Synthesis and characterization of a starch-based cationic flocculant for microalgae harvesting

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Abstract: A process for making a starch-based cationic flocculant for microalgae harvest was studied and the performance of the flocculant was evaluated.   The substituted cationic starch was prepared by reacting corn starch with glycidyltrimethylammonium chloride (GTAC), during which the hydroxyl groups of anhydroglucose units of the starch were partially substituted by the ammonium groups through etherification.   The factors affecting degree of substitution (DS), such as reaction temperature and time, catalyst amount, and the water content were investigated and optimal reaction conditions were determined.   The relationship between DS and microalgae harvest was determined.   A batch of cationic starch with optimal DS was synthesized and used for flocculation experiments.   The flocculation experiment results showed that, for the original microalgae concentration (dry weight) of 1 g/L, the cationic starch-based flocculants can harvest over 90% of the microalgae in pH from 5 to 10, with the aggregation and precipitation time of 10 min to 30 min, and the mass ratios of flocculants to microalgae of 1:8-1:18 (dry mass ratio).   Using starch-based flocculant to harvest microalgae in the effluent is not only efficient, but also nontoxic to the water system, which is very important to the effluent reuse and certain microalgae applications.

Keywords: microalgae, harvest, flocculation, starch-based cationic flocculants

Citation: Cheng Y L, Hua W, Liu W H, Wang W Q, Ye X, Li L, et al.   Synthesis and characterization of a starch-based cationic flocculant for microalgae harvesting.   Int J Agric & Biol Eng, 2016; 9(3): 139－145.

1   Introduction

Mass cultivation of microalgae has been attracting

tremendous attention since last century because microalgae are a potential alternative feedstock to fossil and plant resources for the production of biofuels, chemicals, foods, and feeds[1-5].   Recent development has demonstrated that the production of microalgae can be coupled with flue gas CO_2 mitigation[6], wastewater treatment[7], and fish and livestock farming[4,7-9].   Advances in microalgae cultivation, harvesting and downstream processing are expected to further enhance the cost effectiveness of microalgae-based biofuels and products[10].

Harvesting of microalgae is considered a daunting technical and economic challenge.   Significant efforts have been devoted to developing efficient, environmentally friendly and economical harvesting methods.   The challenge arises from the extremely small

size of live microalgae cells (around 3-40 μm) that do not settle automatically by gravity[11]. Many harvest methods, such as filtration and high speed centrifugation, are either inefficient[12] or impractical in large scale due to its high energy consumption[13].

It is widely believed that flocculation is a convenient, practical and fast harvest method. Microalgae cell surfaces are negatively charged, and therefore it is feasible to flocculate microalgae cells by using electrostatic process, or positively charged reagents[14]. Hence flocculants with cationic groups can be used to aggregate microalgae cells, causing them to settle rapidly. For example, cationic polyacrylamide (CPAM), a flocculant widely used in the industry, is able to flocculate microalgae effectively. However, CPAM contains non-polymerized monomers and additives which are proven to be cancerigenic[15]. AlCl$_3$ flocculant as an alternative flocculant can be used to harvest microalgae with higher dosage but it is not cost effective. Recently, researchers are seeking bioflocculants for algae harvest. Cationic starch[16,17] is synthesized by substituting the −OH groups on the starch chain with positively charged groups (Figure 1), for example, ammonium. If ammonium groups are from non-toxic cationization agents, the synthesized cationic starch would be non-toxic and eco-friendly[16]. The degree of substitution (DS), which indicates the extent of −OH replacement, is an important factor affecting the performance of cationic starch. Since each anhydroglucose unit has three hydroxyl groups, the maximum value of DS is 3. Cationic starch flocculants have been used in paper-making industry to absorb negatively charged cellulose fibers in white water[18]. It could also be used to flocculate negatively charged microalgae. Vandamme et al.[19] reported the use of the starch based flocculants, and the results showed that cationic starch is very effective in fresh water microalgae harvesting[20]. In addition, it worked well under a wider range of pH value than the inorganic flocculants.

