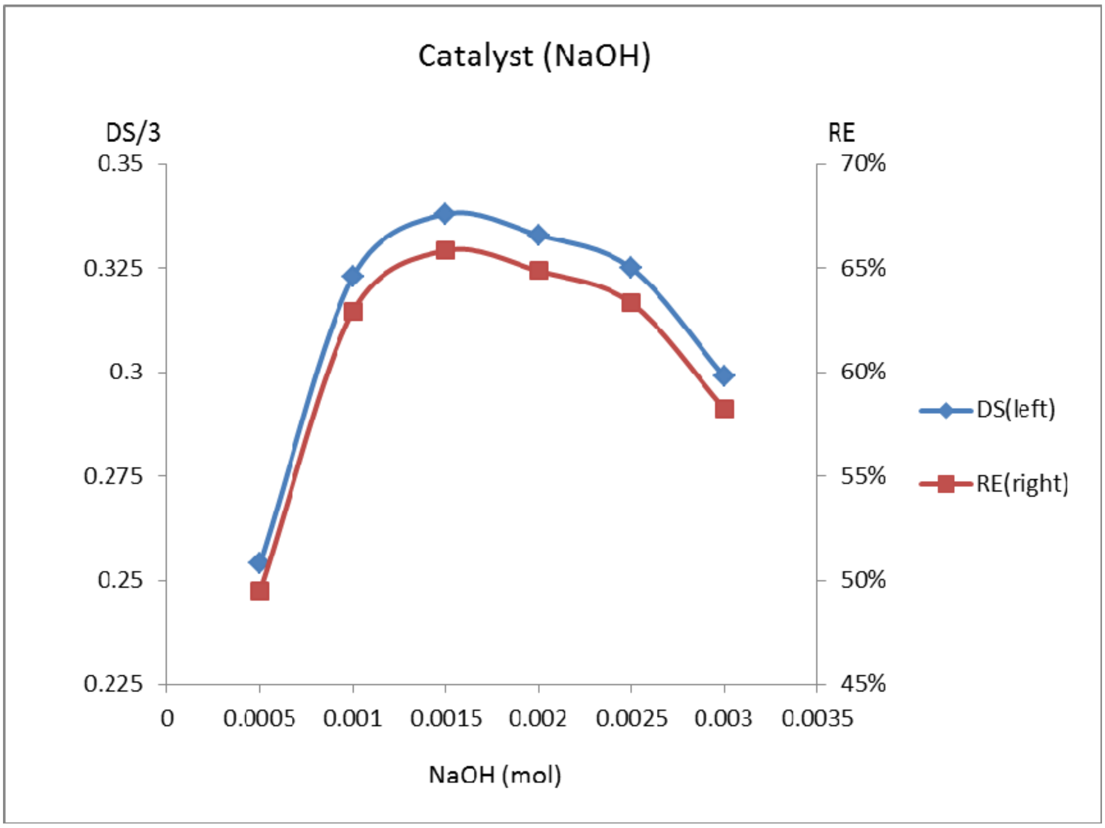


Fig. 6. Change in degree of substitution of cationic starch and reaction efficiency as a function of catalyst dosage while water content (50%), d reaction time (300 minutes), reaction temperature (60 C), and cationization reagent (GTAC) were kept constant.

Water Content

Water acts as a dispersant and medium in many chemical reactions which involve diffusion of reactants for efficient molecular collisions. Further to the reaction leading to starch cationization, water is required for starch swelling. It is therefore necessary to study the effect of water content on the RE and DS in our study.

Figure 7 shows that when the system water content was below 33%, the reaction efficiency increases with increasing water content, which may be attributed to the better mixing, lowered viscosity, increased reactant diffusion, and starch gelatinization. .

Further increase in moisture content led to decrease in RE and DS. Too much water may result in reactant dilution, and is etherifying agent GTAC hydrolysis(Khalil and Farag 1998) and hence reduction in overall RE and DS.

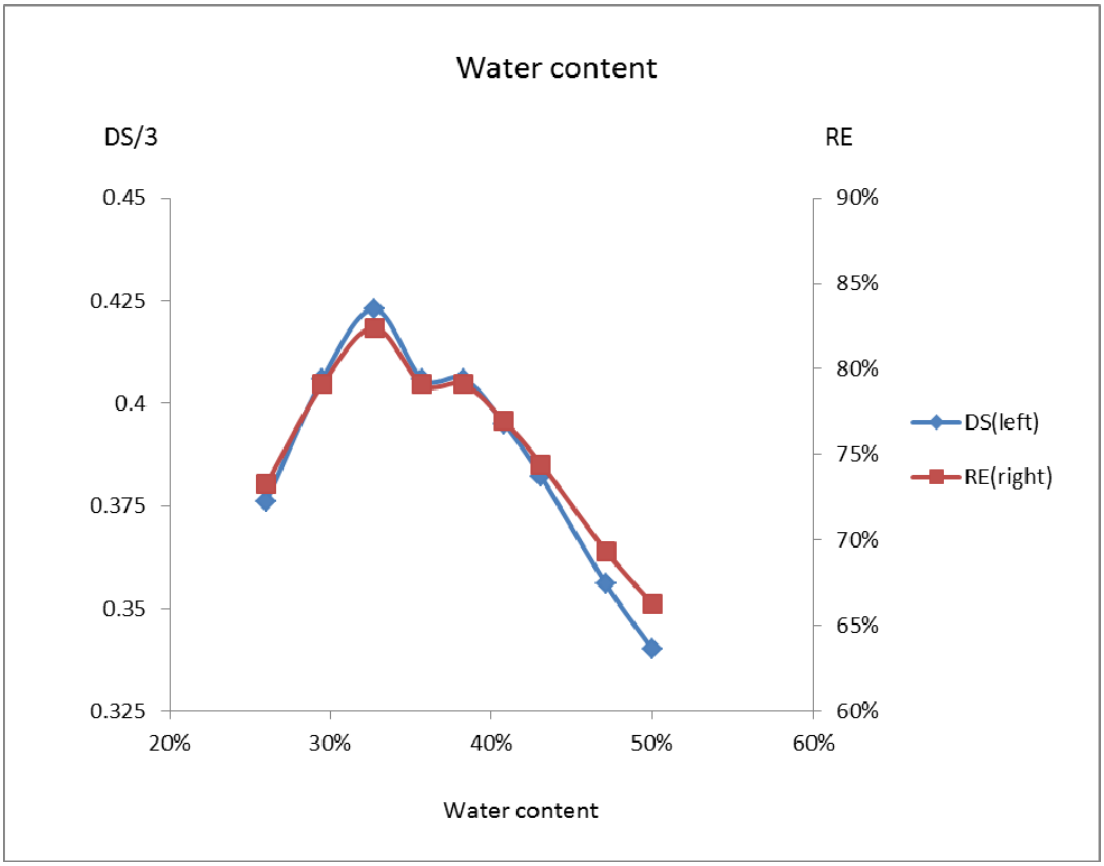


Fig. 7. Change in degree of substitution of cationic starch and reaction efficiency as a function of water content while reaction time (300 minutes), catalyst dosage (0.0015 mol), cationization reagent (GTAC), and reaction temperature (60 ºC) were kept constant.

