BIOMASS AND FLOCCULATION CHARACTERISTICS OF *PICOCHLORUM OKLAHOMENSIS* AND *NANNOCHLOROPSIS OCULATA*

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NOMENCLATURE

v/v	Volume to volume
w/w	Weight to weight
w/v	Weight to volume
rpm	Rotation per minute
%	Percentage
^{o}C	Degree centigrade
d	Day
h	Hour
min	Minute
s	Second
g	Gram
mg	Miligram
m	Meter
cm	Centimeter
mm	Millimeter
µm	Micrometer

nm	Nanometer
L	Liter
mL	Milliliter
μL	Microliter
μmol	Micromole
M	Mole/Liter, a unit of concentration
A	Ampere, a unit of current
V	Volt, a unit of voltage
kWh	Kilowatt-hour, a unit of energy

CHAPTER I

INTRODUCTION

1.1 PROBLEM STATEMENT

Algal biomass shows significant promise as feedstock for biofuel and bio-based product manufacturing. Of all unit operations used in microalgae production, harvesting biomass from culture suspension is of crucial importance. A variety of methods have been studied and applied for algal biomass recovery. These methods include centrifugation, filtration, flocculation, flotation and sedimentation. However, most of these techniques have disadvantages such as high cost, low recovery efficiency, difficulties involved in process scale-up and flocculant toxicity. Further investigation and optimization of existing methods and development of new technologies are needed for efficient harvest of algal biomass from culture medium. Due to significant variations in chemical and physical properties among microalgae strains, biomass production and harvesting process parameters need to be optimized for each strain. Our preliminary research has indicated that biomass from two microalgae strains, *Picochlorum oklahomensis* (PO) and *Nannochloropsis oculata* (NO) can be viable feedstock for bioproduct development. To the best of our knowledge, limited information is available regarding biomass chemical

composition and growth pattern of microalgae strain PO. This information is necessary for evaluation of PO as potential feedstock for value-added bioproduct development. Furthermore, information on effective harvesting methods for PO and NO is lacking.

1.2 HYPOTHESIS

Utilization of pH adjustment, non-toxic biopolymer addition and electro-flocculation conducted under optimized conditions are effective methods for microalgal biomass recovery.

1.3 OBJECTIVES

The main objective of this study is to develop effective flocculation processes for harvesting microalgal biomass produced by NO and PO strains. The specific objectives are as follows:

i. To characterize chemical compositions and growth patterns of two strains of microalgae, PO and NO.

ii. To study the effects of processing parameters on flocculation efficiency.

iii. To optimize the flocculation efficiency with pH adjustment, non-toxic biopolymer addition and electro-flocculation for maximum algal biomass harvesting.

CHAPTER II

LIETRATURE REVIEW

2.1 MICROALGAE

Microalgae, which are unicellular microorganisms, produce biomass that can be used for biofuel and value-added product development. There are a great variety of microalgae strains that vary in their chemical and physical properties. Most microalgae cells contain protein, lipids, polysaccharides, pigments such as chlorophyll and carotenoids, and different inorganic elements such as Cu, Fe, Se, Mn and Zn. Cells of some microalgae strains contain functional bioactive compounds such as lutein and zeaxanthin, the essential components of the macular pigment in the retina of the eye (Weiss et al. 2007a; Weiss et al. 2007b), and polyphenols that possess antioxidant properties and may reduce chronic diseases caused by oxidative damage to cells and cellular molecules (Takeshi et al. 2005).

One of the advantages of microalgae in bioproduct development is their high biomass productivity. Microalgae utilize light and carbon dioxide and have higher photosynthetic efficiency than land plants for biomass production (Benemann 1997; Miao and Wu 2006). Unlike conventional crops, microalgae production does not rely on arable

land and fresh water (Vandamme et al. 2009). Biomass from microalgae can be used as food for people and is a good nutrition source for fish and prawn (Knuckey et al. 2006; Muller 2000; Spolaore et al. 2006a). Some microalgae strains can accumulate functional bioactive compounds, such as fatty acids [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] (Grima et al. 2003; Yang et al. 2009), pigments (zeaxanthin, lutein, β-carotene) (Weiss et al. 2007a; Weiss et al. 2007b) and vitamins (Baker et al. 1981; Bremus et al. 2006; Durmaz 2007). Oil extracted from algal biomass can be used to produce biodiesel (Chisti 2007; Miao and Wu 2006). Microalgae also have environmental applications, for example, wastewater treatment (Aragon et al. 1992; Azarian et al. 2007; Buelna et al. 1990; Ge et al. 2004).

Nannochloropsis oculata (NO) is a green, unicellular marine alga, belonging to the class of Eustigmatophyceae. NO strain has attracted a lot of attention due to its high oil content and high polyunsaturated fatty acids (PUFA) percentage of the total fatty acids (Chiu et al. 2009; Madhu et al. 2004). The oil content of NO may vary between 8 % and 50 % (Brown 1991; Chiu et al. 2009; Converti et al. 2009) depending on the growth phase, temperature, CO_2 and nitrogen concentration in the culture medium. It has been reported that oil content of NO cells increased from 30.8 % at the exponential phase to 50.4% at the stationary phase (Chiu et al. 2009). Oil productivity of the NO cultures grown in a semi-continuous system aerated with 2-15 % CO_2 reached its maximum, 0.142 g L^{-1} d^{-1}, at 2 % CO_2 aeration (Chiu et al. 2009). An increase in temperature from

20 ℃ to 25 ℃ almost doubled the NO oil content (from 7.9 % to 14.9 %), and a 75 % decrease in nitrogen concentration in the medium increased the oil content of NO from 7.9 % to 15.3 % (Converti et al. 2009). Because of its high oil content and biomass productivity, NO is preferred as feedstock for biodiesel production (Umdu et al. 2009) and as feed in aquaculture (Cheng et al. 2004; Madhu et al. 2004).

Currently, there is a great interest in commercial production of PUFA, specifically EPA and DHA, because of their diverse health benefits. Besides fish oil, microalgae is a good source for EPA and DHA (Guschina and Harwood 2006). Zhukova and Aizdaicher (1995) analyzed the fatty acid composition of several microalgae species and concluded that fatty acids of NO were dominated by three components: C16:0, C16:1n-7 and C20:5n-3 (EPA), which together accounted for 74.8 % of the total fatty acids. Among them, EPA made up nearly one-third of the total fatty acids (Zhukova and Aizdaicher 1995). The fatty acid profile of NO was similar to other *Nannochloropsis* species (Hodgson et al. 1991; Volkman et al. 1993; Zhukova and Aizdaicher 1995). *Nannochloropsis* species have been identified as the most promising photoautotrophic producers of EPA for human consumption (Cheng-Wu et al. 2001; Rebolloso-Fuentes et al. 2001; Spolaore et al. 2006b). The fatty acid composition of the same microalgae strain may vary because of the influence of different growth conditions. For example, NO contained higher percentage of total n3 and n6 fatty acids at high CO_2 concentration than at low CO_2 concentration (Roncarati et al. 2004). The percentage of EPA was higher

during the exponential phase than in the stationary phase (Roncarati et al. 2004; Tonon et al. 2002). Hence, the culture conditions of microalgae can be controlled to improve the modified algal biomass composition.

Picochlorum oklahomensis (PO) is a small coccoid, unicellular, green algae strain and was isolated from the Salt Plains National Wildlife Refuge in Oklahoma, USA in 1998 (Henley et al. 2002; Hironaka 2000). At first it was tentatively identified as *Nannochloris sp*., but later after phylogenetic analysis it was designated as *Picochlorum oklahomensis* (Henley et al. 2004). Hironaka (2000) did a lot of characterization work on PO. Scanning electron micrographs showed that PO is a slightly oval shaped green alga with a cell size of 2 μm diameter. Pigments present were chlorophylls a and b, and major carotenoids were lutein, β-carotene, violaxanthin, neoxanthin and vaucheriaxanthin ester.

PO showed a remarkable ability to tolerate a wide range of salinity (0-140 g/L sodium chloride) and temperatures (0-40 ℃) (Hironaka 2000), so PO is a broadly halotolerant algae strain. However, its growth rate decreased with increasing salinity at room temperature (Henley et al. 2002). Low salinity (2 %) combined with high temperature (45 ℃) were the most stressful conditions for PO growth (Henley et al. 2002). In addition, results from Kvíderová and Henley's study (2005) showed that two antibiotics (25 mg/L streptomycin plus 50 mg/L ampicillin) had only a minor effect on the growth and photosynthesis of PO, so the combination of these two antibiotics is

suitable for maintenance of stock cultures and turbidostat cultures of PO to prevent bacteria growth.

To the best of our knowledge, no information is available regarding the growth pattern, oil content and fatty acid composition of microalgae strain PO. This information is necessary for evaluation of PO as potential feedstock for value-added bioproduct development. Furthermore, information on effective harvesting methods for PO and NO is lacking.

2.2 HARVESTING MICROALGAL BIOMASS

Microalgae production involves cultivation, biomass harvesting and recovery of compounds of interest from biomass. Of all unit operations used in microalgae production, harvesting biomass from culture suspension is of crucial importance, both from economic and technological standpoints (Bilanovic and Shelef 1988; Sunkenik et al. 1988). It is estimated that biomass recovery accounts for at least 20-30 % of the total biomass production cost (Gudin and Thepenier 1986). Algal biomass is challenging to harvest due to the small cell size, low specific gravity and low biomass concentration in culture medium (Grima et al. 2003).

Various techniques such as filtration, centrifugation, sedimentation, floatation and flocculation have been used to harvest biomass (Golueke and Oswald 1965; Uduman et al. 2010); however, most of these methods have their own drawbacks. For example, centrifugation is an energy intensive process and consequently the operating cost is high

(Grima et al. 2003). Some flocculants used to harvest biomass are toxic compounds, for example, polyacrylamide and alum, and they are retained in the harvested biomass (Beim and Beim 1994; Buelna et al. 1990; McCollister et al. 1965). Filtration is suitable for recovering algal species with large cell size, but inadequate to recover algal species with sizes approaching bacterial dimensions (in the range of micrometers) (Grima et al. 2003). Small filter pore size required for microalgae filtration increases the cost of operation. Furthermore, it is not feasible to scale up some of these biomass harvesting methods.

Among those methods mentioned above, flocculation is a relatively simple and promising option for algal biomass recovery. Strictly speaking, flocculation involves two processes (Harrison et al. 2003; Knuckey et al. 2006). The first process is coagulation, through which colloidal particles and very fine solid suspensions are destabilized so that they can begin to agglomerate if the conditions are suitable. The second process is flocculation, by which the destabilized particles actually conglomerate into larger aggregates so that they can be separated from the liquid. But the word "flocculation" usually refers to both processes.

2.3 FLOCCULATION BY pH ADJUSTMENT

The term auto-flocculation refers to spontaneous aggregation of algal cells to form flocs and settle down. Auto-flocculation is usually associated with an increase in pH (Uduman et al. 2010). The reason why pH affects microalgae cells is that there are certain functional groups (such as carboxyl and amino groups) on the surface of the cells. These

groups are in a state of ionization in solution. Thus, microalgae cells carry negative charges and stay stable in solution. However, the ionization of these functional groups is very sensitive to pH. Hence, auto-flocculation can be induced by pH adjustment.

The initial pH of microalgae culture medium is usually around 7. However, pH of culture medium changes during algae growth if the medium is not buffered. It was observed by Nigam et al. (1980) and Horiuchi et al. (2003) that flocculation of microalgae could be induced by increasing the pH of culture medium. In other words, flocculation is very sensitive to pH, so pH is an important factor in the optimization of microalgae biomass harvesting.

In general, flocculation by pH adjustment does not introduce hazardous chemical flocculants into the culture medium and the harvested biomass. It is simple, effective and can be a non-toxic and low cost method. Therefore, flocculation by pH adjustment could be a practical method to harvest microalgae biomass.

2.4 FLOCCULATION BY FLOCCULANT ADDITION

Flocculants are chemicals that promote flocculation by causing colloids and other suspended particles in liquids to aggregate and form flocs. The mechanism of flocculation by flocculant addition is mainly based on charge neutralization and bridging of particles (Grima et al. 2003). Flocculant type and concentration are important processing parameters affecting the flocculation by flocculant addition.

Flocculants can be classified into inorganic and organic flocculants. Inorganic

flocculants are often metal salts that have multivalent cations, such as aluminum sulfate and ferric chloride. Organic flocculants are generally long-chain polymers (also referred to as polyelectrolytes) such as chitosan and cationic starch. Based on their charge, polymers are grouped into cationic, nonionic and anionic polymers. Since bio-particles suspended in aqueous solutions are usually negatively charged, cationic polymers are the most common type of flocculants used in biological systems. The ability of flocculants to promote flocculaion mainly depends on the electric charge and the size of the flocculant molecule. Usually the flocculants with larger electric charge and molecular size are more effective in flocculation (Harrison et al. 2003).

Both inorganic and biopolymer flocculants may have potential toxicity. Beim and Beim (1994) did the ecological-toxicological assessment of six flocculants: Magnafloc E1O, Zetag 64 (Germany); Sanfloc N520P, Sanfloc CH009P (Japan); Catfloc (USA); and Polyacrylamide-PAA (Russia). All the six flocculants adversely affected all water ecosystem components and, especially, proto-coccal algae, invertebrates and adult fish (Beim and Beim 1994). Therefore, flocculant properties become very important while choosing a flocculant for a given application, specifically when the harvested biomass to be consumed by people or animals. For example, Knuckey et al. (2006) combined pH adjustment and biopolymer addition to concentrate microalgae to be used as aquaculture feed. Results showed that the flocculation by biopolymer Magnafloc LT-25 addition compared well with flocculation by ferric chloride, with flocculation efficiencies of over

80%. Juvenile Pacific oysters fed on algae concentrates prepared by biopolymer flocculation grew faster than those fed on biomass prepared by ferric chloride flocculation (Knuckey et al. 2006).