However, the cationic starch flocculants used presently in industry normally have DS of 0.1-0.2, and a relatively low harvesting efficiency for microalgae. The objective of this research was to investigate the factors affecting the DS in cationic starch synthesis, and determines the best harvesting conditions for microalgae harvesting using cationic starch synthesized.
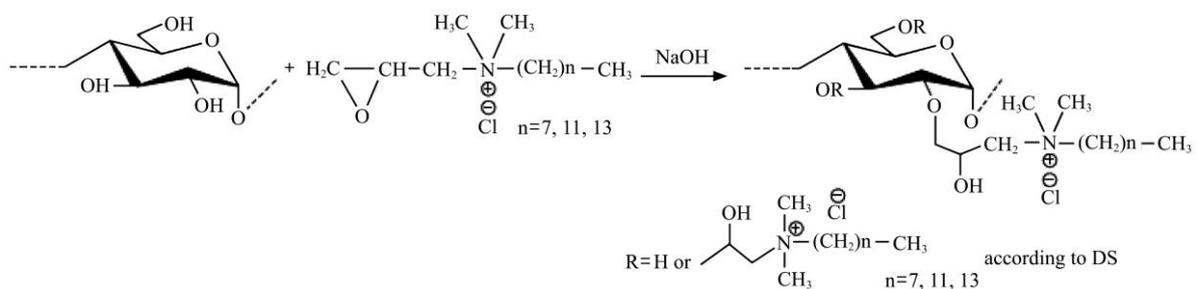

Figure 1   Reaction equation of cationic starch synthesis

2   Methods and materials

In this study, microalgae were cultivated on an artificial medium for use in the flocculation experiments. Cationic starch was prepared under different reaction conditions in order to develop techniques to control the synthesis process. The effect of DS on flocculation was evaluated and the result was used to determine the optimal DS for the microalgae strain used in the present study. A series of experiments were carried out to evaluate the performance of the optimal cationic starch as used for flocculation of microalgae.

2.1   Microalgae culture

Microalgae were cultured on TAP-Medium (Tris-Acetate-Phosphate)[21] consisting of (g/L): NH$_4$Cl 0.4; MgSO$_4$ 7H$_2$O 0.1; CaCl$_2$ 2H$_2$O, 0.05; K$_2$HPO$_4$, 0.108 and KH$_2$PO$_4$, 0.056; Tris (hydroxymethyl aminomethane), 2.42. The medium also included 1 mL/L glacial acetic acid and 1 mL/L trace elements solution consisting of (g/L) Na$_2$EDTA, 50; ZnSO$_4$ 7H$_2$O, 22; CaCl$_2$ 2H$_2$O, 0.05; H$_3$BO$_3$, 11.4; MnCl$_2$ 4H$_2$O, 5.06; FeSO$_4$ 7H$_2$O, 4.99; CoCl$_2$ 6H$_2$O, 1.61; CuSO$_4$ 5H$_2$O, 1.57; (NH$_4$)$_6$Mo$_7$O$_2$ 4H$_2$O, 1.10 and KOH, 16.

A *Chlorella vulgaris* microalgae strain was cultured.

Culturing temperature was 20°C, with an illumination intensity of 80 μmol/(m^2 s). The concentration of algal solution was measured using spectrophotometer (Spectroquant merck Pharo300 Germany) at 550 nm.

2.2 Synthesis of cationic starch flocculants

Cationic starch was prepared by substituting some of the hydroxyl groups on starch molecular chains with the positive groups of a cationization agent[20,22] with NaOH being the catalyst. Corn starch (5 g), 80% glycidyltrimethylammonium chloride or GTAC (3 g, dry weight) solution, 1 mol/L NaOH (0.5-3 mL) and distilled water were added to a flask, and then mixed thoroughly. The mixture in the flask was placed in a water bath from 2 to 7 hours under temperature variations from 45 ℃ to 65 ℃, respectively. After the reaction, excess 95% alcohol was added to the flask to stop the reaction and allow the modified starch to settle, and then 70% alcohol was used to wash the product in order to remove the cationization agent and catalyst NaOH. The resultant paste was dried at 60 ℃ for 6 hours. The dried modified starch cake was milled to fine powder which is the final product.