Interaction of Temperature and Reaction Time

There is usually an inverse relationship between reaction rate and temperature, i.e., a higher reaction rate is expected at a higher temperature. However, as demonstrated earlier RE and DS decreased when temperature was above 60°C, indicating that a high temperature is not always suitable for our case. Figure 8 shows a series of reaction curves (reaction time vs DS) at different temperatures. All the curves share the same parabola shape with one maximum. The curves shift from right to left when temperature was increased, indicating that the reaction reached its maximum quicker when temperatures were higher. Lower temperatures appear to give rise to higher maxima at long reaction time. Highest DS values were observed when temperatures were at 50-60C. However, the fact that a short reaction time of 160 min with DS value of 0.38 at 90°C compared with a long reaction time of 360 min with DS value of 0.42 obtained at 60°C suggests that it is worth considering high temperature short time process in large scale industry production of cationic starch for high productivity. Further study to assess the energy consumption between high temperature short time and low temperature long time process schemes is necessary.

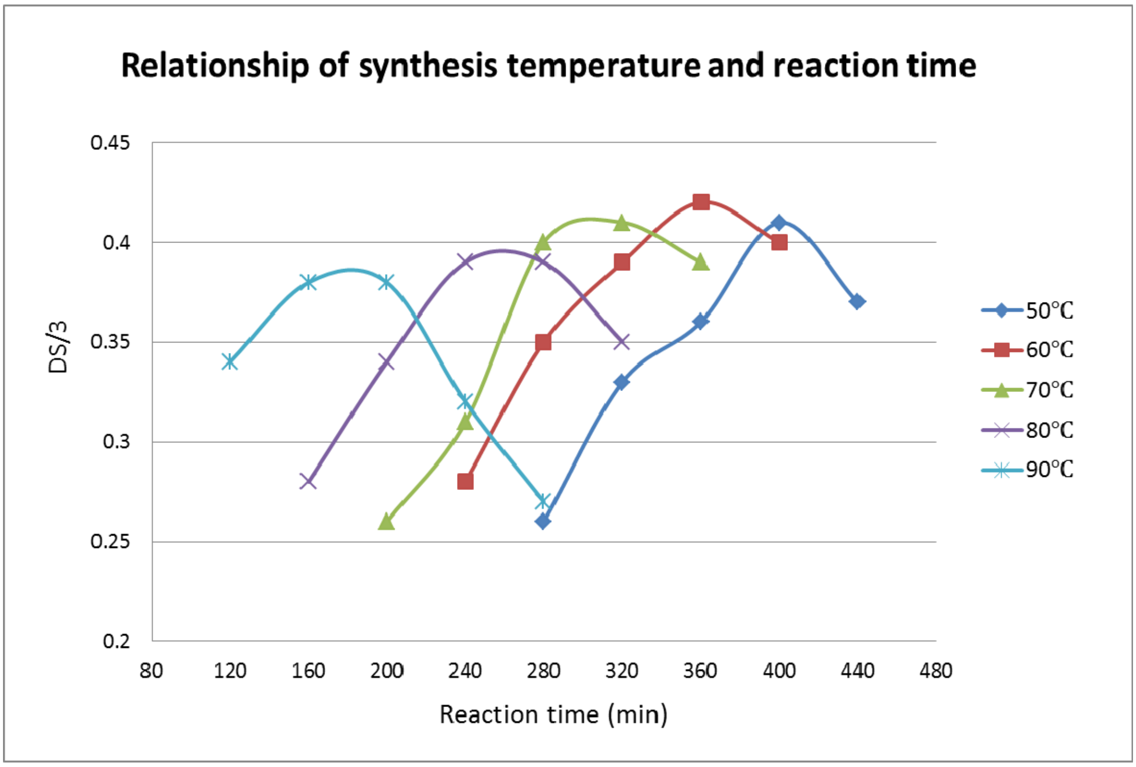


Fig 8. DS affected by the relationship of both synthesis temperature and reaction time.

Cationization Reagent Dosage

Figure 9 shows that the DS increased and RE decreased with increasing cationization reagent. As expected, an increase in the concentration of reactants will shift the reaction equilibrium towards products but on the other hand may lower the efficiency of reactants utilization. For example, the utilization efficiency was about 97% when the GTAC was only 1 gram but dropped down to 71.8% when the GTAC was increased to 4.5 grams.