Unlike inorganic flocculants, flocculation by polymers requires much smaller concentration (Buelna et al. 1990; Morales et al. 1985; Vandamme et al. 2009). Besides, there are non-toxic biopolymers, such as chitosan, sodium alginate and cationic starch, which can produce non-toxic biomass, so these non-toxic biopolymers are preferred as flocculant.

Chitosan is a linear polysaccharide, comprised of randomly distributed D-glucosamine and N-acetyl-D-glucosamine molecules linked by β-(1-4) bonds. It is produced commercially by the deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (crabs, shrimp, etc.) and cell walls of fungi.

Alginate, also referred to as the salts of alginic acid or alginic acid itself, is an anionic polysaccharide extracted from the cell walls of brown algae. It is composed of two hexouronic acids: α-l-guluronic acid and β-d-mannuronic acid, linked by 1-4 bonds.

Cationic starch is a starch derivative obtained by introducing cationic groups to the glucose hydroxyl groups on a starch molecule. The typical degree of substitution (DS) of cationic starch is one to two charged groups per hundred glucose units, i.e. 0.02 to 0.03, and a high DS can contribute to better flocculation performance.

The flocculant concentration is another factor that significantly affects both

flocculation efficiency and rate. In addition to flocculant concentration (mass per volume), flocculant: algal biomass ratio (w/w) is also used to describe the amount of flocculant used for recovery of a unit amount of microalgal biomass. Studies by Buelna et al. (1990) and Morales et al. (1985) reported that the required concentration of biopolymers for a given microalgae suspension was much less than inorganic flocculants to achieve the same flocculation efficiency. Compared to freshwater algae, the marine algae strains usually require higher amounts of flocculant due to the high salinity of the culture medium (Bilanovic and Shelef 1988; Uduman et al. 2010). The optimal dosage of the flocculants alum or ferric chloride required to flocculate marine microalgae was five to ten times higher than that for freshwater strains (Sunkenik et al. 1988). It was indicated that an increase in flocculant concentration may improve the flocculation efficiency, but excessive flocculant addition may result in low flocculation efficiency (Bilanovic and Shelef 1988; Lubian 1989; Vandamme et al. 2009). The reason for this result is not clear. In general, higher flocculant concentration is required for algae solution with higher biomass concentration. Several studies reported that the flocculant and the particle concentrations are linearly correlated (Black and Vilaret 1969; Vandamme et al. 2009) at a given flocculation efficiency, while others found no consistent trend (Divakaran and Pillai 2002).

Flocculation by flocculant addition can be an effective method to recover microalgal biomass. However, the processing parameters, such as flocculant type,

flocculant concentration and the pH of algae culture, will affect the flocculation greatly and thus need to be carefully optimized.

2.5 ELECTRO-FLOCCULATION

Electro-flocculation is another method to recover algal biomass. According to Azarian et al. (2007), the mechanisms of algal biomass recovery by electricity involve: (a) electro-oxidation; (b) electro-flocculation; (c) electro-flotation; or any combination of these three mechanisms. A potential difference is applied between two electrodes placed in a solution containing algae cells. The current generated by the potential difference facilitates oxidation reactions and the metal anode, for example, aluminum anode, generates aluminum ions that react with hydroxyl ions in water and form different types of aluminum hydroxides to flocculate algae. The function of aluminum hydroxide produced during electro-flocculation is similar to that of inorganic flocculants, which is to destabilize microalgae cells and facilitate flocculation. Meanwhile, the cathode generates hydrogen bubbles which entrap some algae and float them up to the surface (Azarian et al. 2007). Power supply, current, voltage, electrode material, effective area of electrodes and the spacing between electrodes affect flocculation efficiency.

The power for electro-flocculation can be supplied via direct current (DC) or alternating current (AC) power supplies. There are a number of studies on utilization of DC power to remove algae from water. Algal biomass was effectively harvested by using DC power (Alfafara et al. 2002; Aragon et al. 1992; Azarian et al. 2007; Gao et al. 2010;

Kumar et al. 1981; Sandbank et al. 1974; Tumsri and Chavalparit 2011; Vandamme et al. 2011). AC power has been used to remove cadmium from water successfully by electro-flocculation (Vasudevan and Lakshmi 2011), but information on electro-flocculation by AC power for algae harvesting is lacking.

Current and voltage are key factors that determine electro-flocculation efficiency. Current mainly depends on voltage as well as on other factors like resistance and spacing between electrodes. Higher current and voltage resulted in not only an increase in the microalgae flocculation efficiency, but also a decrease in the operation time and settling time (Alfafara et al. 2002; Azarian et al. 2007). One reason for it was that the higher power input increased the amount of flocculating agent produced during the process. Decreasing the voltage led to slower flocculation rate, but it had the advantage of consuming less energy (Poelman et al. 1997). Therefore, a balance between the efficiency and energy cost needs to be achieved through optimization.

Electrodes play an important role in electro-flocculation. The materials of electrodes include active material such as aluminum and iron, as well as inactive material such as stainless steel and graphite. Since aluminum and iron anodes produce flocculants during electrolysis, they usually result in high flocculation efficiency and are widely used (Alfafara et al. 2002). Both Gao et al. (2010) and Vandamme et al. (2011) compared the performance of aluminum and iron electrodes and concluded that aluminum electrodes gave a higher efficiency than iron anodes. However, both aluminum and iron are

susceptible to corrosion and have a relatively short life span. Other materials like graphite did not have these disadvantages, but they gave low flocculation efficiency (Alfafara et al. 2002). Hence, development of effective, low-cost and durable electrodes is still a challenging problem.

The effective area of electrodes means the surface area of electrodes submerged in the microalgae suspension through which the current passes. The number, shape and effective area of electrodes and the spacing between electrodes are factors affecting electro-flocculation efficiency. In the experiments carried by Poleman et al. (1997), the processing parameters in electro-flocculation were optimized: nine electrodes placed in a 100 L suspension with 26.5 cm spacing between each other, 26.5 V, 1.0 A current and 75 min operation time. More than 95% of microalgae were easily separated from the dilute suspensions and little energy (0.33 kWh/m^3) was consumed under the optimal conditions (Poelman et al. 1997).

The fact that high flocculation efficiency can be achieved with low energy consumption is one of the main advantages of the electro-flocculation process. Another advantage is that there is no need to add flocculant into the algae culture; thus, it simplifies the process. Also, the electro-flocculation system is easy to set up and control and performs well in a large pH range (Ge et al. 2004). Electro-flocculation is an attractive method, from both technical and economical perspectives, to harvest microalgal biomass.

2.6 EFFECTS OF PROCESSING PARAMETERS ON FLOCCULATION EFFICIENCY

There are several processing parameters that affect flocculation efficiency. Strain type affects harvest efficiency due to the properties like cell size (1-20 μm), cell density, cell wall composition, the charge of cells and various medium compositions. Different strains may have different responses to the same processing parameter. So far, the majority of work done on microalgae flocculation has been on freshwater species (Uduman et al. 2010). Because of the high ionic strength in seawater, however, flocculation of marine microalgae is quite different from freshwater strains.

Biomass concentration of microalgae solutions vary from 0.5 to 5 g dry mass per liter (Vandamme et al. 2009). Biomass concentration in the medium determines the flocculant concentration required to achieve acceptable flocculation efficiencies. A study by Vandamme et al. (2009) showed that the cationic starch concentration required to flocculate 80% algae was linearly correlated with the biomass concentration. Biomass concentration may also affect the conductivity and viscosity of the algae solution, and thus affect the electro-flocculation efficiency.

Mixing facilitates contact among cells as well as among cells and flocculants. However, when mixing speed is too high, flocs that have formed can be destroyed due to high shear forces (Alfafara et al. 2002; Grima et al. 2003). Therefore, stirring speed is also a processing parameter that needs to be optimized.

Operation time (OT) refers to how long the algae suspension is kept stirring when it is treated with flocculant, electricity or pH adjustment. Settling time (ST) is the time that algae cells are allowed to settle out of the suspension. Flocculation efficiency may increase with increasing OT and ST (Alfafara et al. 2002; Horiuchi et al. 2003). However, longer time results in more energy consumption and cost. In addition, long treatment may weaken the floc strength (Alfafara et al. 2002) due to the long stirring time during the treatment. Therefore, OT and ST need to be optimized to achieve high flocculation efficiency.

Temperature of the solution may affect flocculation through changes in the physical and chemical properties like density, viscosity and dielectric constant of the culture medium and the flocculant (Jin 2005). Jin (2005) used a high resolution photographic technique to study the flocculation in water treatment, and he found that low water temperature had a detrimental impact on aggregation. A water temperature of 0 ℃ resulted in slow floc growth and small floc size (Jin 2005). Pan et al. (2009) studied the effects of temperature on flocculation efficiency by using bioflocculant PG.a21 Ca to process kaolin suspension; he found that the flocculation was enhanced with an increase of temperature in the range of 5-60 ℃, but flocculation efficiency decreased at temperatures above 60 ℃. Thus, too high or too low temperature beyond a certain range may result in low flocculation efficiency. The effects of temperature on microalgae flocculation need to be studied in order to find the temperature range and optimal

temperature for each strain and different flocculation system.

CHAPTER III

MATERIALS AND METHODS

3.1 CULTIVATION OF MICROALGAE

Two microalgae strains investigated in this study were obtained from the Culture Collection of Algae at the University of Texas at Austin (UTEX). *Picochlorum oklahomensis* (UTEX B 2795), which is a strain native to Oklahoma, was grown in Modified Artificial Seawater Medium (UTEX). The other strain, *Nannochloropsis oculata* (UTEX LB 2164), was grown in Erdschreiber's Medium (UTEX). The algae cultures in 2 L glass bottles (Kimble Chase Life Science and Research Products LLC, Vineland, NJ) were kept in a closed growth chamber with inner dimensions of 118.8 cm × 58.4 cm × 76.9 cm (Length × Height × Width). The growth chamber was maintained at 23 ± 4 ℃. Four cool white fluorescent bulbs (General Electric Company, Fairfield, Connecticut) installed in the chamber were the light source, and the photosynthetic photon flux (PPF) was 56 ± 4 μmol m^{-2} s^{-1}, measured by a quantum meter (model QMSW-SS, Apogee Instruments Inc., Logan, UT). Cultures were subjected to a 12 h: 12 h cycle in which 12 h was light with aeration while the other 12 h was dark without aeration. The aeration rate was 50 mL/min and the concentration of CO_2 (Industrial

Carbon Dioxide, Airgas, Stillwater, OK) in the air (Grade D Breathing Air, Airgas, Stillwater, OK) was 2 %. The initial cell concentration of the culture medium was 7.6×10^4 cells/mL of medium.

3.2 CHARACTERIZATION OF MICROALGAE

For PO and NO, growth curves were determined as a function of absorbance (ABS) vs. time. The ABS of the sample was measured at 680 nm by a spectrophotometer (model DU 520, Beckman Coulter, Brea, CA). The pH of culture suspensions was measured daily by a pH meter (model AR20, Fisher Scientific, Waltham, MA). Cell density and dry biomass concentration were determined regularly. Two linear associations, cell density vs. ABS and dry biomass concentration vs. ABS, were calculated. Oil content in the biomass of both strains was determined and the fatty acid composition of algae oil samples was analyzed by a gas chromatography (GC) system (model 7890 A, Agilent Technologies, Santa Clara, CA) equipped with a mass spectrometer (MS) (model 5975 C, Agilent Technologies, Santa Clara, CA). Oil content in culture medium was also determined to see whether there was any oil excreted by algae cells. Flocculation tests were carried out when algal biomass concentration reached its maximum in the culture medium (stationary phase).

3.3 FLOCCULATION TECHNIQUES

The following techniques were examined for biomass flocculation: 1) flocculation by pH adjustment; 2) flocculation by non-toxic biopolymers; 3) electro-flocculation.

3.4 FLOCCULATION BY pH ADJUSTMENT

The processing variables for the PO jar tests were set as follows: pH 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and settling time 1 and 2 h. For NO tests pH and settling time settings were the same as those for PO except pH 13 was omitted because of the very high flocculation efficiency achieved at pH 12. The microalgae suspension (120 mL) pH was adjusted to the desired level by using 1 M sodium hydroxide (NaOH) or 1 M hydrochloric acid (HCl) solutions during 5 min stirring at 250 rpm. Then 100 mL of microalgae suspension was placed in a 100 mL graduated cylinder to allow cells to settle, and the initial ABS was measured at the same time. Two layers and an interface appeared in the solution during settling. After 1 h and 12 h of settling time, a 5 mL sample was withdrawn from the supernatant (top portion of the solution) for absorbance measurement (final ABS). A 5 mL sample was withdrawn at the 60 % cylinder height when there was no phase separation. The experimental control was the algae solution without pH adjustment.

Flocculation efficiency was determined as follows:

$$\text{Flocculation efficiency (\%)} = \frac{\text{Initial ABS} - \text{Final ABS}}{\text{Initial ABS}} \times 100\% \qquad (1)$$

3.5 FLOCCULATION BY BIOPOLYMER ADDITION

3.5.1 Biopolymer Screening Test

The biopolymer screening test was carried out using three non-toxic biopolymers: chitosan, sodium alginate, and cationic starch. These biopolymers were selected due to

their long carbon chain length and cationic electric charges on the molecules.