2.3 DS measurement

Cationic starch powder (0.1 g) was first dissolved in 100 mL beakers with 80 ℃ hot deionized water with magnetic stirring at 300 r/min for 30 minutes. The final concentration of cationic starch solution was 1 g/L. After the liquid was cooled to the room temperature, the nitrogen content N (mg/L) was determined using total nitrogen kit (HACH Company, Germany) and spectrophotometer (Spectroquant merck Pharo300 Germany).

The degree of substitution was calculated using Equation (1):

$$DS = \frac{162 \times N}{1400 - 152.5 \times N} \qquad (1)$$

where, N is the nitrogen amount experimentally determined (%); 162 is the molecular weight of nitrogen of the residue in each anhydroglucose monomer of starch; 151.5 is the molecular weight of of GTAC group that was connected on to the starch back bone.

2.4 Flocculation experiment

The original microalgae suspension was adjusted to 1 g/L, mixed with the cationic starch solution in a 50 mL centrifugal tube at a given ratio, and placed on a vortex mixer at speed of 1000 r/min for 10 s. After 30 min setting down, the microalgae particles were aggregated into flocs and settle to the bottom of the tube. The concentration of microalgae in supernatant was measured using Spectrophotometer (Spectroquant merck Pharo300 Germany) and standard curve of algae concentration on culture media was plotted. The harvest rate in percentage can be calculated as following:

$$HR = (1 - CH) / 1 \times 100\% \qquad (2)$$

where, HR is the harvest rate (%), and CH is the concentration of microalgae in supernatant after harvest (g/L).

2.5 Scanning electron spectroscopy

The micro-structure of starch, cationic starch, microalgae and flocculated microalgae was observed using scanning electron microscope (SEM)[23].

3 Results and discussion

3.1 Effects of reaction conditions on the DS in cationic starch

3.1.1 Reaction temperature

Effect of reaction temperature on DS is shown in Figure 2. Higher reaction temperatures were in favor of substitution, which might be due to higher diffusion rate and greater starch gelatinization[24] at higher temperature. In addition, starch structure becomes loser at higher temperature, providing cationic etherifying agent greater access to the -OH groups on molecular chain. The highest DS was observed at 60 ℃. The decline in DS with further increasing temperature may be due to dissociation of GTAC from the starch molecules.
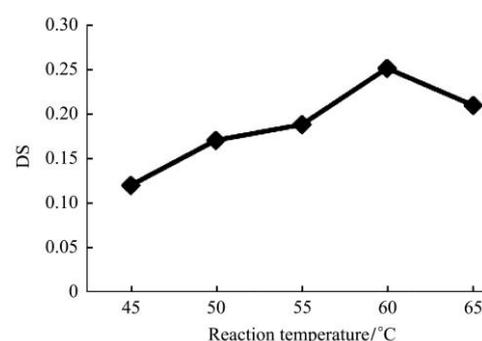

Figure 2   Effect of synthesis temperature on DS of cationic starch (Other reaction conditions: water content 40%, catalyst 0.001 mol (1 mL 1mol/L NaOH solution), and reaction time 5 h)

3.1.2　Reaction time

The etherification reaction was a slow reaction, especially at low temperature.　For example, the copolymerization reaction of starch with epoxide under very low temperature lasted several days to reach a high DS[22].

Figure 3 shows that DS increased with increasing reaction time.　The DS increased steadily from 0.16 to 0.27 with increasing reaction time up to 360 min.　That the decline in DS after 360 min　might be due to dissociation of GTAC from the cationic starch.
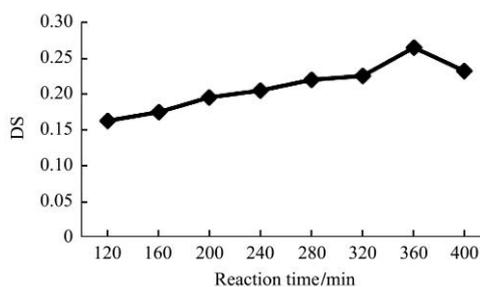

Figure 3　Effect of reaction time on DS of cationic starch (Other reaction conditions: temperature 60 ℃, water content 40%, catalyst 0.001 mol)