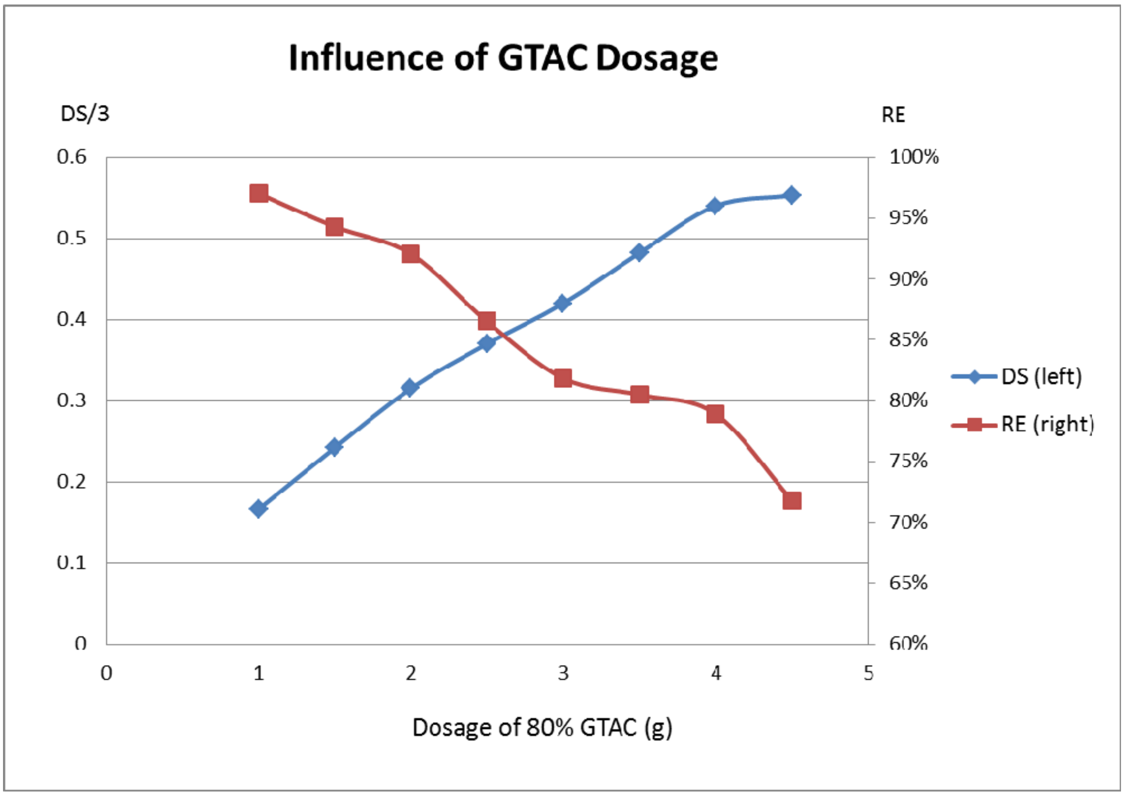


Fig 9. DS and RE affected by dosage of cationization reagent

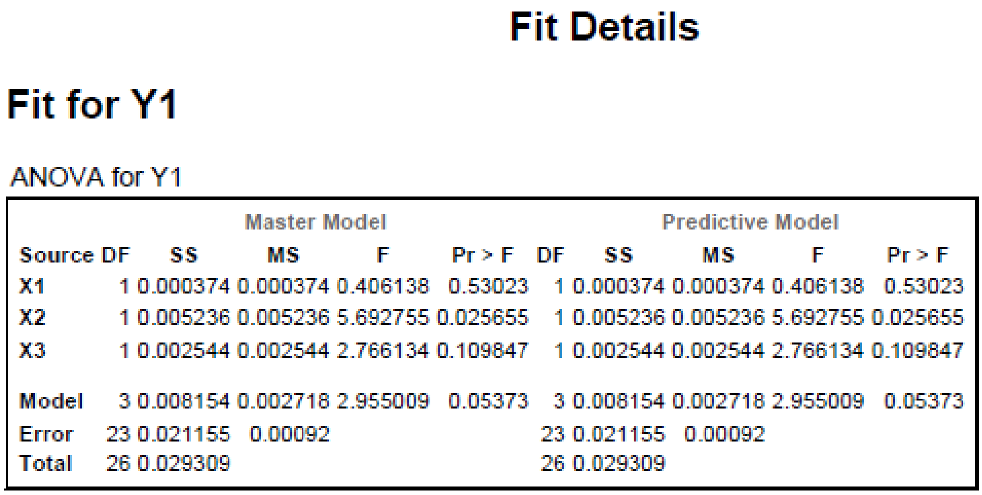
The Most Significant Factor Influencing Cationic Starch Synthesis

Three factors and 27 groups of synthesis samples were set in a full arrest test. Calculation of variance was applied to analysis these data.

#	Water content (mass ratio)	Catalyst (mol)	Reaction time (min)	DS/3	Reaction rate
1	0.37	0.0015	320	0.46	0.672
2	0.41	0.0015	320	0.446	0.651
3	0.45	0.0015	320	0.432	0.631
4	0.37	0.002	320	0.499	0.729
5	0.41	0.002	320	0.519	0.759
6	0.45	0.002	320	0.524	0.766
7	0.37	0.0025	320	0.524	0.766

8	0.41	0.0025	320	0.489	0.715
9	0.45	0.0025	320	0.479	0.7
10	0.37	0.0015	360	0.437	0.638
11	0.41	0.0015	360	0.432	0.632
12	0.45	0.0015	360	0.504	0.736
13	0.37	0.002	360	0.509	0.744
14	0.41	0.002	360	0.54	0.789
15	0.45	0.002	360	0.535	0.781
16	0.37	0.0025	360	0.479	0.7
17	0.41	0.0025	360	0.489	0.715
18	0.45	0.0025	360	0.484	0.707
19	0.37	0.0015	400	0.428	0.625
20	0.41	0.0015	400	0.428	0.625
21	0.45	0.0015	400	0.455	0.665
22	0.37	0.002	400	0.479	0.7
23	0.41	0.002	400	0.494	0.722
24	0.45	0.002	400	0.489	0.715
25	0.37	0.0025	400	0.465	0.679
26	0.41	0.0025	400	0.46	0.672
27	0.45	0.0025	400	0.46	0.672

Table 1. Table of the most three significantly influence factors of cationic starch synthesis

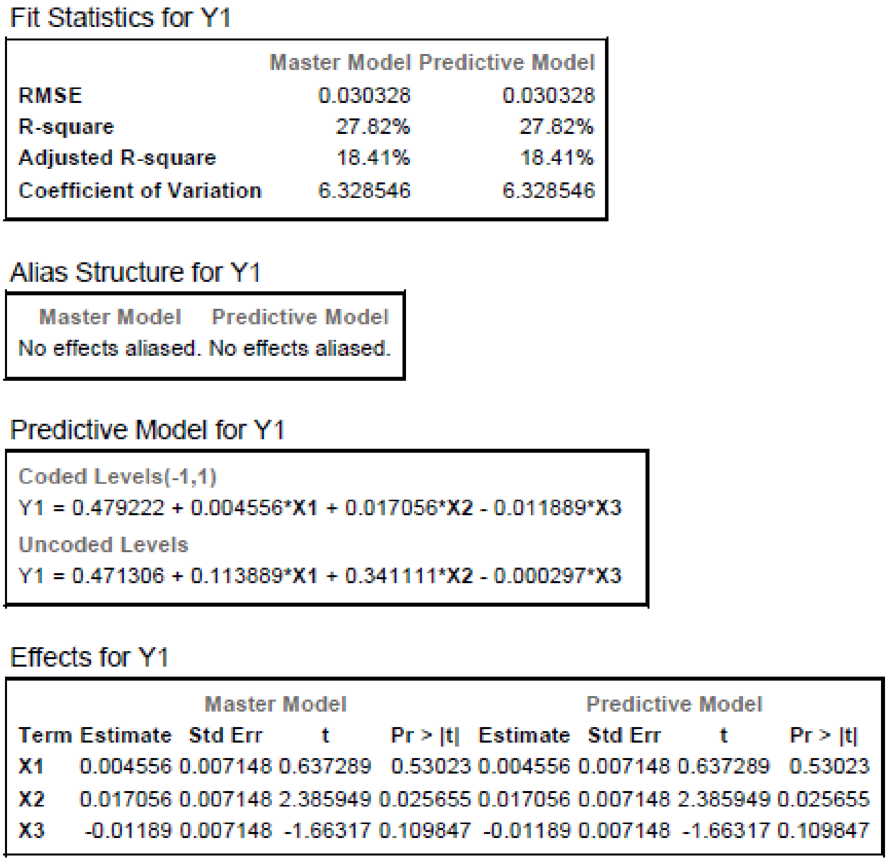


Fit Details

Fit for Y1

ANOVA for Y1

	Master Model					Predictive Model				
Source	**DF**	**SS**	**MS**	**F**	**Pr > F**	**DF**	**SS**	**MS**	**F**	**Pr > F**
X1	1	0.000374	0.000374	0.406138	0.53023	1	0.000374	0.000374	0.406138	0.53023
X2	1	0.005236	0.005236	5.692755	0.025655	1	0.005236	0.005236	5.692755	0.025655
X3	1	0.002544	0.002544	2.766134	0.109847	1	0.002544	0.002544	2.766134	0.109847
Model	3	0.008154	0.002718	2.955009	0.05373	3	0.008154	0.002718	2.955009	0.05373
Error	23	0.021155	0.00092			23	0.021155	0.00092		
Total	26	0.029309				26	0.029309			



Fit Statistics for Y1

	Master Model	Predictive Model
RMSE	0.030328	0.030328
R-square	27.82%	27.82%
Adjusted R-square	18.41%	18.41%
Coefficient of Variation	6.328546	6.328546

Alias Structure for Y1

Master Model	Predictive Model
No effects aliased.	No effects aliased.