Chitosan (medium molecular weight) used in this study was obtained from Sigma-Aldrich/ Acros Organics (St. Louis, MO). Chitosan solution with a concentration of 5 g/L was prepared by dissolving 5 g chitosan in 1 L of 1 % acetic acid solution. The pH of the chitosan solution was around 3.5.

Sodium alginate (GRINDSTED Alginate FD 155) was obtained from DANISCO (Copenhagen, Denmark). Sodium alginate solution with a concentration of 10 g/L was prepared and its pH ranged 5.5 to 7.5.

CHARGEMASTER® L360 liquid cationic starch paste was used in this study and obtained from Grain Processing Corporation (Muscatine, IA). The starch paste contained about 41 % solids and a very high level of cationic charge (DS of 0.53-0.65). Slurry with 10 % (w/w) cationic starch was prepared by diluting the starch paste with deionized water.

A jar test similar to the one described in section 3.4 of this thesis was carried out and the flocculation efficiency was calculated by using Eq. (1). The difference between the jar tests described in section 3.4 and this section was, the flocculant addition which was done while stirring the solution at 250 rpm for 5 min before or no pH adjustment. Final ABS was measured at settling time of 1 h. The experiments were carried out at room temperature. One strain, PO, was used for the biopolymer screening test. Control was the treatment without flocculant addition. Flocculant amounts 5, 10, 20, 40 and 60 mg

corresponded to flocculant: biomass ratio of 0.06, 0.13, 0.25, 0.51 and 0.76, respectively.

3.5.2 Optimization of chitosan flocculation

Based on the preliminary results of the biopolymer screening test, the most efficient flocculant was chitosan. Hence, chitosan was used for the optimization experiments. The optimization experiments were jar tests as described in Section 3.5.1 of this thesis. Chitosan solution with a concentration of 10 g/L was used.

Levels of the experimental variables for the PO tests were as follows: pH 4, 5, 6, 7, 8, 9, 10, 11 and 12 and flocculant (chitosan) amounts of 20, 40, 60, 80 and 100 mg which corresponded to chitosan: biomass ratio (mg/mg) (CAR) of 0.09, 0.18, 0.27, 0.36 and 0.45, respectively. Of the possible forty-five pH × chitosan combinations for PO treatment, twenty-eight treatment combinations were selected as the design points based on the statistical method used in this study (Figure 1). The treatment at pH 8 and chitosan amount of 60 mg was the “center point” at which four replications were run. Four design points closest to the center had three replications, and all the others had two replications. Final ABS was measured at settling time of 1 h and 12 h. The control was the treatment without chitosan addition.

Figure 2 shows the experimental design points selected for NO. The effects of chitosan amount [10, 20, 30, 40, 50 and 60 mg which corresponded to chitosan: biomass ratio (mg/mg) (CAR) of 0.08, 0.17, 0.25, 0.33, 0.42 and 0.50, respectively] and pH (6, 7, 8, 9, 10 and 11) on flocculation efficiency were examined. The treatment at pH 8 and

chitosan amount of 30 mg was the “center point”, at which four replications were run. Six design points close to the center also had four replications, and all the others had two replications. Final ABS was measured at settling time of 1 h and 12 h. The control was the treatment without chitosan addition. In all the chitosan flocculation experiments, the biomass content of the solution used for each treatment was kept constant.

3.6 ELECTRO-FLOCCULATION

A schematic diagram and a picture of the electro-flocculation system that was used for the experiments are shown in Figure 3 and Picture 1. Electro-flocculation tests were conducted at room temperature and 100 rpm stirring rate using 250 mL microalgae suspension. Two aluminum plates each having a 40 cm^2 effective surface area were used as electrodes and the spacing between them was 4 cm. Initial ABS of microalgae suspension was measured before treatment. Current, provided by a DC power supply (model 1710, BK PRECISION, Yorba Linda, CA), was applied to the microalgae suspension for varying operation time. Cells were allowed to settle after treatment. Two layers and an interface appeared in the solution during settling. After 0.5 h, 1 h and 12 h of settling time, a 5 mL sample was withdrawn from the supernatant (top portion of the solution) for absorbance measurement (final ABS). Flocculation efficiency was determined by Eq. (1).

The processing parameters in optimization experiments for PO were set as follows; operation time of 5, 10, 15 and 20 min, current of 0.2, 0.5 and 0.8 A and settling

time of 0.5, 1 and 12 h,. Since flocculation efficiencies were close to 100 % at 0.5 and 0.8 A and 15 min OT, these conditions were not included in the experimental design. Thus, an incomplete 3 × 4 factorial design was used to optimize electro-flocculation of PO. For NO, operation time of 2, 3, and 4 min and current value of 0.1, 0.3, and 0.5 A were chosen for the study. A 3 × 3 factorial experimental design was utilized to optimize electro-flocculation of NO. Final ABS was measured at settling time of 0.5 h, 1 h, and 12 h.

3.7 ANALYTICAL METHODS

3.7.1 Dry Biomass Concentration

Aluminum dishes with glass microfiber filters (GF/C 90 mm diameter, Whatman, ME14 2LE, UK) were pre-dried in a forced-air oven (model 1370 FM, VWR Science, Bristol, CT) at 105 ^{o}C for 2 h and then cooled to room temperature in desiccators and weighed. Sample (50 mL) was filtered by vacuum filtration by using the pre-weighed filter paper. Aluminum dish and filter paper with retained biomass were dried in the oven at 105 ^{o}C for 12 h, then cooled in desiccators to room temperature and weighed. The biomass concentration of the sample was reported as weight difference / volume of sample.

3.7.2 Oil Content in Biomass

The procedure to determine the oil content of algal biomass was largely appropriated from Lee et al. (1998). The biomass was harvested from the culture medium by

centrifugation at 8000 rpm for 10 min. About 120 mg biomass (dry weight) was used for lipid extraction. The cells were suspended in 5 mL of phosphate buffer (pH 7.4) and treated in a bead-beater (model HBB908, Hamilton Beach, Richmond, VA) for 1 min using 1 mm glass beads. The raptured cells were transferred to a separation funnel and chloroform/methanol (2:1, v/v) of 30 mL was added for lipid extraction. The mixture containing algal cells was shaken vigorously for 20 min and left to stand for 30 min. After phase separation, the organic layer was decanted. The residual cells were treated with 20 mL solvent once again and the organic layer was recovered. The combined chloroform/ethanol extract was washed with 20 mL 5 % (w/v) sodium chloride solution. The solvent was evaporated from the extract in RapidVap (LABCONCO Corporation, Kansas City, KS). The total lipids were measured gravimetrically. The oil content of the sample was reported as extract weight / dry biomass of sample.

3.7.3 Oil Content in Culture Medium

After the separation of microalgae biomass and culture medium by centrifugation, oil content in culture medium was determined. Petroleum ether, 25 mL, was added to 250 mL culture medium placed in a 500 mL separation funnel. After phase separation, the organic layer was decanted. The same procedure was repeated twice with 25 mL of petroleum ether. The following washing and evaporation steps were same as the steps described in section 3.7.2. The total lipids were measured gravimetrically. The oil content in the culture medium was reported as weight difference / weight of the culture medium.

3.7.4 Fatty Acid Composition

The oil sample was first converted to its fatty acid methyl esters (FAME) according to the following method. About 20 mg oil sample was weighed into a 10 mL tube, and about 1.9 mL GC grade heptane, 100 μL of 10 mg/mL undecanoic acid methyl ester (internal standard) (99% GC grade, Sigma-Aldrich, St. Louis, MO) in GC grade heptane and 200 μL of 2 M potassium hydroxide in methanol solution were added. After mixing and centrifugation at 3000 rpm for 3 min, the lower layer was discarded using a Pasteur pipette. Saturated ammonium acetate solution (500 μL) was added into the methylated oil sample, mixed and centrifuged at 3000 rpm for 3 min. Then the lower layer was discarded. After adding deionized water (500 μL), mixing and centrifugation, the lower layer was discarded again. The organic layer was dried with some anhydrous sodium sulfate. After centrifugation and filtration, the organic liquid was transferred in a GC vial. Separation of fatty acids was carried out using a CP-Sil 88 column with 100 m x 0.25 mm inner diameter and 0.20 μm film thickness (CP7489, Agilent Technologies, Santa Clara, CA). Chromatographic peaks were detected by a mass spectrometer detector (MSD) on electron impact (EI) mode (model 5975 C, Agilent Technologies, Santa Clara, CA). The carrier gas, helium, flow rate was 1.0 cm/s. The initial oven temperature, 80 ^{o}C, was increased to 220 ^{o}C at a rate of 4 ^{o}C/min and maintained at 220 ^{o}C for 5 min. Then the temperature was increased from 220 ^{o}C to 240 ^{o}C at a rate of 4 ^{o}C/min and held at 240 ^{o}C for 10 min. The total run time was 55 min. Solvent delay was set at 11.0 min. The

conditions of the MS detector were as follows: temperature of the EI ion source was 230 ^{o}C, electron energy was 70 eV and mass data were collected in full scan mode (m/z 35-550). Oil samples (1 μL) were injected manually. The injector temperature was maintained at 250 ^{o}C. The split ratio was 10: 1. Data were analyzed using the Agilent GC/MSD Productivity Chemstation software (Revision. G1701 B.02.00 sp2, Agilent Technologies, Santa Clara, CA) with the Automated Mass Spectral Deconvolution and Identification System (AMDIS) software and library search [National Institute for Standards and Technology (NIST), Gaithersburg, MD]. Chromatographic peaks were identified by comparison of the retention times of a 37 component FAME standard mix (Supelco, Bellefonte, PA) and a GLC 403 standard (Nu-Chek Prep Inc., Elysian, MN) to the peak retention times on sample chromatograms. Undecanoic acid methyl ester (C11:0) was used as the internal standard.

3.8 STATISTICAL ANALYSIS

Except the biopolymer screening test, all analytical tests and flocculation experiments were carried out at least in duplicate and in randomized order with the mean values being reported. Data from flocculation by pH adjustment, chitosan addition and electro-flocculation were analyzed using a generalized linear mixed model for responses fit by a beta distribution. Comparisons to the maximum response mean were done using Dunnett's multiple comparisons with a "best" mean. These analyses were performed using SAS 9.3 and SAS 9.2 (SAS Institute Inc., Cary, NC). The two linear associations,

dry biomass concentration vs. ABS, and cell density vs. ABS, were calculated using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA). The coefficient of determination, R^2, indicated the percent of the data that was closest to the best fit line and how well the regression line represented the data.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 CHARACTERIZATION OF MICROALGAE

4.1.1 Characterization of PO strain

The shape and size of microalgae cells were observed under microscope (Picture 2). PO strain had either round or oval green cells. The observed cell size of PO, 1-2.5 μm, was in agreement with the literature, 2 μm (Hironaka 2000). Unlike some algae strains that form cell clusters, PO cells were dispersed in the culture medium, which makes the biomass recovery harder. Thus, it is necessary to develop effective processes to harvest PO cells.

The maximum absorbance was observed at 680 nm for PO cultures (Figure 4). This is due to the presence of chlorophyll a in the cells of PO which is a green alga. The maximum absorption peak of chlorophyll a at 680 nm can be used to estimate chlorophyll and indirectly algal biomass concentrations in a solution (Erokhina et al. 2002; Erokhina et al. 2004). As microalgae grew and the biomass concentration in microalgae culture suspension improved, the content of chlorophyll a in algae culture increased, and as a result, the intensity of the peak increased, indicating a good association between

absorbance at 680 nm and algal biomass concentration in solution (Figure 5).

The linear association of dry biomass concentration vs. ABS for PO is shown in Figure 5. The following regression equation was obtained:

$y = 0.6528\ x + 0.0274\ (R^2 = 0.85)$

where y = dry biomass concentration (g/L), x = ABS at 680 nm.

The linear association of cell density vs. ABS for PO was calculated (Figure 6). The regression equation is as follows:

$y = 721.88\ x - 15.586\ (R^2 = 0.94)$

where y = cell density ($\times 10^5$ cells/mL) and x = ABS at 680 nm.

Algae growth curve was determined as a function of ABS vs. time (Figure 7). Four typical growth stages were observed for PO: lag phase, exponential phase, stationary phase and death phase. It took about 18 days for the PO cultures to reach maximum biomass concentration in the medium. The highest biomass concentration was approximately 2.1 g/L. PO biomass concentration in the medium started decreasing shortly after it reached the maximum, indicating that biomass needs to be harvested as soon as maximum biomass concentration is reached in the medium. Delay in harvest would cause biomass degradation.

The pH of the culture suspensions was monitored during the growth of PO (Figure 8). The initial pH of the PO culture suspension was adjusted to 8.0. Then pH slightly fluctuated at around 7.5 during the entire growth period.

Oil contents in algae strains may vary significantly depending on the growth conditions and stage. The oil content in PO biomass harvested during stationary phase was about 20.5 % (w/w, dry basis). Oil content in culture medium was analyzed after separation of intact PO cells. The PO culture medium did not contain significant amount of oil (0.01 %, w/w, as is basis), indicating that PO did not excrete oil to the medium and all the oil was accumulated within the cells. Hence, efficient biomass harvesting is critical for the feasibility of algal oil production.