3.1.3　Catalyst dosage

Catalyst dosage in reaction system is an important factor which might affect the reaction rate and activation energy.　Our experimental results (Figure 4) show that the DS of cationic starch increased with increasing catalyst dosage between 0.0005 and 0.0015.　In the catalytic process, NaOH provides additional hydroxyl ions in the system, promoting the forward reaction and decreasing the reaction activity energy.　The highest DS appears when the NaOH dosage was 0.0015 mol.　The drop in DS with further increase in NaOH was probably due to higher base concentration causing a reverse reaction resulting in hydrolysis of the product. Other side reactions may take place between NaOH and GTAC[22].
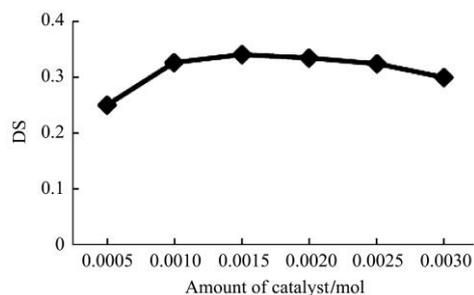

Figure 4　Effect of catalyst amount on DS of cationic starch (Other reaction conditions: reaction time 360 min, and water content 40%)

3.1.4　Water content

Figure 5 shows DS as a function of water content of the reaction systems.　When water content was below 33%, the DS increased with increasing water content. Water plays a key part in swelling or gelatinization of starch particles.　Water also acts as a required medium for catalysts and cationic agent to efficiently and homogeneously diffuse into starch structure[22]. However, too much water dilutes reactants and may decrease molecular collision probability leading to a reduced reaction rate.　Furthermore, too high a water content may induce hydrolysis, causing cleavage of the bonds between the starch and cationization reagent[22]
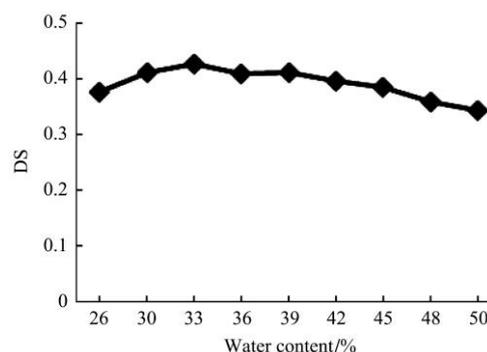

Figure 5　Effect of water content on DS of cationic starch (Other reaction conditions: reaction temperature 60 ℃, reaction time 360 min, and catalyst dosage 0.0015 mol)

3.2　Flocculation of microalgae with the cationic starch synthesized

Flocculation of microalgae cells with cationic starch relies on the interactions between the negative charges on microalgae cells and the cations on the cationic starch. Therefore, the DS of cationic starch, which represents the positive charge strength, is expected to have strong influence on flocculation and hence the harvesting rate (HR).　The effect of DS on HR is showed in Figure 6. The results were obtained from experiments in which microalgae broth and cationic starch were mixed in 16:1 ratio (dry weight).

The data show that the optimum DS for the harvesting of the chlorella strain is around 0.41 with about 94% of the microalgae harvested.　Cationic starch with low DS had limited positive charges to flocculate microalgae particles from culture solution while too high DS may cause the stiffness of starch chains[25] due to the mutual exclusion between positive charges as well as the steric

clash on ammonium groups, which will reduce their ability to attract the negatively charged microalgae cells.
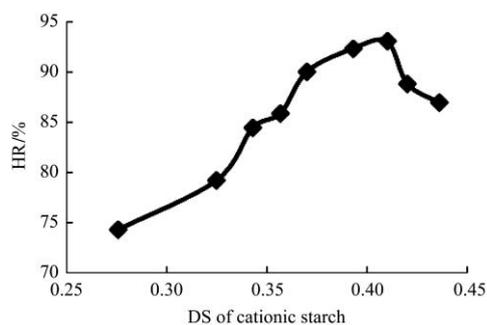

Figure 6   Effect of DS on HR of cationic starch

Since the optimal DS for flocculation of the chlorella strain was determined to be 0.41, a new batch of cationic starch was synthesized under following conditions to have a DS value of around 0.41: corn starch 5 g, GTAC 2.85 g, water content 33%, reaction time 6 h, reaction temperature 60°C, catalyst dosage 0.0015 mol based on previous experiments.   This cationic starch was used for the following algae flocculation experiments.