Predictive Model for Y1

Coded Levels(-1,1)

Y1 = 0.479222 + 0.004556***X1** + 0.017056***X2** - 0.011889***X3**

Uncoded Levels

Y1 = 0.471306 + 0.113889***X1** + 0.341111***X2** - 0.000297***X3**

Effects for Y1

	Master Model				Predictive Model			
Term	**Estimate**	**Std Err**	**t**	**Pr > |t|**	**Estimate**	**Std Err**	**t**	**Pr > |t|**
X1	0.004556	0.007148	0.637289	0.53023	0.004556	0.007148	0.637289	0.53023
X2	0.017056	0.007148	2.385949	0.025655	0.017056	0.007148	2.385949	0.025655
X3	-0.01189	0.007148	-1.66317	0.109847	-0.01189	0.007148	-1.66317	0.109847

Table 2. Forms of fit details of the most significant affect condition

Data was analyzed through ANOVA using the master model in SAS software. The usage of catalyst significantly influenced the DS ($P<0.05$) while the other two factor including water content and reaction time didn't show obivous effect on DS ($P>0.05$). The order significance was: catalyst dosage > reaction temperature > system water content.

Effects of Pretreatments on RE and DS

Acid, alkaline and microwave pretreatment may cause structural change of starch chains, which may influence molecular weight and viscosity alternation and other effects on flocculation experiment, so it needs to be discussed separately.

We tried three methods of corn starch pretreatments to test improvement of degree of substitution and reaction efficiency of cationic starch. Firstly, the acid and base pretreatment showed only a slight improvement of reaction efficiency but was not significant. Acid pretreatment was better than base (Table. 1). In the table, DS is the dimensionless value, and all the values in this form are average data from 3 parallel experiments.

Method	DS/3	RE
Control	0.423	82.4%
Acid	0.437	85.1%
Alkaline	0.428	83.3%
Microwave 10s	0.410	79.8%
Microwave 15s	0.416	81.0%
Microwave 20s	0.414	80.2%

Table 3. Reaction effected by different pre-treating ways

Flocculation of Microalgae with Cationic Starch

Relationship Between Harvesting Efficiency and Degree of Substitution (DS)

Flocculation using the cationic starch was carried out with a dry mass ratio of algae to cationic starch at 16:1, under the pH of 6.67, which is the original pH of culture broth, and flocculation time 40 minutes. Figure 10 shows that the harvesting efficiency increased with increasing DS up to 0.41 where 94% of algae cells were removed and then decreased. A higher DS gave rise to higher amount of positively charged groups which are capable of binding negatively charged algae cells, resulting in a higher harvesting efficiency. When the DS is too high, starch chains may become too stiff (Bratskaya, Schwarz et al. 2005), due to the mutual exclusion between positive charge as well as the steric clash on ammonia groups, which will reduce their ability of capturing the negative charged algae cells.

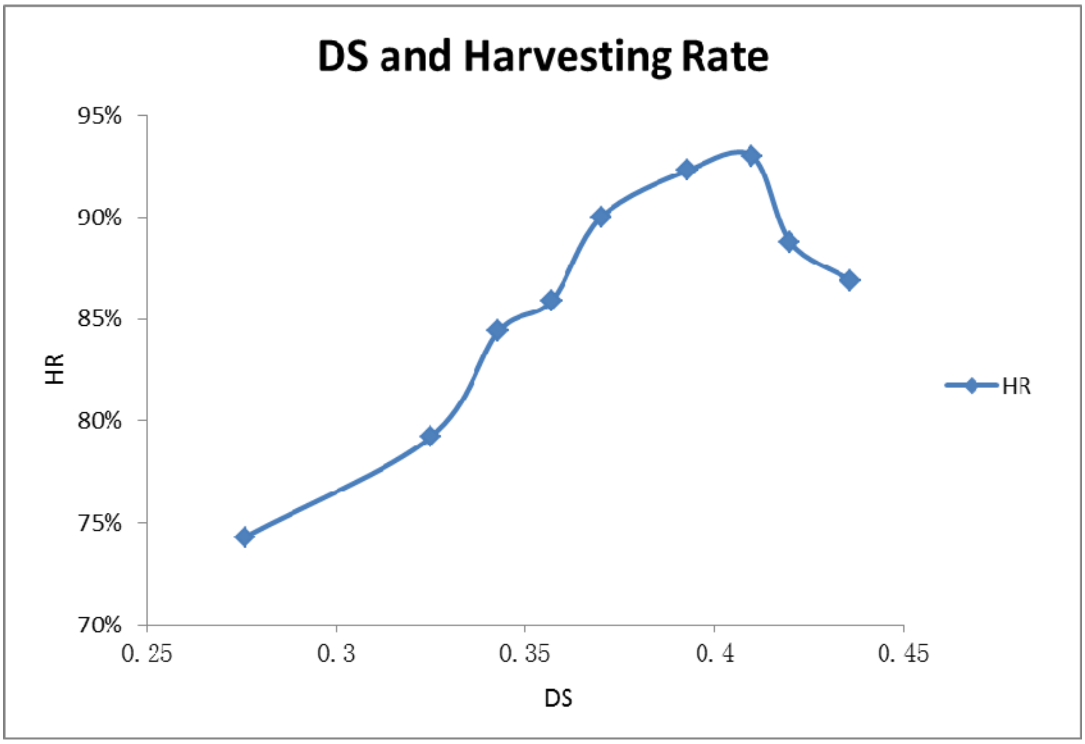


Fig. 10. Harvesting efficiency affected by degree of substitution of cationic starch

Since DS of 0.41 is the best for microalgae harvesting, an optimized reaction condition (reaction time: 360 minutes, catalyst dosage: 0.0015 mol, GTAC: 2.85g, and reaction temperature: 60°C) was used to produce cationic starch with DS value of 0.41, which was used for the rest of algae flocculation and harvest experiments.

Effect pH of Culturing Medium on Flocculation and Harvest

Cationic starch can be used in a wide range of pH values but perform better at weak alkaline conditions than neutral and acid condition because the quaternary ammonium group is stable under high pH value condition. In our study, the harvesting efficiency was above 90% at around the natural pH value (6.67) of the algae culture broth and the highest harvesting efficiency was obtained at pH around 8.3-8.5 (Figure 11). A solution’s pH is known to affect the surface charges of the particles in the solution. It is quite possible that the changes in the electrical properties of the surfaces of algae cells and cationic starch at different pH values affect the interactions between algae cells and cationic starch, and hence the flocculation efficiency.