Linoleic (C18:2n6c) (26.2 %), palmitic (C16:0) (23.8 %) and linolenic (C18:3n3) (13.5 %) acids accounted for 63.5 % of the total fatty acids in PO oil (Table 1). Significant amounts of palmitoleic (C16:1) (8.2 %), oleic (C18:1n9c) (8.0 %) and cis-11-octadecenoic (C18:1n11c) (5.8 %) acids were also detected in the samples. Although we did not have the standard to verify the peak retention time, a NIST AMDIS library search indicated the possibility of the presence of 7,10-hexadecadienoic acid (C16:2n6) (6.9 %) in the oil. PO oil contained a high level of PUFA (47 % of the total fatty acids) which comprised of 13.5 % n-3 and 33.2 % n-6 fatty acids. Two fatty acids essential for human nutrition, linoleic (C18:2n6c) and linolenic (C18:3n3) acids, made up more than one third of the total fatty acids (40 %). Thus, PO oil could be a good source of essential fatty acids. High PUFA content may lower the oxidative stability of biodiesel derived from PO oil. However, PO oil can still be utilized for production of renewable diesel by using hydrodeoxygenation process. Fatty acid composition of the feedstock does not affect the

oxidative stability of the renewable diesel produced by this process.

4.1.2 Characterization of NO strain

Similar to PO, NO cells were either round or oval, green and dispersed in the culture medium (Picture 3). However, the cell size of NO, 5-9 μm, was significantly larger than that of PO, 1-2.5 μm. The large cell size of NO may promote easy floc formation. The diameter of NO cells reported in the literature was 1-6 μm (Andersen et al. 1998; Madhu et al. 2004; NCMA; Yufera and Pascual 1985). The variation in NO cell size reported in this study and the literature was probably due to the different growth conditions, for example, composition of culture medium.

As expected, there was an absorption peak at 680 nm for NO cultures (Figure 9) because of the presence of chlorophyll a in the NO cells. Likewise, good associations could be calculated between algal biomass concentration in solution and absorbance at 680 nm (Figure 10), and between algal cell density in solution and absorbance at 680 nm (Figure 11).

The linear association of dry biomass concentration vs. ABS for NO is shown in Figure 10. The following regression equation was obtained:

$y = 1.0097 x - 0.0541$ ($R^2 = 0.97$)

where y = dry biomass concentration (g/L), x = ABS at 680 nm.

The linear association of cell density vs. ABS for NO was calculated (Figure 11). The regression equation is as follows:

$y = 30.96 x + 0.4588$ ($R^2 = 0.95$)

where y = cell density ($\times 10^5$ cells/mL) and x = ABS at 680 nm.

Growth curve of NO was determined as a function of ABS vs. time (Figure 12). The four typical growth stages were observed: lag phase, exponential phase, stationary phase and death phase. Biomass concentration in NO culture medium reached the maximum at around the 11^{th} day, about a week earlier than PO. However, the highest biomass concentration for NO, 1.2 g/L, was significantly lower than that for PO. The stationary phase for NO was about 12 days. The biomass concentration of algae in the medium varies depending on the strain type, medium composition and growth conditions. Similar to the findings reported in this thesis, *Nannochloropsis sp*. biomass concentrations of 0.8-3.8 g/L in various media and growth conditions were reported by other research groups (Rodolfi et al. 2003; Xu et al. 2004).

When both strains had the same ABS and the same volume of culture suspensions, the number of PO cells was more than 20 times higher than that for NO. However, the difference in dry biomass was less than double. That was because PO cells were significantly smaller than NO cells. Compared to NO, the smaller cell size and higher cell density makes PO cells harder to harvest.

For NO culture suspension, the initial pH was 6.5. As NO began to grow, the pH increased slightly (Figure 13). When the cell concentration of NO reached its maximum, pH of the culture medium was around 7.2. Then the medium pH slowly decreased to 6 as

NO stayed in the stationary phase. During the death phase, the pH decreased steadily.

The oil content in NO biomass harvested during stationary phase was about 36.4 % (w/w, dry basis). This value was within the range of NO oil content (8-50 %) reported in literature (Brown 1991; Chiu et al. 2009; Converti et al. 2009). Oil content in NO biomass was two times higher than that for PO (20.5 %). But at the same time, the maximum biomass concentration of PO (2.1 g/L) was nearly twice as much as that of NO (1.2 g/L). Therefore, considering both biomass concentration and oil content in biomass, PO and NO contained the same amount of oil per volume of culture solution (0.431-0.436 g oil/L of culture solution). The oil contents of PO and NO biomass are comparable to the oil content in common oilseeds, 18-70 % (Inchbald 2000; McKevith 2005; Salunkhe et al. 1992), indicating that PO and NO can be potential feedstocks for oil production.

Linolenic (C18:3n3) (25.8 %) and palmitic (C16:0) (19.5 %) acids were the most abundant fatty acids in NO (Table 1). The other major components of NO oil were linoleic (C18:2n6c) (9.0 %), oleic (C18:1n9c) (8.8 %), γ-linolenic (C18:3n6) (5.3 %), cis-11-octadecenoic (C18:1n11c) (4.0 %), and palmitoleic (C16:1) (2.0 %) acids. Similar to PO, 7,10-hexadecadienoic acid (C16:2n6) (2.2 %) was identified from the NIST AMDIS library search. In NO oil, there was one unknown component which consisted of 12.5 % of the total GC-MS peak area. Based on the NIST AMDIS library search, this compound could be either cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n3) (EPA) or cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6n3) (DHA). However, the retention time

of this compound was 8 min earlier than the retention times of EPA and DHA as determined by the available standard FAME mixture. Consequently, identification of this peak requires further analytical tests to be carried on the oil samples.

The most abundant saturated fatty acid in NO was palmitic acid (C16:0) (19.5 %), while all the other saturated fatty acids accounted for only 2 % altogether. The saturated fatty acids content of NO oil was similar to that of PO. NO contained 9.0 % linoleic acid (C18:2n6c) and 25.8 % linolenic acid (C18:3n3). The PUFA (45 %) and total n-3 and n-6 fatty acid (44 %) contents of NO oil examined in this study were within the range reported in the literature, 20.2-50.1 % and 20.1-49.8 %, respectively (Dunstan et al. 1993; Hodgson et al. 1991; Mourente et al. 1990; Renaud and Parry 1994; Renaud et al. 1991; Roncarati et al. 2004; Zhukova and Aizdaicher 1995). Essential fatty acid content of NO oil reported in this study, 35 %, is significantly higher than that reported in literature, 0-6 %.

The fatty acid composition in soybean oil is quite different from that of algae oil (Table 1). Typically soybean oil (Hammond et al. 2005) contains higher amounts of linoleic (C18:2n6c) (54.5 %) and oleic (C18:1n9c) (23.0 %) acids, but lower amounts of palmitic (C16:0) (10.6 %) and linolenic (C18:3n3) (7.23 %) acids than PO and NO oils examined in this study.

4.2 FLOCCULATION BY pH ADJUSTMENT

The pH flocculation efficiency of PO was below 10% between pH 4 and pH 10

(Figure 14). A sharp increase in flocculation efficiency was observed over pH 10. Between pH 11 and 13, the flocculation efficiency was as high as 90.5 - 97.0 %. The highest flocculation efficiency, 97.0 %, was obtained at pH 13 and ST of 12 h. The same trend of flocculation efficiency vs. pH was observed in the flocculation of *Chlorella* strain by pH adjustment without flocculant addition (Vandamme et al. 2010; Yahi et al. 1994). The reason for the sharp increase in flocculation efficiency is not clear. Lubian (1989) and Morales et al. (1985) have indicated that cell size, cell density, cell wall composition and the charges on cell surface are all contributing variables of flocculation efficiency.

In pH flocculation of NO (Figure 15), there was not such a sharp increase as the one observed in pH flocculation of PO. The flocculation efficiency of NO increased with increasing pH and reached over 99 % at pH 11 (Figure 15). NO flocculation efficiency by pH adjustment was always higher than that for PO at the same pH level and settling time. Hence, NO biomass is easier to harvest than PO. This might be due to the larger cell size of NO and the lower biomass concentration in culture medium than PO.

The effect of pH on flocculation efficiency was significant for both strains (Table 2). The electrical charge originating from the ionization of functional groups on the cell surface stabilizes the suspended cells. The mechanism of flocculation by pH adjustment is explained by the reduction of electric charge on the surface of algae cells (zeta potential) with increasing pH which destabilizes the cells in the solution (Horiuchi et al.

2003; Montgomery 1985). Another reason is that the salts in culture medium may precipitate out of the solution with increasing pH. Algae cells may be trapped within the salt precipitate (Blanchemain and Grizeau 1999).

For both PO and NO, the interactions between pH and ST were significant (Table 2). There were significant differences in the flocculation efficiencies achieved between ST of 1 h and 12 h at the same pH level, except at pH 10 and 11 for PO and at pH 11 for NO. These results indicate that in general high pH over 10 not only induces extensive flocculation, but also accelerates flocculation. In a study where 80 % of algal biomass of *Skeletoma costatum* was flocculated by adjusting suspension pH to 10.2, algae cells lost their viability (Blanchemain and Grizeau 1999) and cell lysis was observed at extreme pH. Hence, the effects of high pH on biomass properties need to be examined carefully.

4.3 FLOCCULATION BY BIOPOLYMER ADDITION

4.3.1 Biopolymer Screening Test

Three nontoxic biopolymers, chitosan, sodium alginate and cationic starch, were tested for flocculation of PO. The first step was to test the flocculation performance of different biopolymers at medium pH (about 7-8) (Figure 16). No flocculation was observed when up to 60 mg of sodium alginate or cationic starch was added into 120 mL of culture medium. Flocculation efficiency of PO reached to around 70 % when the chitosan amount in the medium was increased to 40 mg, but the efficiency did not change significantly when chitosan amount was further increased from 40 mg to 60 mg.

Since the pH flocculation test showed that increasing pH may help flocculation, the flocculant amount was fixed and pH was increased in the second step of biopolymer screening test. Chitosan amount was maintained at 40 mg because no increase in flocculation efficiency was observed beyond this point. After chitosan addition, pH of the algae suspension decreased from about 7.5 to 4.4. An increase in flocculation efficiency was observed for cultures containing chitosan at pH 11. The effect of pH on flocculation performance of sodium alginate and cationic starch (60 mg in 120 mL culture) were also investigated (Figure 17). Sodium alginate or cationic starch addition did not affect the pH of the solution and pH of the medium was about 7.5. No flocculation was detected after the pH of the sodium alginate or cationic starch containing culture medium was adjusted to 8-10. When pH of the sodium alginate containing culture medium was raised to 11, PO flocculation efficiency was still very low (less than 5 %). PO flocculation efficiencies in the presence and absence of cationic starch were similar at pH 11. The preliminary results from biopolymer screening test indicated that chitosan was more effective in flocculation of microalgae cells than the other biopolymers (sodium alginate and cationic starch) tested in this study. Hence, optimization tests were carried out using chitosan for PO and NO flocculation.

4.3.2 Optimization of Chitosan Flocculation for PO

The flocculation efficiency of PO varied between 0.2 % and 98.4 % (Figures 18 and 19). The highest flocculation efficiency, 98.4 %, was obtained at chitosan amount of

80 mg (CAR of 0.36), pH 9 and ST of 12 h. The three-way interaction of processing parameters, pH, chitosan amount and settling time, on flocculation efficiency was significant ($p < 0.0001$) (Table 3).

The effect of pH on flocculation efficiency was very complex and there was no consistent trend (Figure 20). A similar finding was reported for chitosan flocculation of freshwater algae (Divakaran and Pillai 2002). The importance of pH on flocculation of microalgae by chitosan addition was also demonstrated by several other authors (Divakaran and Pillai 2002; Lubian 1989; Morales et al. 1985; Sunkenik et al. 1988). It was argued that the lack of a consistent trend was due to the complex response of chitosan to pH changes. Interpretation of the flocculation mechanisms involved in polymer flocculation is further obscured by the complex physicochemical reactions between chitosan and the algal cells (Morales et al. 1985). The ionic strength of the culture medium changes with addition of NaOH or HCl used for pH adjustment. The ionic strength of the medium may affect the configuration and dimensions of the chitosan polymer, which in turn affects the chitosan flocculation efficiency (Sunkenik et al. 1988). At low ionic strength, the polymer is highly hydrated and linearly extended; hence it is effective in bridging the particles in the medium. High ionic strength can result in formation of randomly coiled molecular configurations with less molecular hydration, thus reducing the effectiveness of the polymer for flocculation (Pelton and Allen 1983). Furthermore, increasing pH may lead to auto-flocculation of algae cells at the same time.

The effect of chitosan amount on flocculation efficiency varied with pH (Figure 21). Except pH 6 × ST of 1 h, an increase in chitosan amount resulted in an improvement in the flocculation efficiency at both settling times of 1 h and 12 h between pH 4 and 9. The response to chitosan amount at pH 10, 11 and 12 was not consistent. It has been reported that chitosan was quite effective in freshwater algae flocculation (Divakaran and Pillai 2002; Knuckey et al. 2006; Sunkenik et al. 1988), but not for marine microalgae (Sunkenik et al. 1988; Vandamme et al. 2009). A similar trend was observed in flocculation of microalgae using cationic starch (Vandamme et al. 2009). This is because of the high ionic strength of seawater resulting from salinity. As mentioned earlier, at high ionic strength some biopolymers partially collapse, reducing the possibility of their interaction with algal cells (Lubian 1989). Thus, the salinity of seawater requires higher flocculant dosages and renders flocculation less effective than in freshwater algae media (Sunkenik et al. 1988). The adverse effect of salinity diminished at reduced salinity levels, less than 5 g/L (Knuckey et al. 2006; Sunkenik et al. 1988). It is also important to point out that there are studies reporting 100% flocculation efficiency for chitosan flocculation of marine microalgae (Morales et al. 1985). In this study, PO and NO were grown in seawater medium. The lack of a consistent trend with pH and chitosan amount can be attributed to the very complex interactions among the chemical components in culture medium and microalgae cells. The presence of the significant three-way interaction among the variables, chitosan amount × pH × ST, also supports the latter argument.