3.2.1   Effect of pH on microalgae harvesting

To investigate how the cationic starch performed under different ionic environment, flocculation was carried out at different pH values ranging from 2.79 to 10.66.   The results (Figure 7) showed that better performances were achieved in weak base conditions. When the pH was higher than 10, the high HR could be mainly due to microalgae auto-flocculation under strong based environment[19].
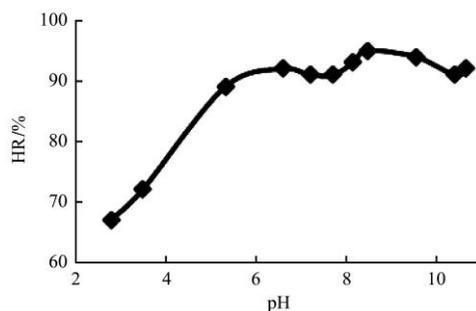

Figure 7   Effect of pH on HR in microalgae culture

3.2.2   Effect of flocculation time on microalgae harvesting

After the cationic starch was added to the microalgae solution, the microalgae cells settled quickly compared with the control test.   In the previous experiments, the flocculation time was 30 minutes.   In this experiment, the flocculation time was chosen as a variable with the ratio of cationic starch over microalgae of 1/18 (dry

weight).   The amount of flocs was a function of flocculation time (Figure 8).   After 20 min of flocculation, more than 90% of microalgae was flocculated.
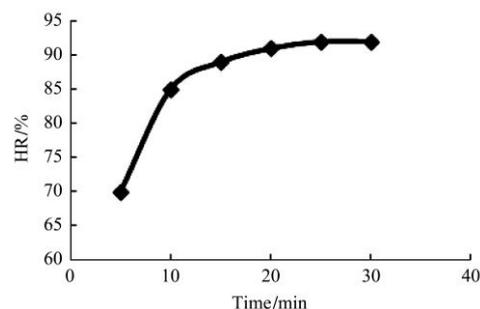

Figure 8   Change in harvest rate as a function of flocculation time (microalgae to cationic starch ratio: 18:1)

3.2.3   Effect of cationic starch weight/microalgae weight on HR

To determine the optimal cationic starch dosage (minimum dosage to achieve highest HR), flocculation experiments with different microalgae to cationic starch ratios were carried out.   The results (Figure 9) showed that the dry mass ratios of cationic starch to microalgae weight could be as low as 1:18 and still achieve over 90% HR.   However when the ratio was higher than 1:13, the clarity decreased because of the unconsumed cationic starch in the water, and the increased viscosity slowing down flocs sedimentation.
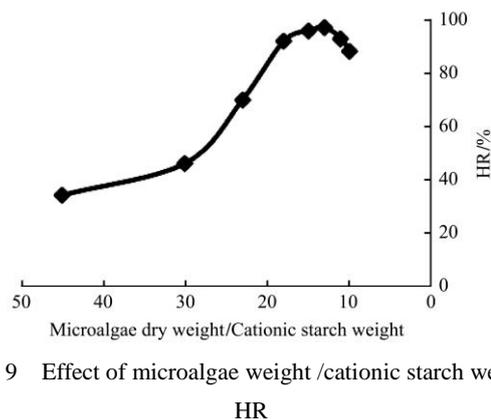

Figure 9   Effect of microalgae weight /cationic starch weight on HR

3.2.4   Micro-structure of particles using Scanning Electron Microscope

Scanning electron microscope (SEM) was used to observe delicate morphology of microalgae[23,26] and the starch granules[27].   The micrographs in Figure 10 show the difference between original corn starch and cationic starch synthesized (Figure 10), and those in Figure 11 show as the morphology of suspended and the flocculated