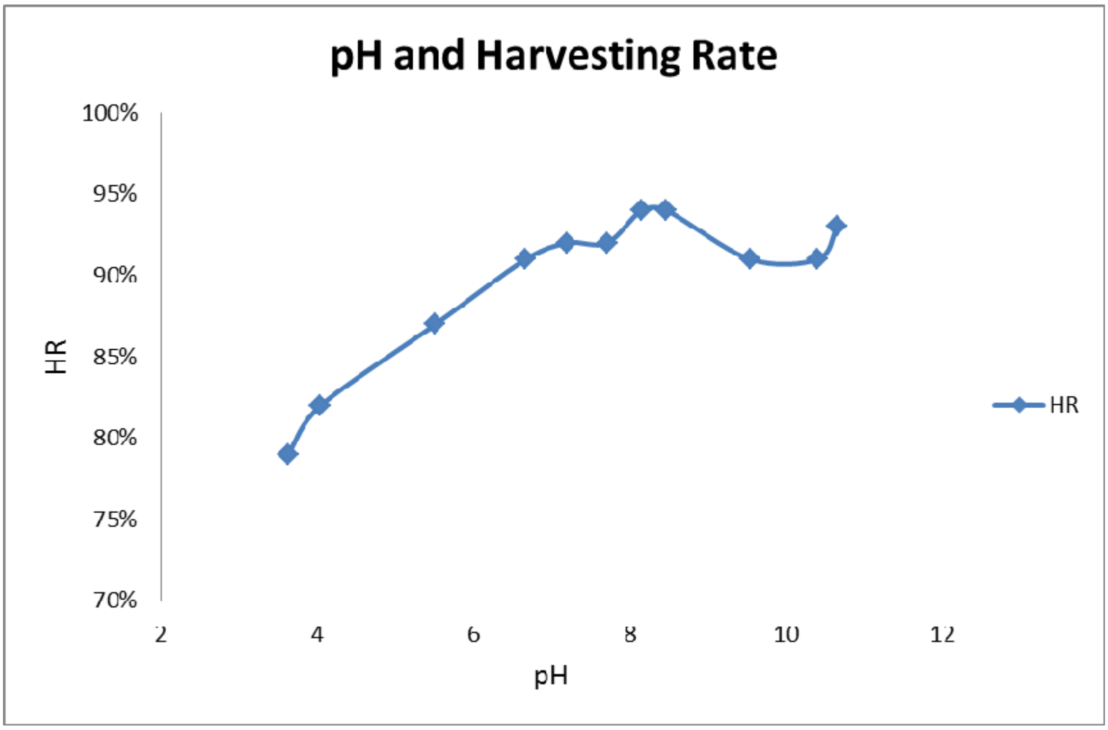


Fig. 11 Flocculation ability affected by pH value of the algae broth

For the pH higher than 10, the high HR is mainly because the algae surface charge has been destroyed under strong base environment. The repulsion force between algae fade away and then algal cell could agglograte together.

Compared to a flocculation experiment with industrial cationic starch (Greenfloc 120) in figue 12, algal biomass and cationic starch at ratio of 6:1 dry base and yielded highest harvest rate at about 80% under mild alkaline condition. Our cationic starch can get much higher harvest rate (95%) at the ratio of 16:1, and the dosage is about 200% lower than the Greenfloc 120.

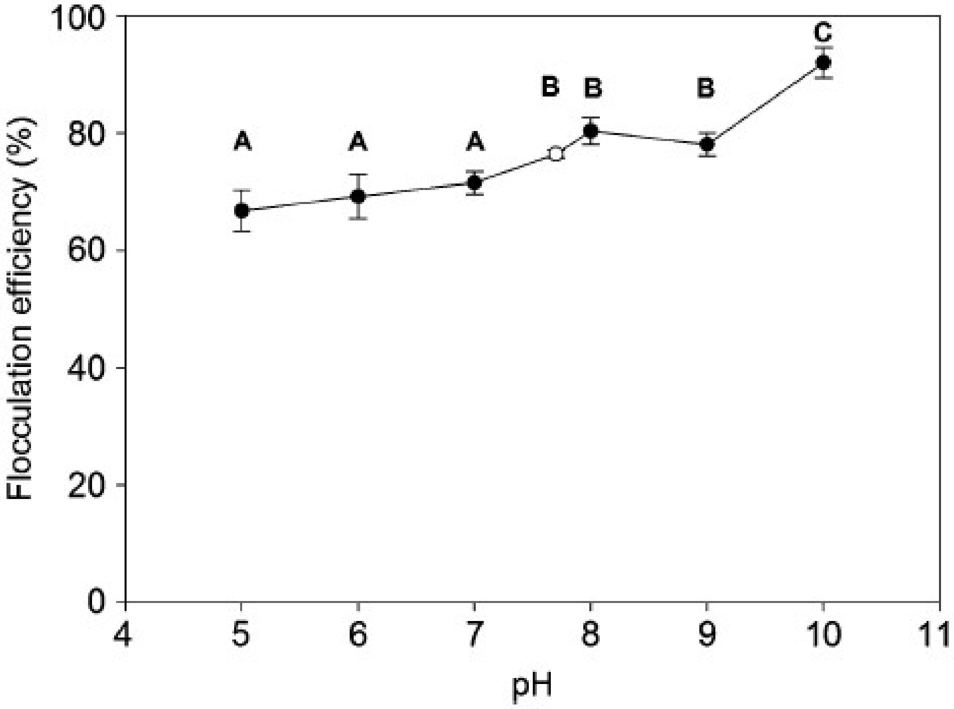


Fig 12. Microalgae harvest efficiency by industrial cationic starch affected by pH of algal culturing broth (Vandamme, Foubert et al. 2010)

Effect of Flocculation Time on Harvest Efficiency

After the cationic starch was added to an algae culture broth, the algae cells settled quickly to the bottom. The data in Fig. 13 illustrates that harvest efficiency increased rapidly in the first 15 min of flocculation and rose only slightly afterwards. With a dry mass ratio of cationic starch: algae at 1:18, a flocculation time in the range of 15-20 min should be sufficient to achieve over 90% harvest rate in industrial setting.