Comparisons of the treatment efficiency means to the highest PO flocculation efficiency were made using Dunnett's multiple comparisons test (Figures 22 and 23). The treatment condition of the highest PO flocculation efficiency is shown as a blue triangle on the figures. There were eleven other treatment combinations of chitosan × pH × ST (the red dots on the figures) that can be recommended for maximizing flocculation efficiency, because the flocculation efficiencies they gave were not significantly different from the highest efficiency. The eleven combinations were: no chitosan × pH 12 × 12 h, no chitosan × pH 13 × 1 h, no chitosan × pH 13 × 12 h, 20 mg × pH 12 × 12 h, 60 mg × pH 8 × 12 h, 60 mg × pH 10 × 12 h, 80 mg × pH 11 × 12 h, 100 mg × pH 8 × 1 h, 100 mg × pH 8 × 12 h, 100 mg × pH 10 × 12 h, and 100 mg × pH 12 × 12 h.

Desirable flocculation efficiency is defined as the observed highest flocculation efficiency plus the other observed flocculation efficiencies that were not significantly different from the highest efficiency. In chitosan flocculation of PO, there were twelve treatment combinations of the processing parameters that gave the desirable flocculation efficiency. The range of the flocculation efficiency for this group was 93.5 - 98.4 %. All 12 treatment combinations were at alkaline conditions (pH 8-13). Selection of the optimal treatment conditions for PO flocculation has to be based on an economic feasibility study that would take into account the cost of chitosan, NaOH used for pH adjustment and the settling time. However, it appears that depending on the cost of chitosan, the treatment condition with 100 mg chitosan, pH 8 and 1 h settling time could be favorable. Relatively

low pH would minimize the adverse effects of high alkalinity on equipment and biomass, and short settling time could improve the production capacity of a commercial operation.

4.3.3 Optimization of Chitosan Flocculation for NO

The flocculation efficiency of NO varied between 15.8 % and 99.8 % (Figures 24 and 25). The highest flocculation efficiency for NO, 99.8 %, was obtained at pH 11 and ST of 1 h with no chitosan addition into the culture medium. Similar to the PO flocculation results, there was no consistent trend with either pH or chitosan amount added (Figures 26 and 27). There was also the significant three-way interaction of the processing variables (pH, settling time and chitosan amount) on flocculation efficiency (Table 2). The reasons for the lack of a trend in flocculation efficiency with varying processing parameters discussed earlier for PO would be applicable to NO flocculation. The difference is that over 95 % NO flocculation could be achieved at lower pH and chitosan: algal biomass ratio than those for PO.

Flocculation efficiencies of NO at different treatment conditions were compared to the highest NO flocculation efficiency using Dunnett's multiple comparisons test (Figures 28 and 29). The treatment condition of the highest NO flocculation efficiency was referred to as "Highest" and shown as a yellow star on the figures. There were seven other treatment combinations that can be recommended for maximizing flocculation efficiency. They gave flocculation efficiencies that were not significantly different from the "Highest". These seven combinations (referred to as "Not Sig_1" and shown as red

triangles on the figures) were as follows in the order of chitosan amount × pH × ST: 0 mg × 11 × 12 h, 20 mg × 11 × 1 h, 20 mg × 11 × 12 h, 40 mg × 11 × 1 h, 40 mg × 11 × 12 h, 60 mg × 11 × 1 h, and 60 mg × 11 × 12 h. From the first comparison, eight treatment combinations of the processing parameters were selected. They gave desirable flocculation efficiency that ranged 99.3 - 99.8 %, and they were all at pH 11.

Because the range of the desirable NO flocculation efficiency given by the above comparison test was narrow (within 0.5 %), and their treatment conditions were very limited (all at pH 11), a second comparison test was performed to find out more treatment combinations resulting in the desirable flocculation efficiency. Data used in the second comparison were the NO chitosan flocculation data excluding the eight flocculation efficiencies and their treatment combinations obtained from the first comparison. The maximum flocculation efficiency among the data used in the second comparison was 97.7 % (referred to as "Max_2"), achieved at chitosan amount of 50 mg, pH 10 and ST of 1 h (shown as a green diamond on the figures). NO flocculation efficiencies were compared to "Max_2", 97.7 %, using Dunnett's multiple comparisons test (Figures 28 and 29). There were five other treatment conditions where the flocculation efficiencies were not significantly different from the **"**Max_2". The five combinations (referred to as "Not Sig_2" and shown as blue triangles on the figures) were as follows in the order of chitosan amount × pH × ST: 10 mg × 8 × 12 h, 30 mg × 10 × 1 h, 40 mg × 9 × 1 h, 50 mg × 8 × 12 h, and 60 mg × 9 × 1 h. From the second comparison, six treatment

combinations of the processing parameters were selected to give the desirable flocculation efficiency, whose range was 94.7 - 97.7 %. These treatments were at pH 8-10.

Combining the results from two comparison tests, there were a total of 14 treatment combinations of the processing parameters that gave the desirable flocculation efficiency in chitosan flocculation of NO. The range of the desirable flocculation efficiency for NO was 94.7 - 99.8 %. All 14 combinations were at alkaline conditions (pH 8-11). It appears that depending on the cost of chitosan, the treatment condition with 40 mg chitosan, pH 9 and 1 h settling time could be favorable due to the relatively low pH and short settling time. Nevertheless, the selection of the optimal treatment conditions would be based on an economic feasibility study. The selection criteria discussed earlier for PO flocculation would be applicable to NO flocculation.

4.4 ELECTRO-FLOCCULATION

4.4.1 Optimization of Electro-Flocculation for PO

The electro-flocculation efficiency of PO varied between 65.9 % and 99.7 % (Figures 30-32). The highest flocculation efficiency for PO, 99.7 %, was obtained at current of 0.8 A, OT of 15 min and ST of 12 h. The three-way interaction of the processing variables, current, OT and ST, was significant in electro-flocculation of PO (Table 3).

Flocculation efficiency improved with increasing current, OT and ST (Figures

33-35). However, current and OT had little effect on flocculation efficiency when they exceed certain values (0.5 A and 10 min for PO) (Figure 35). At higher current value, less OT was required to attain the desirable flocculation efficiency (Figure 35). These findings are in agreement with the previously reported results for electro-flocculation of algae (Bayar et al. 2011; Gao et al. 2010; Tumsri and Chavalparit 2011; Vandamme et al. 2011). Faraday's law is the generally accepted theoretical basis for explaining these observations. Increasing either current or OT resulted in increased generation of aluminum ions from the aluminum anode, and thus more aluminum hydroxide was available for algae flocculation.

Dunnett's multiple comparisons with a "best mean" were employed and 7 other treatment combinations were found to be not significantly different from the "best mean". The 7 treatment combinations that are shown as red dots on Figures 36-38 in the order of current × OT × ST were 0.5 A × 10 min × 12 h, 0.5 A × 15 min × 0.5 h, 0.5 A × 15 min × 1 h, 0.5 A × 15 min × 12 h, 0.8 A × 10 min × 12 h, 0.8 A × 15 min × 0.5 h, and 0.8 A × 15 min × 12 h.

In total, there were eight combinations of the processing parameters that can give the desirable electro-flocculation efficiency. The range of the desirable electro-flocculation efficiency was 97.8 - 99.7 %. The eight combinations were either at current value not less than 0.5 A, or at OT not shorter than 10 min.

Although the processing condition at 0.5 A, 15 min OT and 0.5 h ST appears to be

a good option with short ST, the selection of the optimum conditions should be based on an economic feasibility study.

4.4.2 Optimization of Electro-Flocculation for NO

The electro-flocculation efficiency for NO varied between 85.1 % and 99.4 % (Figures 39 – 41). The highest flocculation efficiency for NO, 99.4 %, was obtained at current of 0.5 A, OT of 4 min and ST of 12 h. The effects of all processing parameters and their interactions on flocculation efficiency were significant except current*ST, OT*ST and current*OT*ST (Table 3). Flocculation efficiency improved with increasing current, OT and ST (Figures 42-44). The highest increase in flocculation efficiency with increasing current was observed at the lowest OT, 2 min (Figure 42). At a given ST, highest improvements were observed at low OT, 2 min and low current, less than 0.3 A (Figure 43).

The results of Dunnett's multiple comparisons with a "best mean" are shown in Figures 45-47. The condition of the highest NO flocculation efficiency is shown as a blue triangle. Seventeen flocculation efficiencies that were not significantly different from the highest one were shown as the red dots. Following were the 17 combinations of current × OT × ST: 0.1 A × 4 min × 12 h, 0.3 A × 2 min × 1 or 12 h, 0.3 A × 3 min × 0.5, 1 or 12 h, 0.3 A × 4 min × 0.5, 1 or 12 h, 0.5 A × 2 min × 0.5, 1 or 12 h, 0.5 A × 3 min × 0.5, 1 or 12 h, 0.5 A × 4 min × 0.5 or 1 h. The flocculation efficiencies for this group ranged from 97.1 % to 99.4 %. The 18 combinations were all at current value of 0.3 A or greater. It

appears that the treatment at 0.3 A, 3 min OT and 0.5 h ST could be a favorable option because of the short ST. However, selection of the optimum treatment conditions needs to be based on a feasibility study.

CHAPTER V

CONCLUSION

In this study, two microalgae strains, *Picochlorum oklahomensis* (PO) and *Nannochloropsis oculata* **(**NO), were examined for their biomass properties and harvesting characteristics. Both strains had either round or oval and green cells, but the cell size of PO (1-2.5 μm) was significantly smaller than that of NO (5-9 μm). The maximum biomass concentration of PO (2.1 g/L) was nearly twice as much as that of NO (1.2 g/L), but PO reached the maximum biomass level a week later than NO. Both PO and NO cells had significant oil contents comparable to common oilseeds, but the oil content in NO biomass (36.4 %) was almost double the oil content in PO biomass (20.5 %). Volumetric oil productivity (amount of oil/volume of culture at stationary phase) was similar for PO and NO. In summary, this study demonstrated that PO and NO can be potential feedstock for biofuel production.

Three flocculation methods, pH adjustment, biopolymer addition and electro-flocculation, were examined for algal biomass recovery from the culture medium. To the best of our knowledge, this is the first study on biomass harvesting of PO strain. The smaller cell size and higher maximum biomass concentration makes PO harder to

harvest than NO. This was demonstrated by the results that the flocculation efficiency by pH adjustment for NO was always higher than that for PO at the same pH level and settling time. Over 95 % NO flocculation could be achieved at a lower pH and chitosan: algal biomass ratio than those for PO.

There were significant interactions among the processing parameters in all three flocculation techniques for both PO and NO. Comparing all the three techniques above, the simplest way to effectively harvest PO and NO biomass by flocculation is to adjust the pH of culture medium to 11 or 12. However, it is important to further evaluate the potential adverse effects of high pH on harvested biomass and waste water generated during the process which needs to be neutralized prior to reuse or discard. High flocculation efficiency (over 93.5 %) could be achieved at lower pH (pH 8-10) by adding chitosan to the culture medium. In such a case the harvested biomass will contain chitosan. The effects of the presence of chitosan in the medium on downstream processing need to be considered while evaluating process options.

In conclusion, this study demonstrated that pH adjustment, chitosan addition and electro-flocculation were all effective methods to flocculate PO and NO cells. However, selection of the most suitable technique and the optimum treatment conditions needs to be based not only on the application of algal biomass, but also on an economic feasibility study.

FUTURE WORK

It is apparent that microalgae growth systems are very complex involving numerous interactions among algae cells, components of the culture medium and environment, i.e. light, CO_2-air supply. Further research is needed to understand these complex interactions. A better understanding of the entire system would certainly lead to design of more efficient systems. Although this study demonstrated the technical viability of the three different flocculation techniques for algal biomass recovery, physical separation of the flocs from the supernatant was not examined. Future work on floc properties, such as compactness and strength, should be conducted. Suitability of the harvested algal biomass for specific applications needs to be evaluated. The effects of presence of biopolymers in the system and high pH on downstream processes involved in microalgae production need to be further investigated. Additionally, further research is needed to determine the economic feasibility and scalability of the flocculation techniques examined in this study.

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Table 1: Fatty acid composition of microalgae oil from PO and NO as compared to soybean oil.

Fatty Acid	Weight Percentage (%)		
	PO oil	NO oil	Soybean oil
C10:0	0.05 ±0.00	0.04 ±0.00	-
C12:0	0.03 ±0.00	0.04 ±0.01	-
C14:0	0.64 ±0.01	0.34 ±0.03	0.04
C15:0	0.33 ±0.01	0.02 ±0.00	-
C16:0	23.81 ±0.08	19.46 ±0.59	10.57
C16:1	8.20 ±0.06	2.02 ±0.14	0.02
C17:0	0.15 ±0.00	0.07 ±0.01	-
C16:2n6*	6.93 ±0.04	2.15 ±0.12	-
C17:1	-	0.07 ±0.00	-
C18:0	1.49 ±0.02	0.81 ±0.13	4.09
C16:3n6*	-	1.67 ±0.12	-
C18:1n11t	-	0.05 ±0.00	-
C18:1n9t	0.06 ±0.02	-	-
C18:1n9c	8.02 ±0.04	8.76 ±0.31	22.98
C18:1n11c	5.77 ±0.03	3.98 ±0.18	-
C19:0	-	0.19 ±0.03	-
Not identified*	-	12.50 ±0.33	-
C18:2n9*	-	1.06 ±0.10	-
C18:2n6c	26.19 ±0.19	9.03 ±0.22	54.51
C20:0	0.10 ±0.00	0.23 ±0.04	0.33
C18:3n6	0.09 ±0.01	5.26 ±0.27	-
C20:1	0.15 ±0.01	0.10 ±0.01	0.18
C18:3n3	13.52 ±0.07	25.78 ±1.52	7.23
C20:2	0.05 ±0.01	1.29 ±0.10	-
C22:0	0.06 ±0.01	0.13 ±0.04	0.25
C22:1n9	-	0.15 ±0.11	-
C20:3n3	-	0.09 ±0.01	-
C22:2	-	0.01 ±0.01	-
C24:0	0.01 ±0.01	0.12 ±0.01	0.10
C22:6n3	-	0.18 ±0.16	-
C26:0*	0.55 ±0.02	-	-

Table 1: Fatty acid composition of microalgae oil from PO and NO (continued from previous page).