microalgae, respectively. The original starch granules had irregular shapes with average size around 15-20 μm. The smaller immature ones had a size 10 μm or smaller. After the synthesis reaction, the starch particles changed and became flatter and connected, and lost their three-dimensional shape and the crystalline structures.
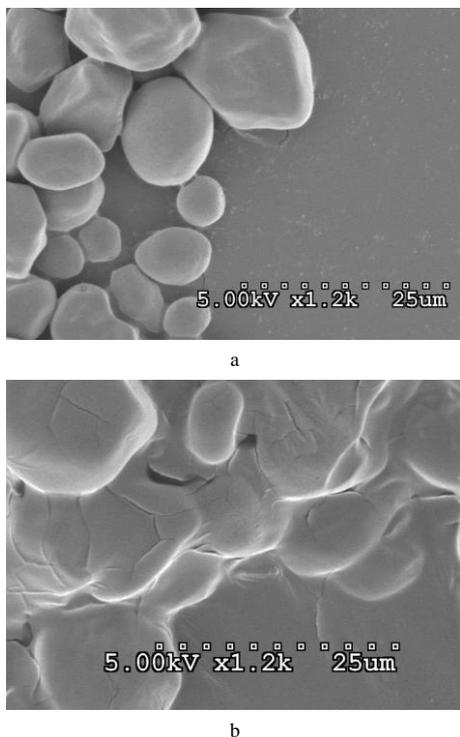

a

b

Figure 10   Morphology of microalgae under SEM (a) Original corn starch granules, (b) Cationic starch structure
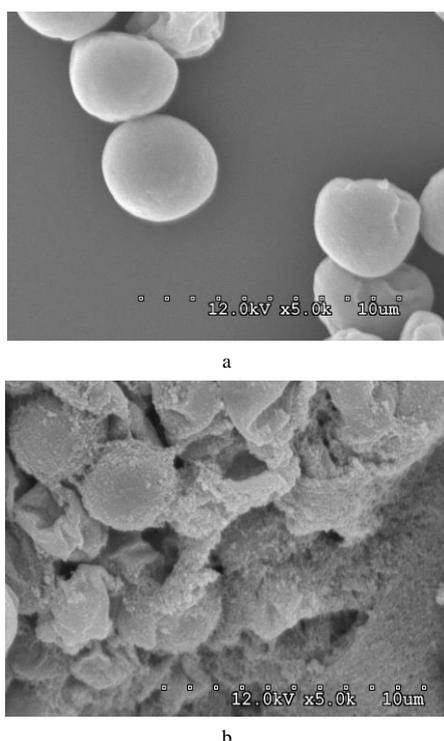

a

b

Figure 11   Morphology of harvested microalgae under SEM (a) centrifugation harvested algae, (b) Cationic starch flocculated algae

The size of the microalgae is around 3-4 μm in Figure 11a with smooth cell wall surface and individual cells. Because the original microalgae were harvested with centrifugation, some of them seem to stay together, but not actually "connected". Whereas, microalgae flocculated by cationic starch adhered to each other and formed large flocs with size up to 3 cm. Also, under SEM photos, cationic starch granules formed many tiny "bridges" between the microalgae cells holding the cells together.

4   Conclusions

After evaluation of the effects of key reaction conditions on the degree of substitution (DS) of cationic starch and the effect of DS on flocculation performance, an optimized cationic starch based flocculant was produced and investigated in a series of flocculation experiments. At microalgae to cationic starch ratios ranging from 18:1 to 13:1, more than 90% microalgae in the culture broth can be flocculated within 20 min. This non-toxic bioflocculant can be used in production of microalgae for food and feed applications. Further study is needed to shorten the flocculation time and evaluate the economic performance of the cationic starch based flocculant.

[References]

[1]   Chisti Y.   Biodiesel from microalgae.   Biotechnology Advances, 2007; 25(3): 294−306.

[2]   Barrow C, Shahidi F.   Marine nutraceuticals and functional foods.   CRC Press, Taylor & Francis Group, 2008.

[3]   Brown M R, Jeffrey S W, Volkman J K, Dunstan G A. Nutritional properties of microalgae for mariculture. Aquaculture, 1997; 151(1-4): 315–331.

[4]   Barclay W R, Meager K M Abril J R.   Heterotrophic production of long chain omega-3 fatty acids utilizing algae

and algae-like microorganisms. Journal of Applied Phycology, 1994; 6(2): 123−129.

[5] Becker E. Micro-algae as a source of protein. Biotechnology Advances, 2007; 25(2): 207−210.

[6] Sawayama S, Minowa T, Yokoyama S Y. Possibility of renewable energy production and CO_2 mitigation by thermochemical liquefaction of microalgae. Biomass and Bioenergy, 1999; 17(1): 33−39.