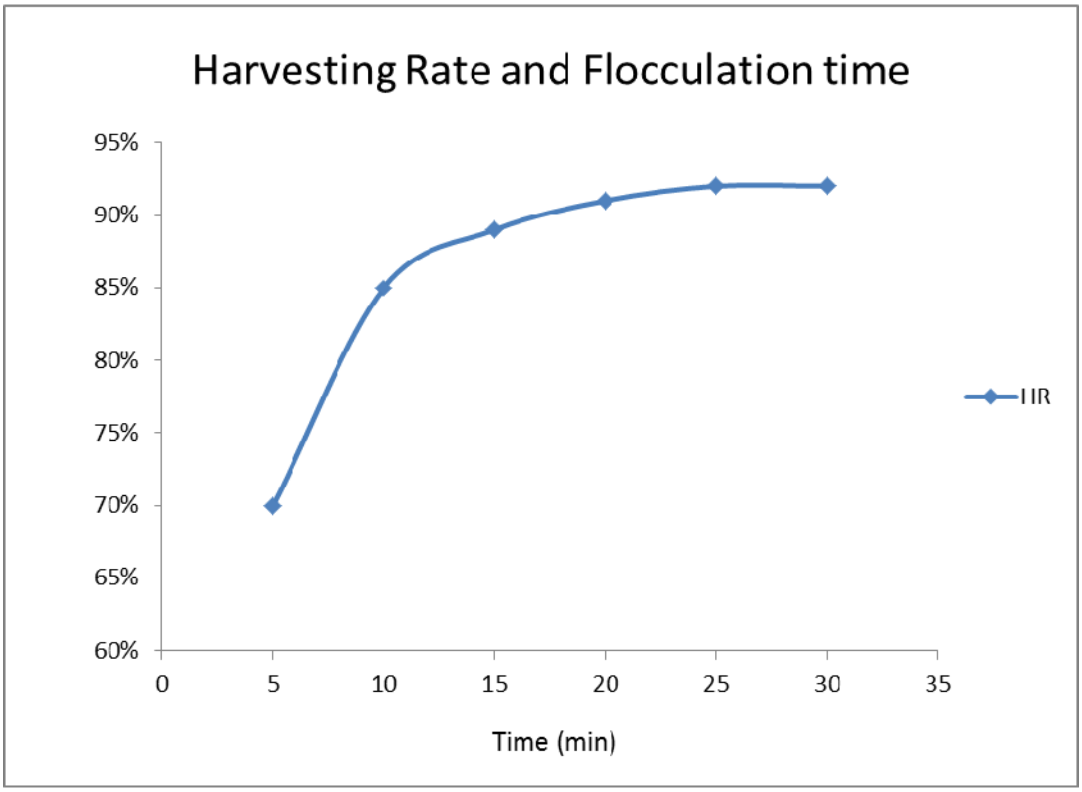


Fig 13. Microalgae harvest efficiency influenced by flocculation time

Comparison Between CPAM and Cationic Starch for Harvesting Fresh Water Grown Algae

To provide a meaningful assessment of our cationic starch in perspective, we compared the performance of our cationic starch with that of CPAM (cationic polyacrylamide), a commercial flocculant which is widely used in wastewater treatment and being tested for algae harvest. The flocculation time was set to 30 min, and pH broth was set to 8.5. The ratio of algae to flocculant varied from 10 to 45 (a higher ratio indicates a lower usage of flocculant). Figure 14 shows that our cationic starch performed better (about 10%-15% higher) than CPAM at all ratios. The algae:flocculant ratios in the range from 20:1 to 13:1 are best. The decline in harvest efficiency with increasing flocculant at the high flocculant concentration end is probably due to increase in viscosity and charges which slowed down the sedimentation of algae flocs. Too high a concentration of flocculant may adversely affect the recycle of water for algae cultivation. Our data suggest that a ratio of 15:1 is suitable for harvest of algae grown in fresh water in lab scale.

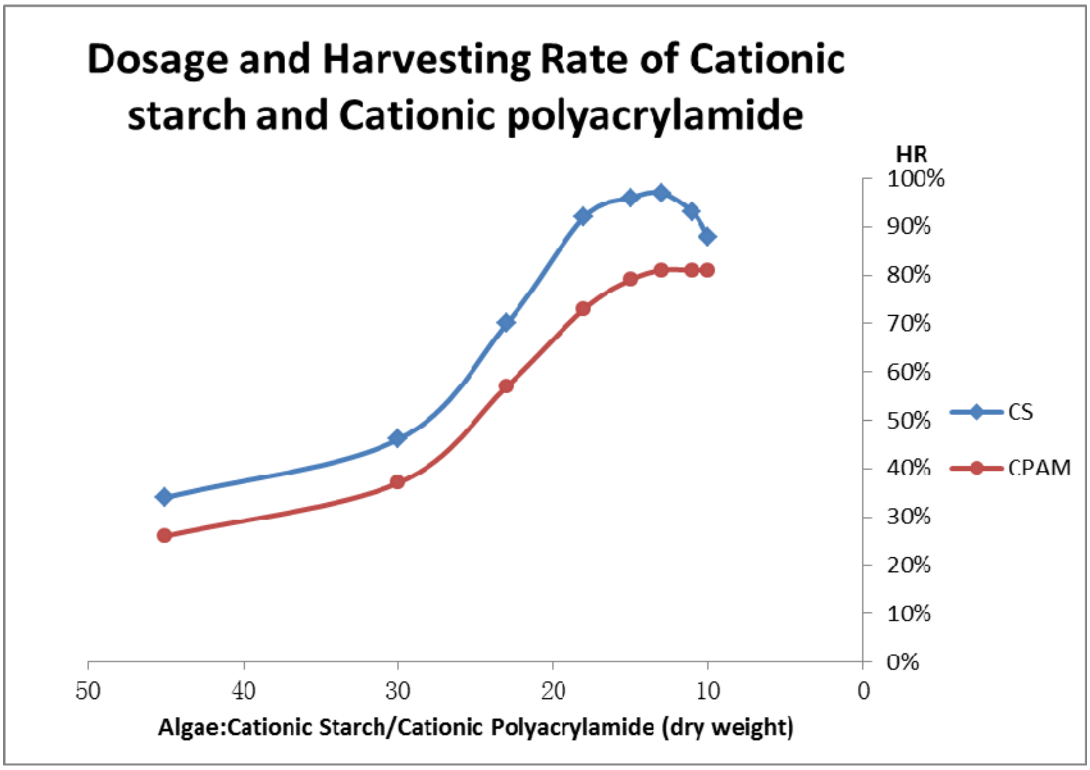


Fig 14. Harvest efficiency as a function of algae to flocculant ratio.

Comparison Between CPAM and CS for Harvesting Manure Grown Microalgae

Growing algal biomass on wastewater is considered an economically and environmentally viable approach. While we have demonstrated the excellent performance of our cationic starch in harvesting fresh water grown algae, this cationic starch's performance in wastewater grown algae is unknown. Due to the variation in manure nutrient profile, it was difficult to measure the component ratio of algae and manure, so we can not use algae concentration and dry weight of biomass here to reflect the harvest efficiency. So we only OD value was used to show the clarification capability of flocculants: the lower the OD stands for the higher biomass clarification efficiency.

The result (Figure 15) shows that the cationic starch has a better ability than CPAM to clarify the manure cultured algal suspension, which agrees with the result obtained with fresh water grown algae. Considering the excellent performance and biodegradability and low toxicity of the cationic starch, we concluded that our cationic starch is an excellent replacement for CPAM.