Fatty Acid	Weight Percentage (%)		
	PO oil	NO oil	Soybean oil
Total n-3	13.52 ± 0.07	26.05 ± 1.70	7.23
Total n-6	33.21 ± 0.23	18.11 ± 0.74	54.51
Total n-3 and n-6	46.73 ± 0.30	44.16 ± 2.43	61.74
Essential FA	39.71 ± 0.25	34.81 ± 1.75	61.74
Saturated FA	27.22 ± 0.17	21.45 ± 0.89	15.38
Monounsaturated FA	22.20 ± 0.17	15.13 ± 0.75	23.18
PUFA	46.73 ± 0.30	45.22 ± 2.54	61.74

The data of soybean oil came from Hammond et al. (2005).

The "-" means that this component was not detected in this study and the "*" means the possible components from library search.

FA and PUFA represent fatty acids and polyunsaturated fatty acids, respectively.

C10:0 = Capric Acid; C12:0 = Lauric Acid; C14:0 = Myristic Acid; C15:0 = Pentadecanoic Acid; C16:0 = Palmitic Aicd; C16:1 = Palmitoleic Acid; C17:0 = Heptadecanoic Acid; C16:2n6 = 7,10-Hexadecadienoic Acid; C17:1 = cis-10-Heptadecenoic Acid; C18:0 = Stearic Acid; C16:3n6 = 4,7,10-Hexadecatrienoic Acid; C18:1n11t = trans-11-Octadecenoic Acid; C18:1n9t = Elaidic Acid; C18:1n9c = Oleic Acid; C18:1n11c = cis-11-Octadecenoic Acid; C19:0 = Nonadecanoic Acid; C18:2n9 = 6,9-Octadecadienoic Acid; C18:2n6c = Linoleic Acid; C20:0 = Arachidic Acid; C18:3n6 = γ-Linolenic Acid; C20:1 = cis-11-Eicosenoic Acid; C18:3n3 = Linolenic Acid; C20:2 = cis-11,14-Eicosadienoic Acid; C22:0 = Behenic Acid; C22:1n9 = Erucic Acid; C20:3n3 = cis-11,14,17-Eicosatrienoic Acid; C22:2 = cis-13,16-Docosadienoic Acid; C24:0 = Lignoceric Acid; C22:6n3 = cis-4,7,10,13,16,19-Docosahexaenoic Acid; C26:0 = Pentacosanoic Acid.

Table 2: Statistical analysis of the fixed effects in pH flocculation for PO and NO.

PO					NO				
Effect	Numerator DF	Denominator DF	F Value	Pr > F	Effect	Numerator DF	Denominator DF	F Value	Pr > F
pH	11	12	177.88	<0.0001	pH	8	11	274.40	<0.0001
ST	1	12	73.43	<0.0001	ST	1	11	110.25	<0.0001
pH*ST	11	12	4.32	0.0091	pH*ST	8	11	90.42	<0.0001

DF and ST represent degree of freedom and settling time, respectively.

$P < 0.05$ indicates statistical significance.

The denominator degrees of freedom were determined in the mixed model analysis of these repeatedly measured experiments.

Table 3: Statistical analysis of the fixed effects in chitosan flocculation for PO and NO.

PO					NO				
Effect	Numerator DF	Denominator DF	F Value	Pr > F	Effect	Numerator DF	Denominator DF	F Value	Pr > F
chitosan	5	41	89.83	<0.0001	chitosan	6	42	150.31	<0.0001
pH	9	41	128.26	<0.0001	pH	5	42	631.11	<0.0001
ST	1	41	1886.26	<0.0001	ST	1	42	24.62	<0.0001
chitosan*pH	18	41	58.09	<0.0001	chitosan*ST	12	42	46.81	<0.0001
chitosan*ST	5	41	41.58	<0.0001	chitosan*ST	6	42	10.90	<0.0001
pH*ST	19	41	59.12	<0.0001	pH*ST	5	42	154.91	<0.0001
chitosan*pH *ST	18	41	32.43	<0.0001	chitosan*pH * ST	12	42	21.03	<0.0001

DF, chitosan and ST represent degree of freedom, chitosan amount and settling time, respectively.

$P < 0.05$ indicates statistical significance.

The denominator degrees of freedom were determined in the mixed model analysis of these repeatedly measured experiments.

Table 4: Statistical analysis of the fixed effects in electro-flocculation for PO and NO.

PO					NO				
Effect	Numerator DF	Denominator DF	F Value	Pr > F	Effect	Numerator DF	Denominator DF	F Value	Pr > F
Current	2	10	172.91	<0.0001	Current	2	9	65.55	<0.0001
OT	3	10	96.44	<0.0001	OT	2	9	13.84	0.0018
Current *OT	4	10	15.38	0.0003	Current *OT	4	9	11.26	0.0015
ST	2	10	50.94	<0.0001	ST	2	9	56.79	<0.0001
Current *ST	4	10	4.13	0.0313	Current *ST	4	9	0.80	0.5561
OT*ST	6	10	2.50	0.0961	OT*ST	4	9	1.88	0.1983
Current *OT*ST	8	10	4.18	0.0191	Current *OT*ST	8	9	1.23	0.3786

DF, OT and ST represent degree of freedom, operation time and settling time, respectively.

$P < 0.05$ indicates statistical significance.

The denominator degrees of freedom were determined in the mixed model analysis of these repeatedly measured experiments.

Figure 1: Experimental design points selected for chitosan flocculation of PO.

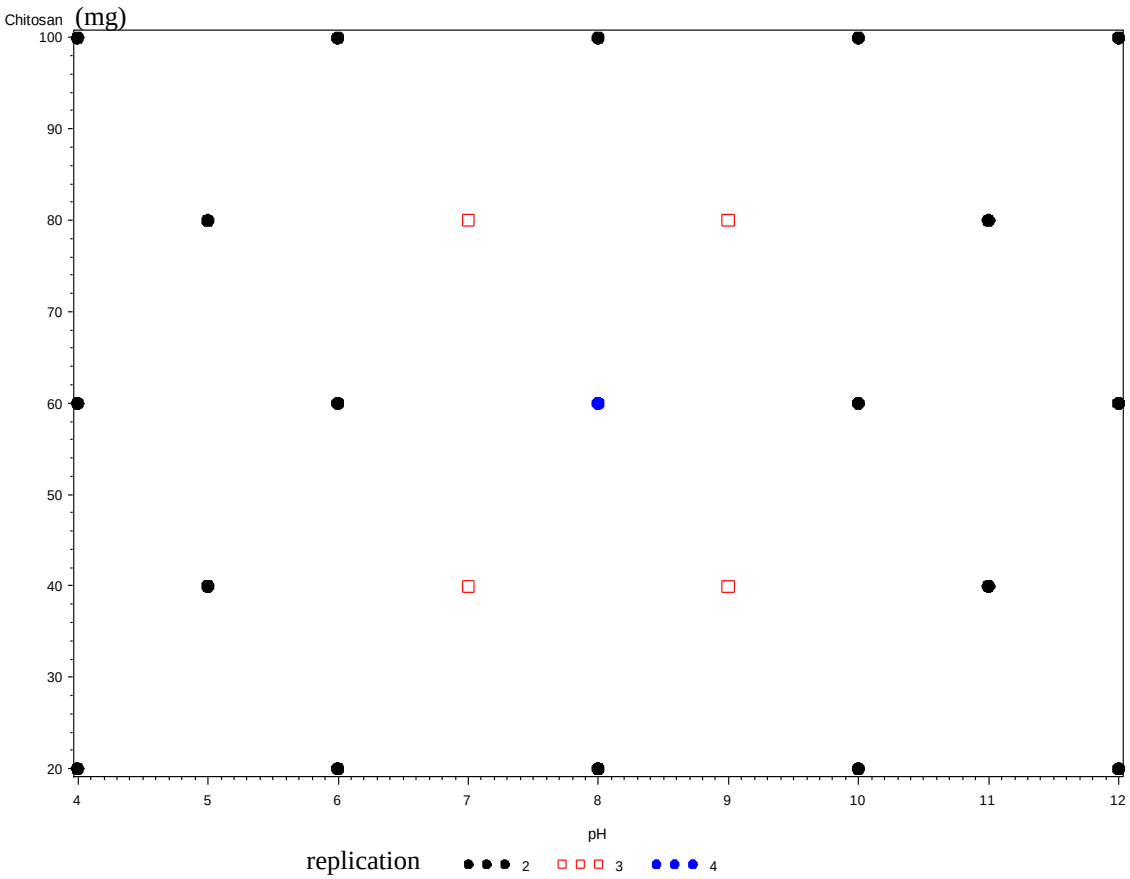


Figure 2: Experimental design points selected for chitosan flocculation of NO.

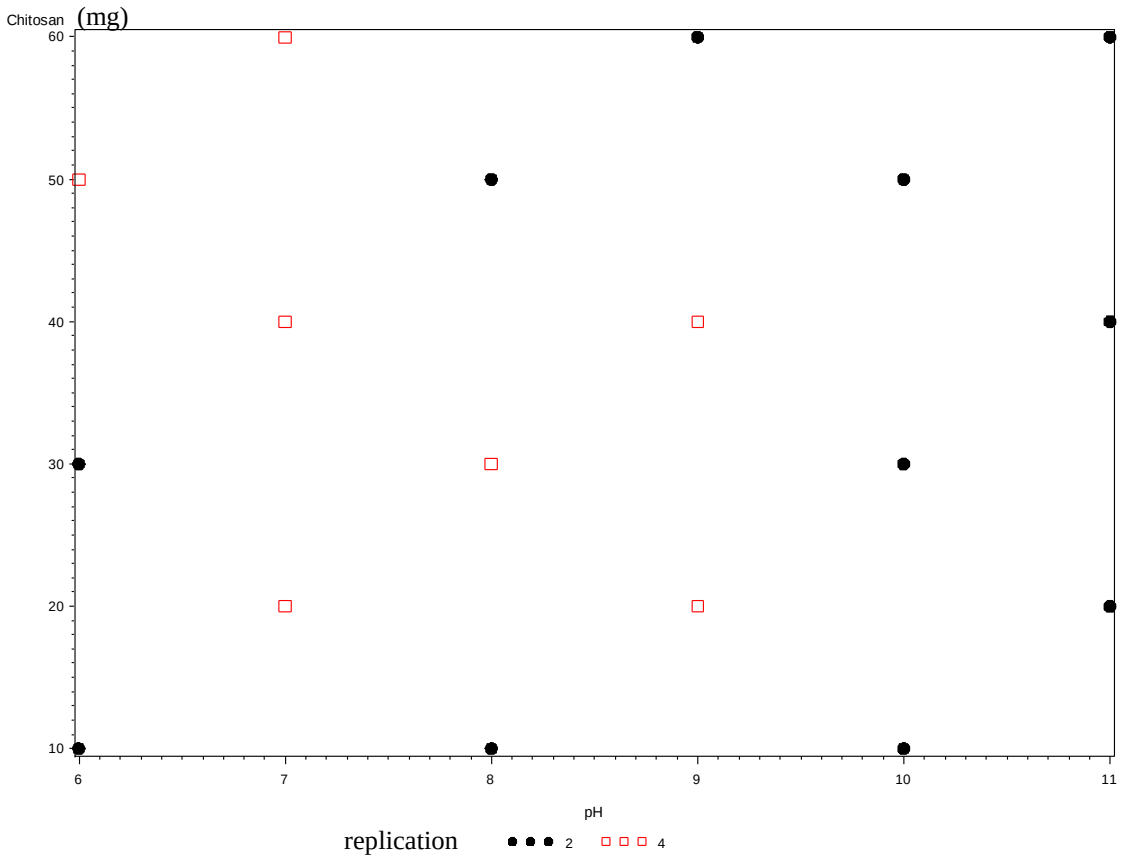


Figure 3: Schematic of an electro-flocculation device for harvesting microalgae.

(1 – treatment tank, 2 – magnetic stirrer, 3 – electrodes, 4 – wire, 5 – power transformer)

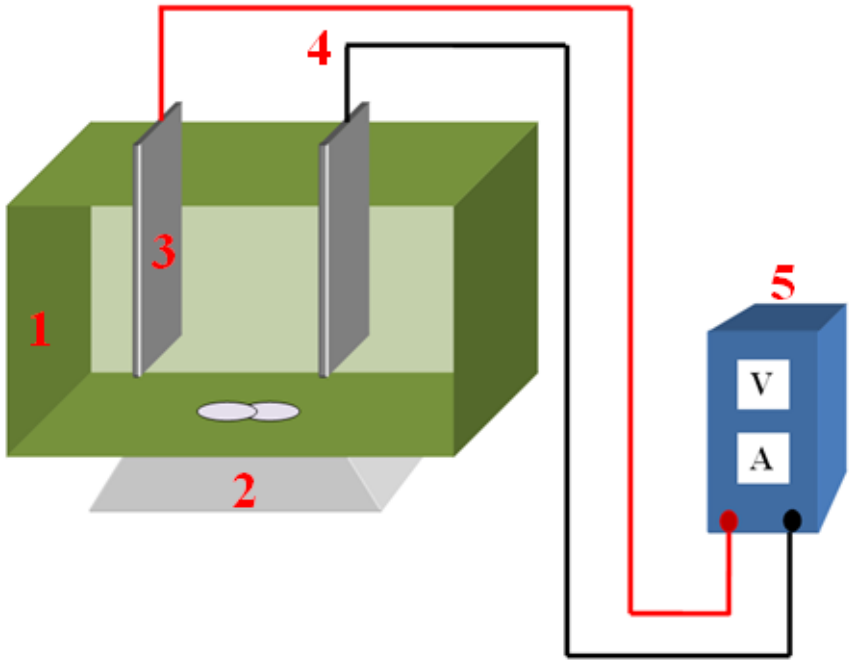


Figure 4: Absorption spectra for PO.