[7] Wang L, Min M, Li Y C, Chen P, Chen Y F, Liu Y H, et al. Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. Applied Biochemistry and Biotechnology, 2010; 162(4): 1174−1186.

[8] He M, Hollwich W, Rambeck W A. Supplementation of algae to the diet of pigs: a new possibility to improve the iodine content in the meat. Journal of Animal Physiology and Animal Nutrition, 2002; 86(3-4): 97−104.

[9] Schmid S, Ranz D, Schmid S, Ranz D, He M L, Burkard S, et al. Marine algae as natural source of iodine in the feeding of freshwater fish-a new possibility to improve iodine supply of man. Revue de Médecine Vétérinaire. 2003; 154(10): 645−648.

[10] Li Q, Du W, Liu D. Perspectives of microbial oils for biodiesel production. Applied Microbiology and Biotechnology, 2008; 80(5): 749−756.

[11] Shelef G, Sukenik A, Green M. Microalgae harvesting and processing: a literature review. Technion Research and Development Foundation Ltd., Haifa (Israel), 1984.

[12] Papazi A, Makridis P, Divanach P. Harvesting *Chlorella minutissima* using cell coagulants. Journal of Applied Phycology, 2010; 22(3): 349−355.

[13] Knuckey R M, Brown M R, Robertc R, Frampton D M F. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacultural Engineering, 2006; 35(3): 300−313.

[14] Ives K. The significance of surface electric charge on algae in water purification. Journal of Biochemical and Microbiological Technology and Engineering, 1959; 1(1): 37−47.

[15] Rudén C. Acrylamide and cancer risk−expert risk assessments and the public debate. Food and Chemical Toxicology, 2004; 42(3): 335−349.

[16] Dencs J, Nos G, Marton G. Investigation of solid-phase starch modification reactions. Chemical Engineering Research and Design, 2004; 82(2): 215−219.

[17] Gugliemelli L, Weaver M, Russell C R, Rist C E. Base‐hydrolyzed starch-polyacrylonitrile (S-PAN) graft copolymer. S-PAN-1: 1, PAN MW 794,000. Journal of Applied Polymer Science, 1969; 13(9): 2007−2017.

[18] Nachtergaele W. The benefits of cationic starches for the paper industry. Starch-Stärke, 1989; 41(1): 27−31.

[19] Vandamme D, Foubert I, Meesschaert B. Flocculation of microalgae using cationic starch. Journal of Applied Phycology, 2010; 22(4): 525−530.

[20] Kato M, Gyoten Y, Sakai-Kato K, Nakajima T, Toyo'oka T. Cationic starch derivatives as dynamic coating additives for analysis of amino acids and peptides using poly (methyl methacrylate) microfluidic devices. Analytical Chemistry, 2004; 76(22): 6792−6796.

[21] Harris E H, Stern D. The chlamydomonas sourcebook. Cambridge Univ Press. 1989.

[22] Kavaliauskaite R, Klimaviciute R, Zemaitaitis A. Factors influencing production of cationic starches. Carbohydrate Polymers, 2008; 73(4): 665−675.

[23] Collins S. P, Pope R K, Scheetz R W. Advantages of environmental scanning electron microscopy in studies of microorganisms. Microscopy Research and Technique, 1993; 25(5-6): 398−405.

[24] Lelievre J. Starch gelatinization. Journal of Applied Polymer Science, 1974; 18(1): 293−296.

[25] Bratskaya S, Schwarz S, Laube J Liebert T, Heinze T, Krentz O, et al. Effect of polyelectrolyte structural features on flocculation behavior: cationic polysaccharides vs. synthetic polycations. Macromolecular Materials and Engineering, 2005; 290(8): 778−785.

[26] Adebowale K O, Lawal O S. Microstructure, physicochemical properties and retrogradation behaviour of Mucuna bean (*Mucuna pruriens*) starch on heat moisture treatments. Food hydrocolloids.2003; 17(3): 265−272.

[27] Jane J L, Kasemsuwan T, Leas S, Zobel H, Robyt J F. Anthology of starch granule morphology by scanning electron microscopy. Starch-Stärke, 1994; 46(4): 121−129.