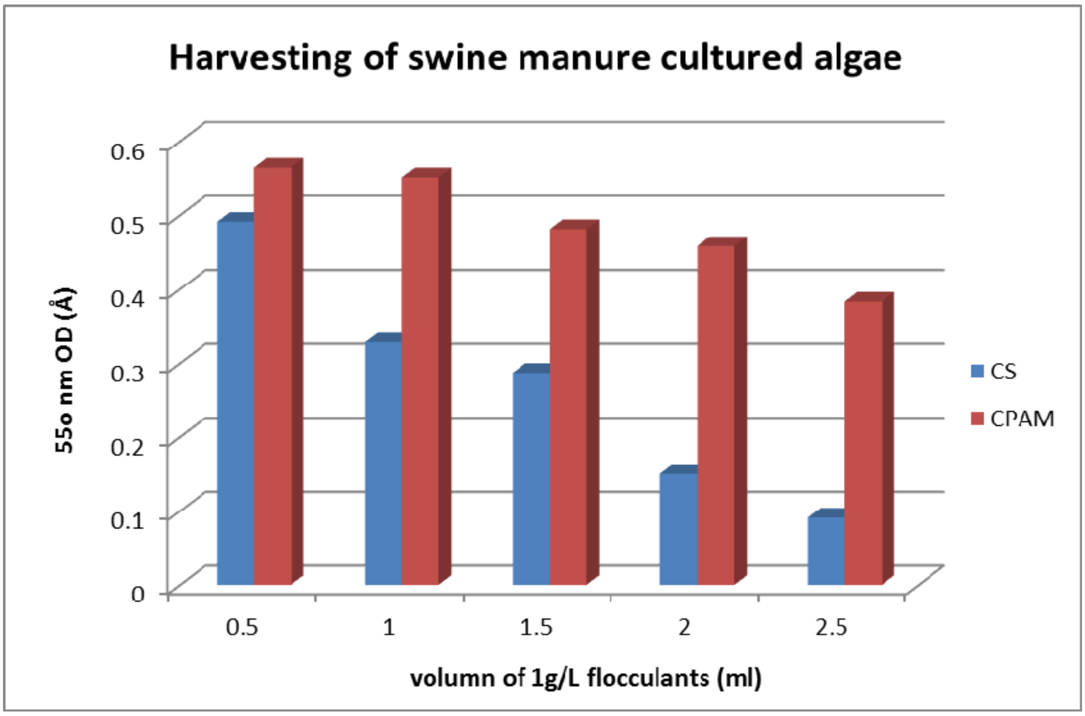


Fig 15. Comparison of cationic starch and CPAM of swine cultured algae harvesting.

Pilot scale Microalgae Harvesting Experiment

We further compared the performance of cationic starch and CPAM in pilot scale. The algae cultivation was carried out with a multilayer photobrioreactor housed within a greenhouse. The reactor had a culture volume of 500 liters to flocculate, flocculants were added to the 500 liter algae culture broth (0.9g dry algae/L) at a ratio of algae to flocculant of 13:1. Experimental variables, observations and harvesting efficiency data are shown in Table 4. The cationic starch performed slightly better than CPAM. The cationic starch required slower but longer mixing and produced smaller flocs compared with CPAM. Mixing in large scale algae production is energy intensive. Further research and development efforts should aim to reduce the mixing requirement for the cationic starch.

Large scale flocculation comparison	Cationic Starch	CPAM
Stable time	30 min	15-20 min
Clumps Size	3-10mm	8-30mm
Average OD of supernatant liquid (Å)	0.252	0.306

Table 4. Comparison of cationic starch and CPAM on large scale algae harvesting

Scanning Electron Microscope

SEM (Scanning electron microscope) is an excellent tool for study of morphological features of microorganisms (Collins, Pope et al. 1993) and starch granules (Jane, Kasemsuwan et al. 1994). In this project, SEM was used to study the morphological features of original corn starch, cationic starch, naturally suspended algae, and microalgae flocculated by the cationic starch. The SEM micrographs are shown in Figures 16-18. The algae cells harvested through centrifugation are around 3-4 μm with smooth cell wall surface and disperse individually (Figure 17). However, the cationic starch flocculated algae are covered by a coat of starch and appear to be bridged together by starch to form some large flocs of 2mm to 3cm in size which are visible the macroscopically (Figure 18).