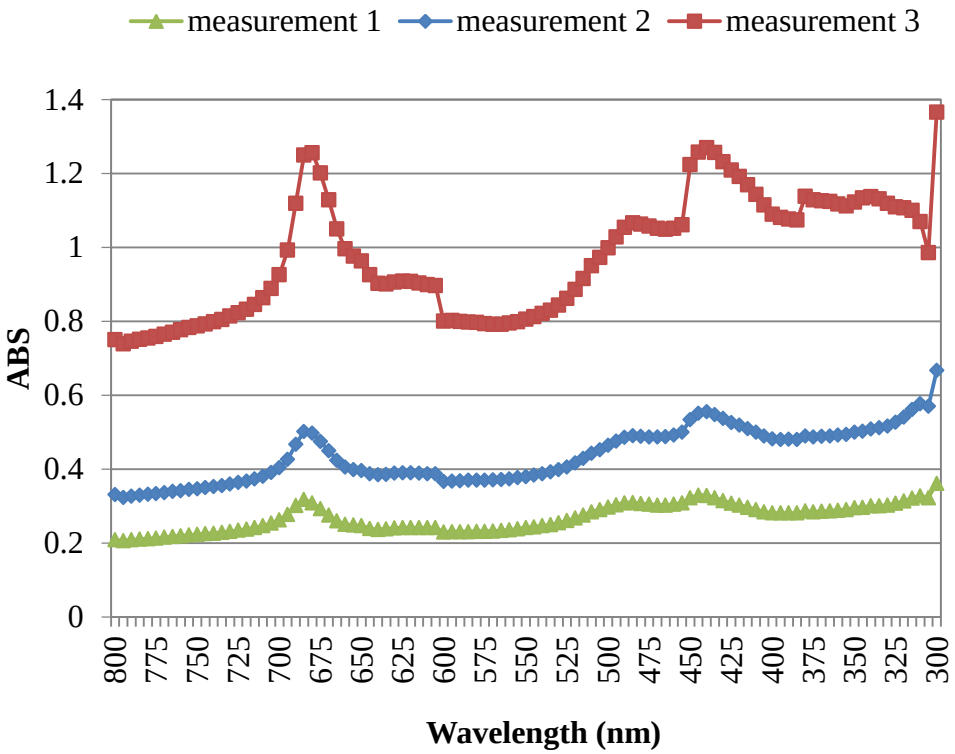


Figure 5: Linear association of dry biomass concentration vs.ABS for PO.

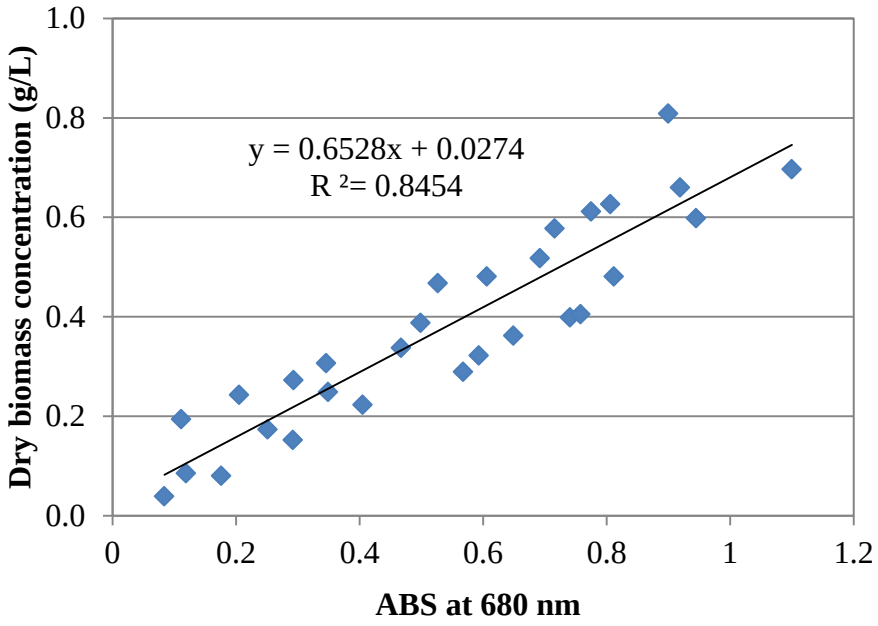


Figure 6: Linear association of cell density vs.ABS for PO.

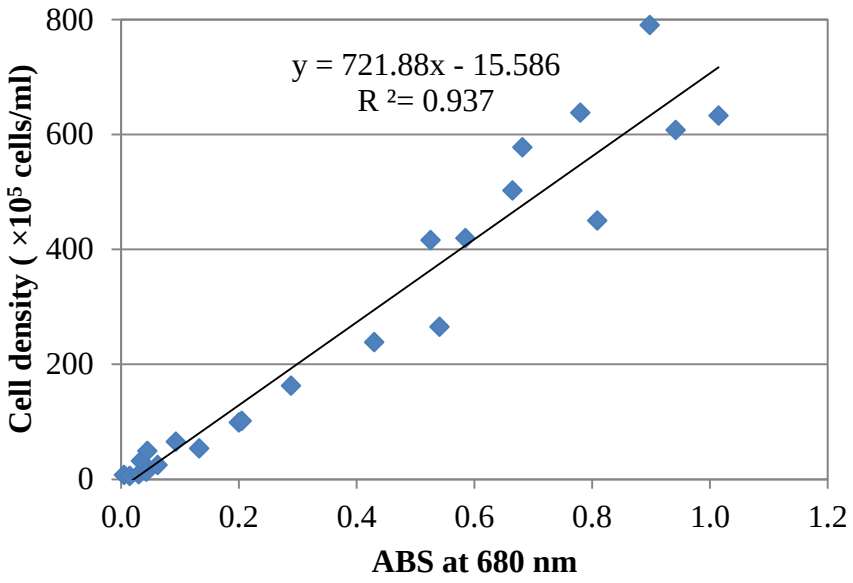
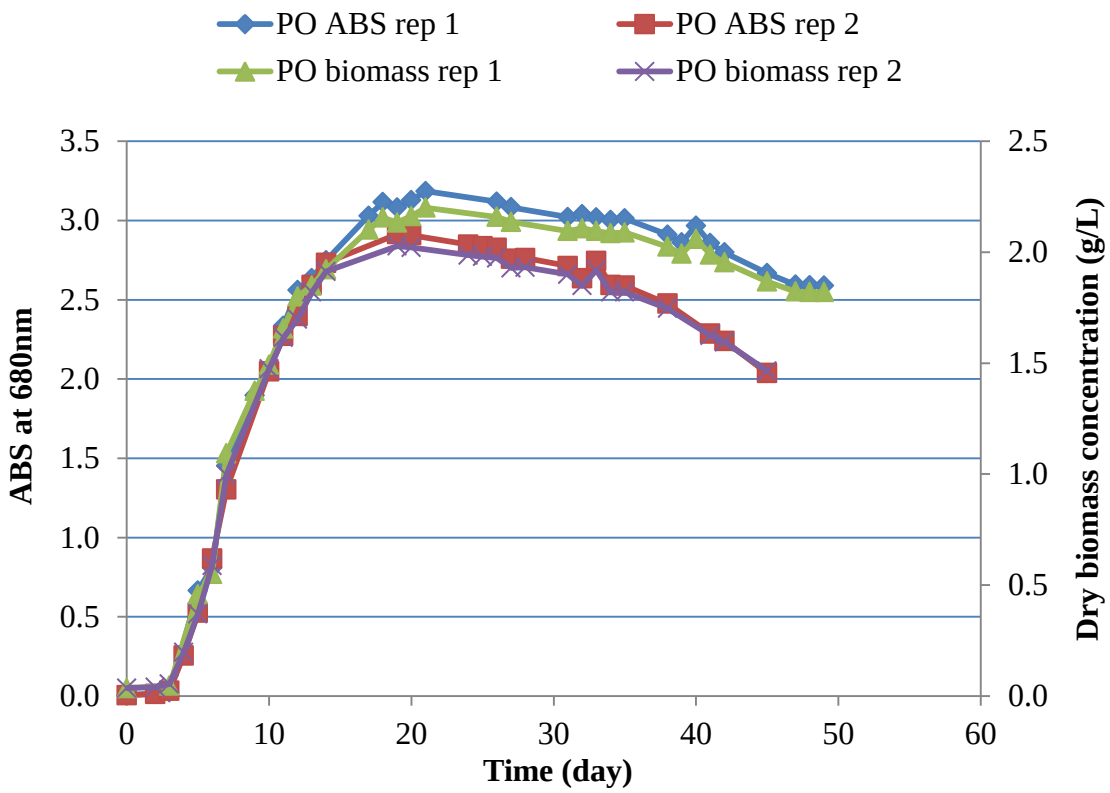


Figure 7: Growth curves for PO.



The data of dry biomass concentration was not actually measured. It was calculated using the linear association between dry biomass concentration and ABS.

Figure 8: pH of the culture suspension during the growth of PO.

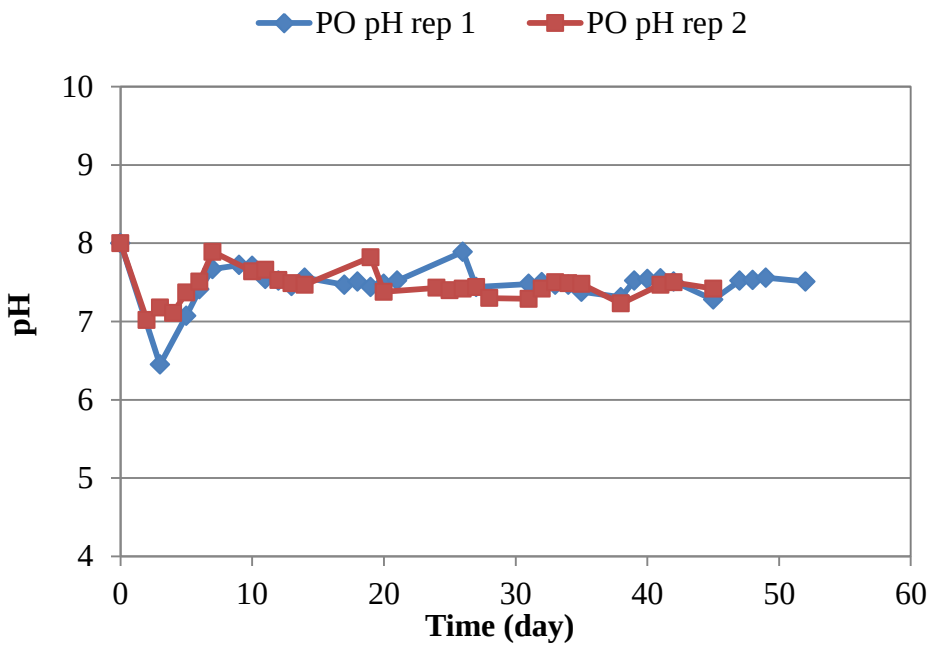


Figure 9: Absorption spectra for NO.

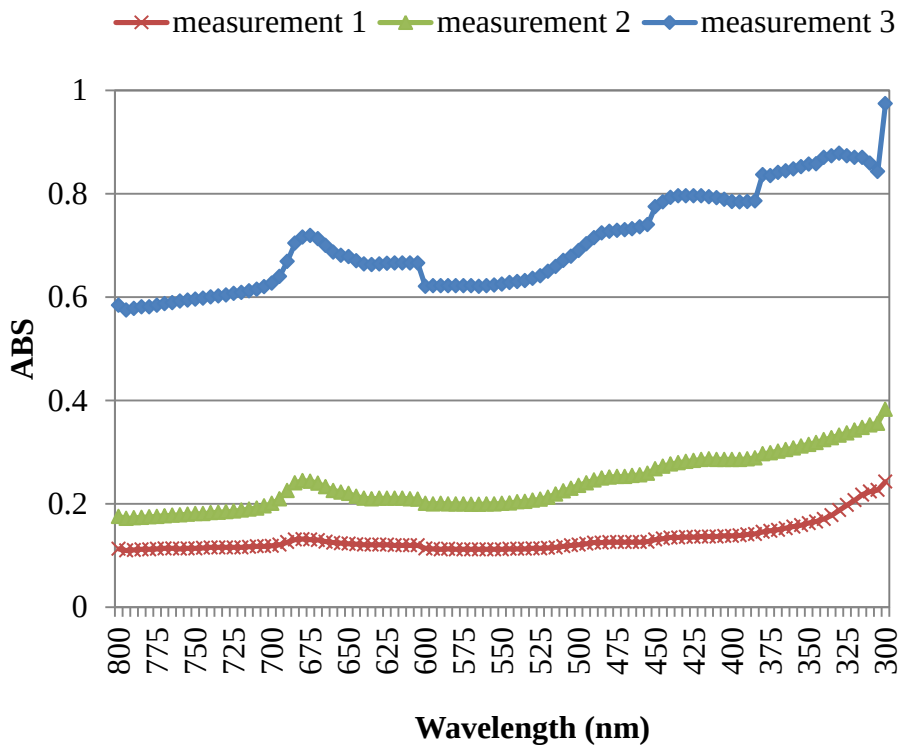


Figure 10: Linear association of dry biomass concentration vs.ABS for NO.