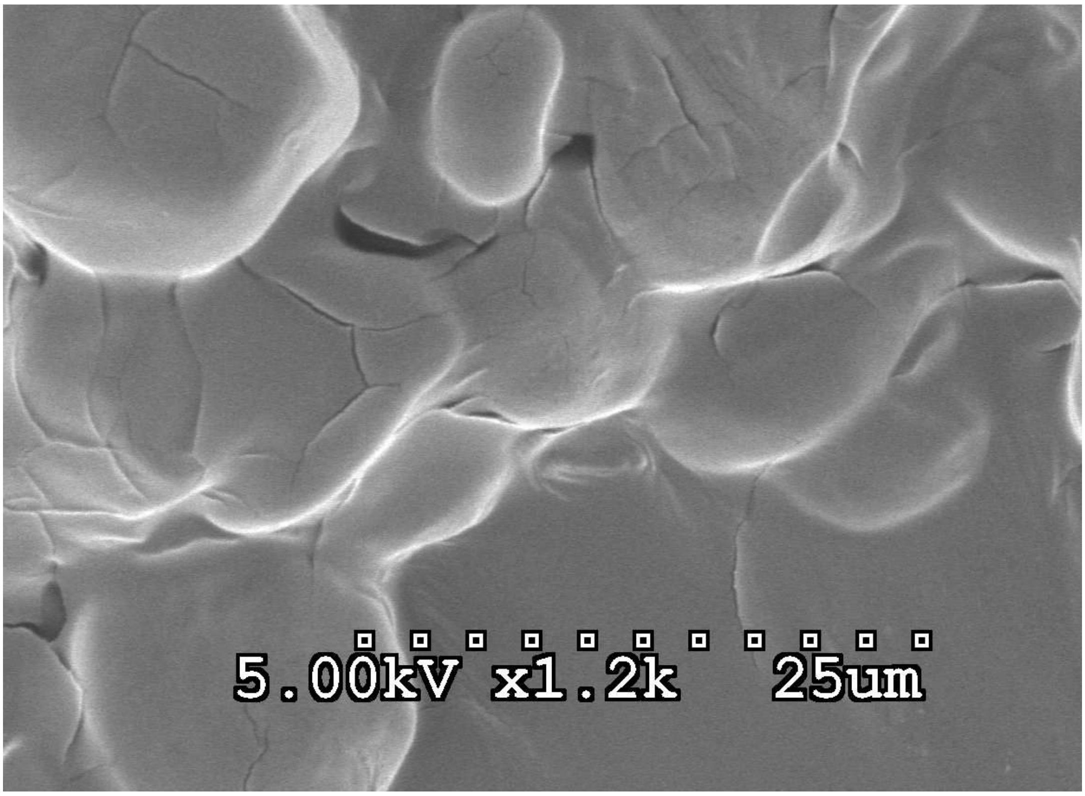


Fig 16. Cationic starch structure under SEM

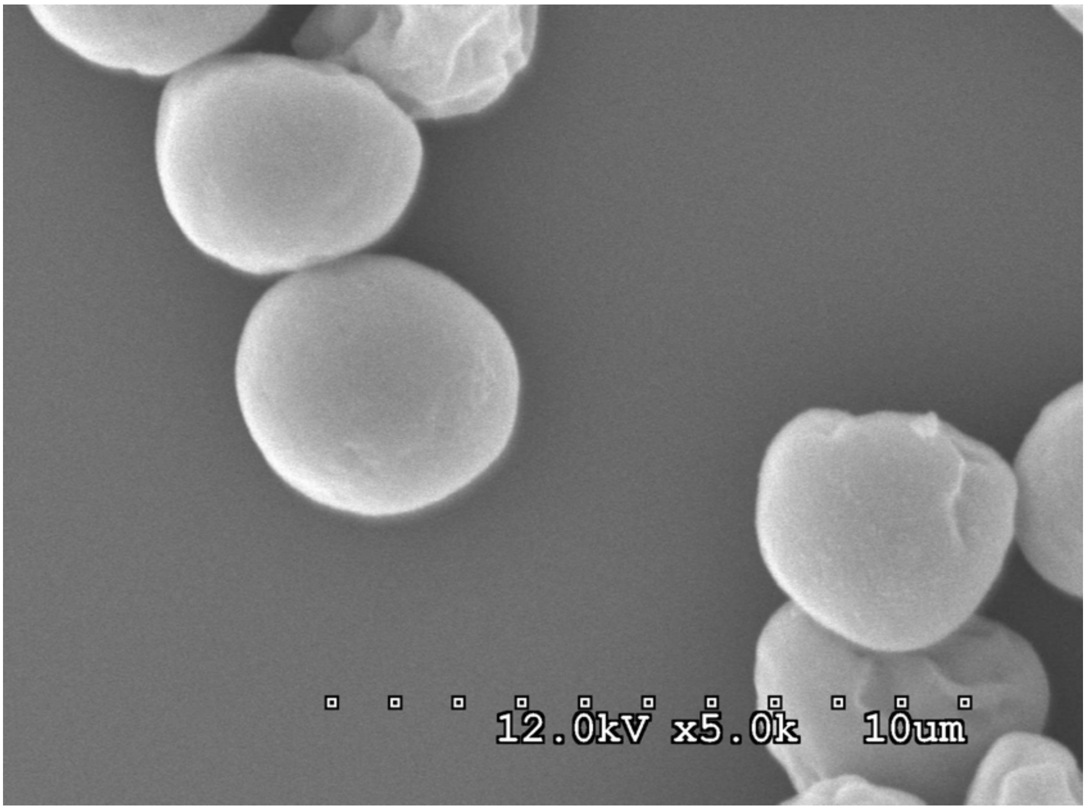


Fig 17. The shape of centrifugation harvested algae under SEM

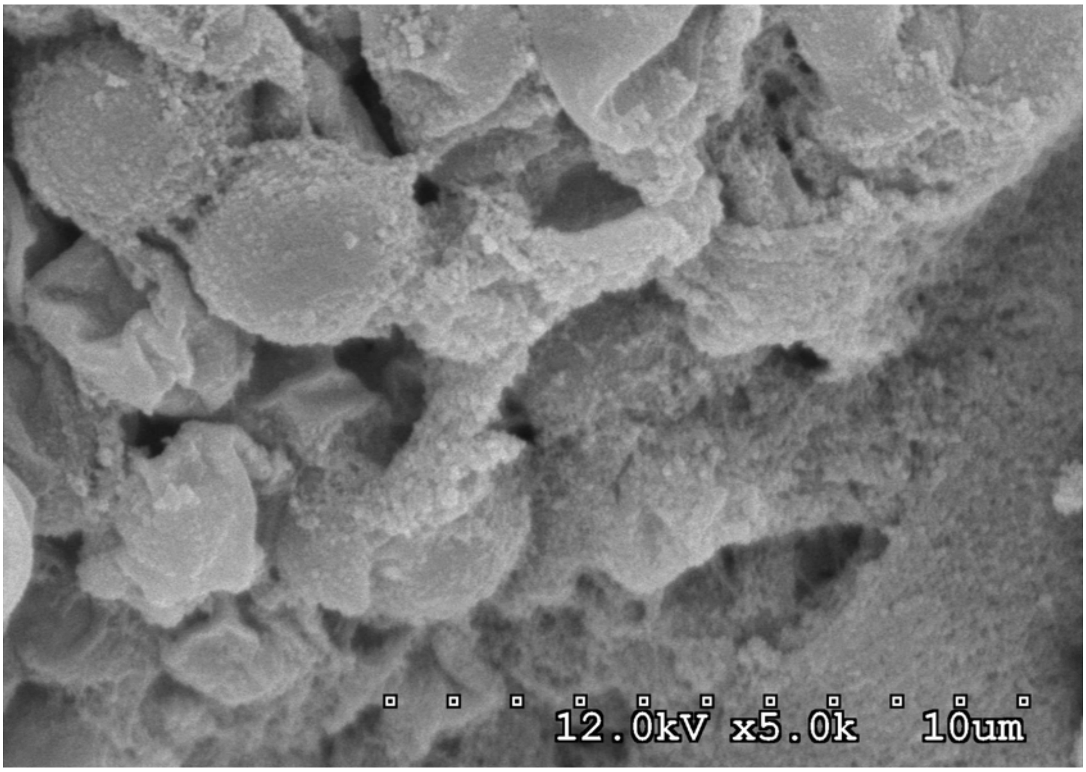


Fig 18. Cationic starch flocculated algae under SEM

Summary and Conclusions

Flocculation for microalgae harvest was studied. Specific emphasis on this research was on cationic starch flocculant synthesis. The behavior of cationic starch flocculant was evaluated in term of microalgae harvest effiency. The effects of cationic starch flocculant with different DS on microalgae harvest were investigated. The influence of synthesizing variables such as reaction time, reaction temperature, and catalyst content for cationic starch flocculant on harvest effiency were studied. The specific conclusions of this study are:

1. The optimized cationic starch synthesis condition in our lab are: synthesis temperature at 60℃, the reaction time is 360 min, with total system moisture content at 32.7%, and catalyst is 0.0015 mol when the corn starch is 5 grams. And the order of effect significancy is: catalyst dosage > reaction temperature > water content;
2. Acid and alkaline pretreatment could bring some slight improvement to the reaction rate but not dramatically. However, microwave pretreatment shows no improvement to DS;
3. The best DS for c*hlorella* harvesting is 0.41 and the optimal flocculation pH is 8.0-9.0. And 95% of the algal biomass in culturing broth could be flocculated which is a very effective harvesting rate;
4. After cationic starch was added, the separation of algal broth could reach a relatively stable situation in 20 minutes;
5. By direct comparison, CS performed better than CPAM on fresh water microalgae harvesting, as well as manure cultured algae. This development is

expected to further enhance the cost effectiveness of biofuel and other products from microalgae;

For future studies, more bio-molecules will be investigated for the development of biodegradable flocculants. Microalgae harvesting by cationic starch will be applied into larger scale microalgae production and water will be recycled in actual algae culture operation. The influence of cationic starch to further downstream algae utilization process will be explored also.

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> The objective of this report is to present a discussion of the literature review performed on methods of harvesting microalgae. There is no single best method of harvesting microalgae. The choice of preferable harvesting technology depends on algae species, growth medium, algae production, end product, and production cost benefit. Algae size is an important factor since low-cost filtration procedures are presently applicable only for harvesting fairly large

microalgae. Small microalgae should be flocculated into larger bodies that can be harvested by one of the methods mentioned above. However, the cells' mobility affects the flocculation process, and addition of nonresidual oxidants to stop the mobility should be considered to aid flocculation. The decision between sedimentation or flotation methods depends on the density difference between the algae cell and the growth medium. For oil-laden algae with low cell density, flotation technologies should be considered. Moreover, oxygen release from algae cells and oxygen supersaturation conditions in growth medium support the use of flotation methods. If high-quality algae are to be produced for human consumption, continuous harvesting by solid ejecting or nozzle-type disc centrifuges is recommended. These centrifuges can easily be cleaned and sterilized. They are suitable for all types of microalgae, but their high operating costs should be compared with the benefits from their use. Another basic criterion for selecting the suitable harvesting procedure is the final algae paste concentration required for the next process. Solids requirements up to 30% can be attained by established dewatering processes. For more concentrated solids, drying methods are required. The various systems for algae drying differ both in the extent of capital investment and the energy requirements. Selection of the drying method depends on the scale of operation and the use for which the dried product is intended.

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