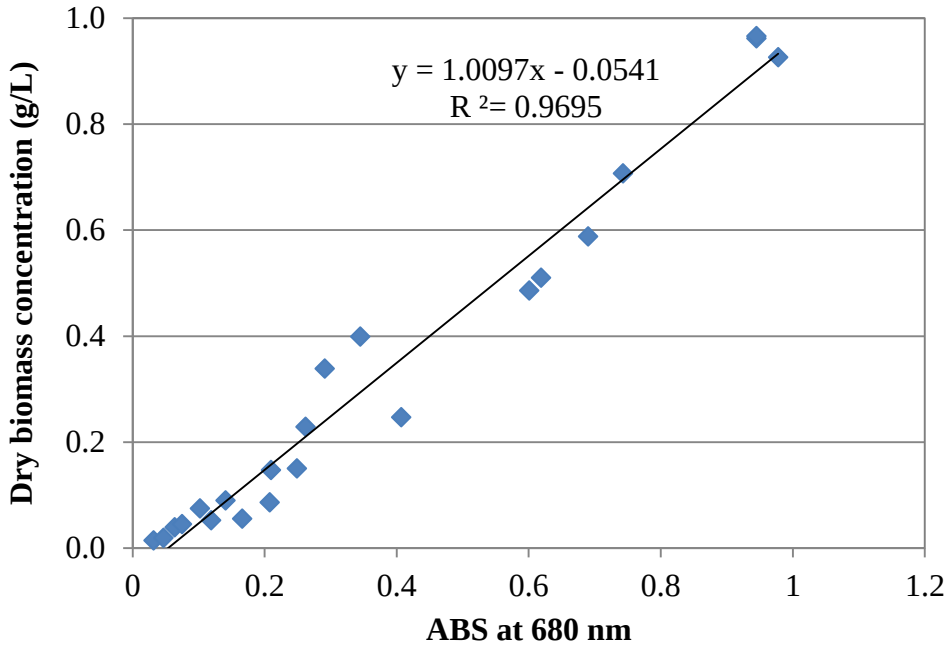


Figure 11: Linear association of cell density vs.ABS for NO.

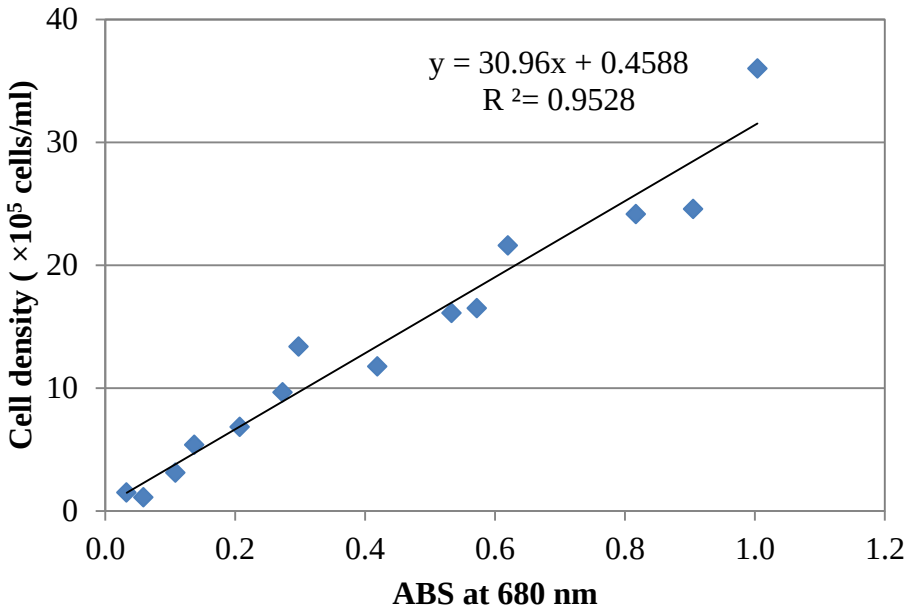
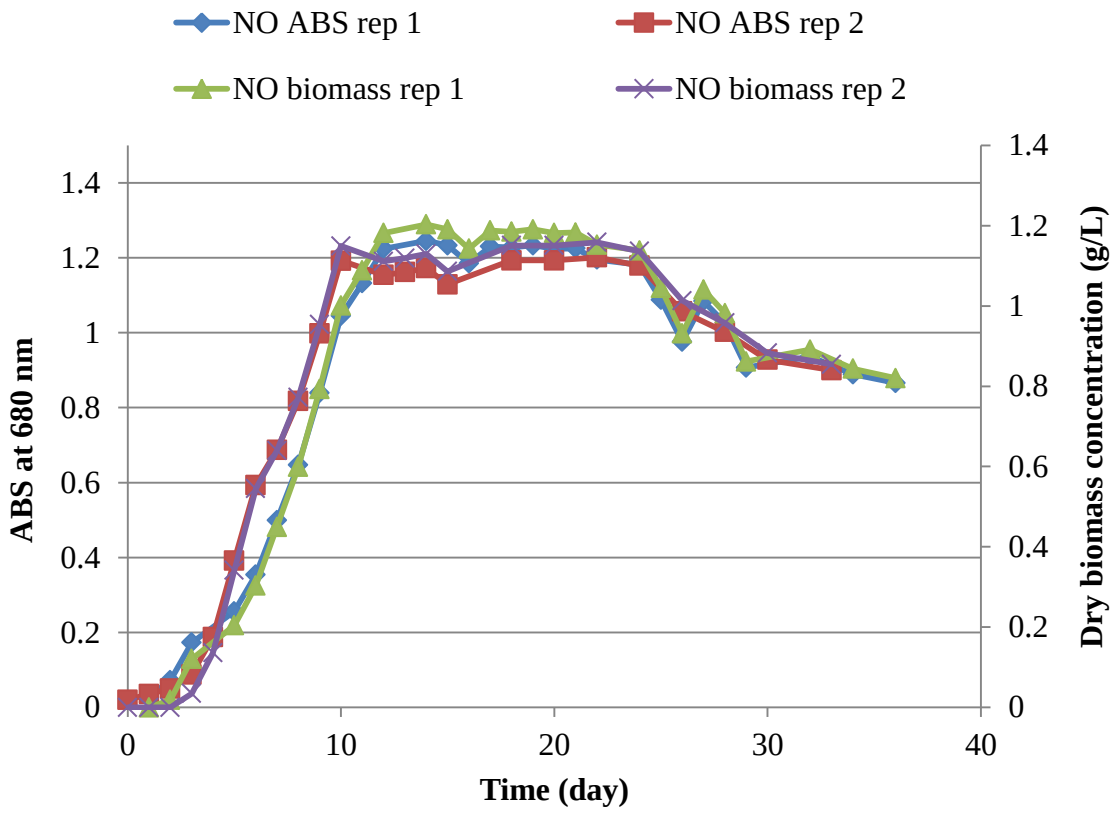


Figure 12: Growth curves for NO.



The data of dry biomass concentration was not actually measured. It was calculated using the linear association between dry biomass concentration and ABS.

Figure 13: pH of the culture suspension during the growth of NO.

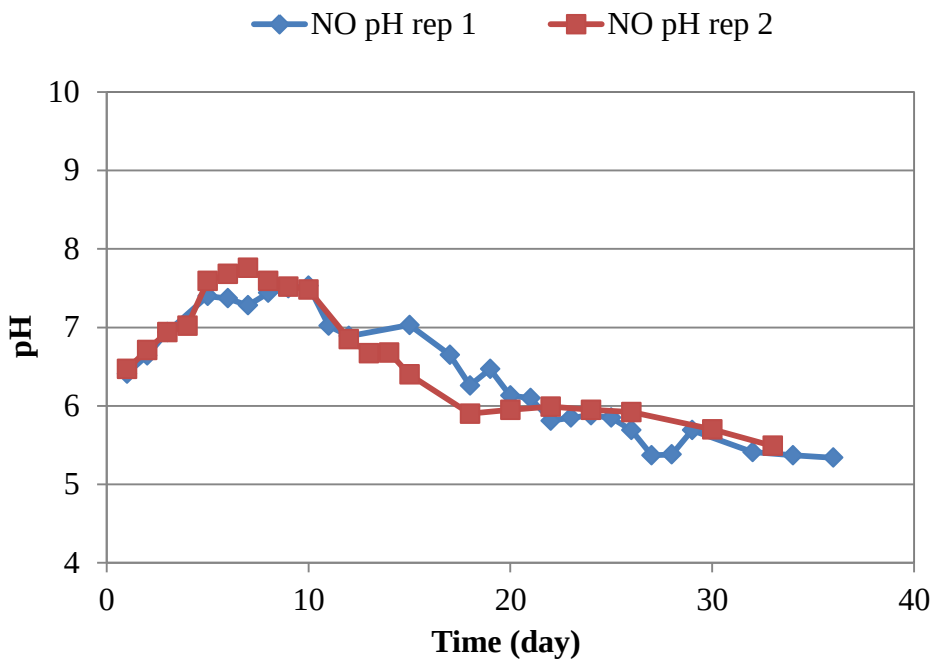


Figure 14: Flocculation efficiency for PO obtained from flocculation by pH adjustment.

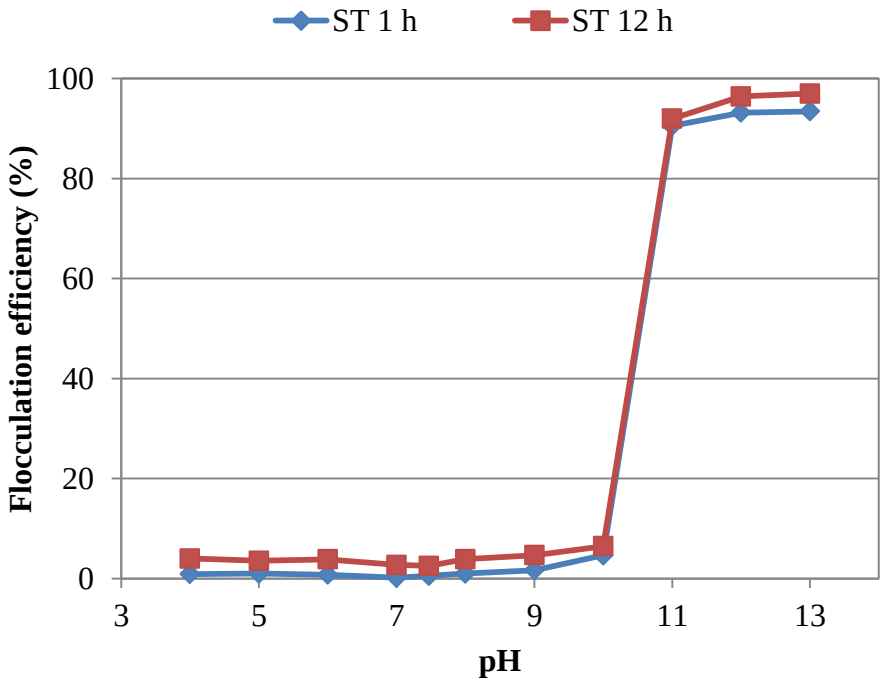


Figure 15: Flocculation efficiency for NO obtained from flocculation by pH adjustment.

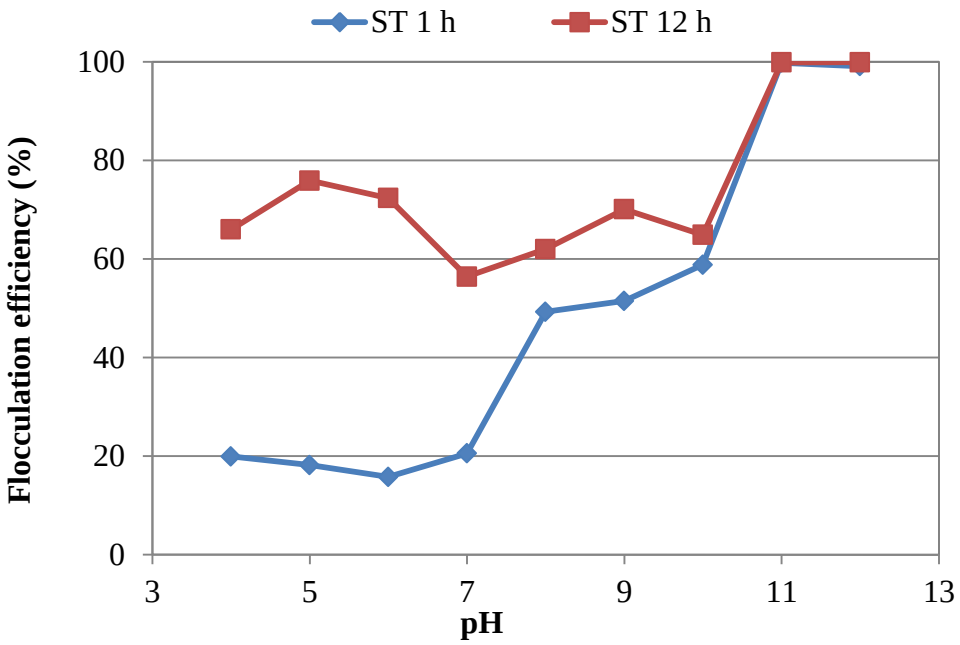


Figure 16: Flocculation of PO by different biopolymers addition at medium pH.

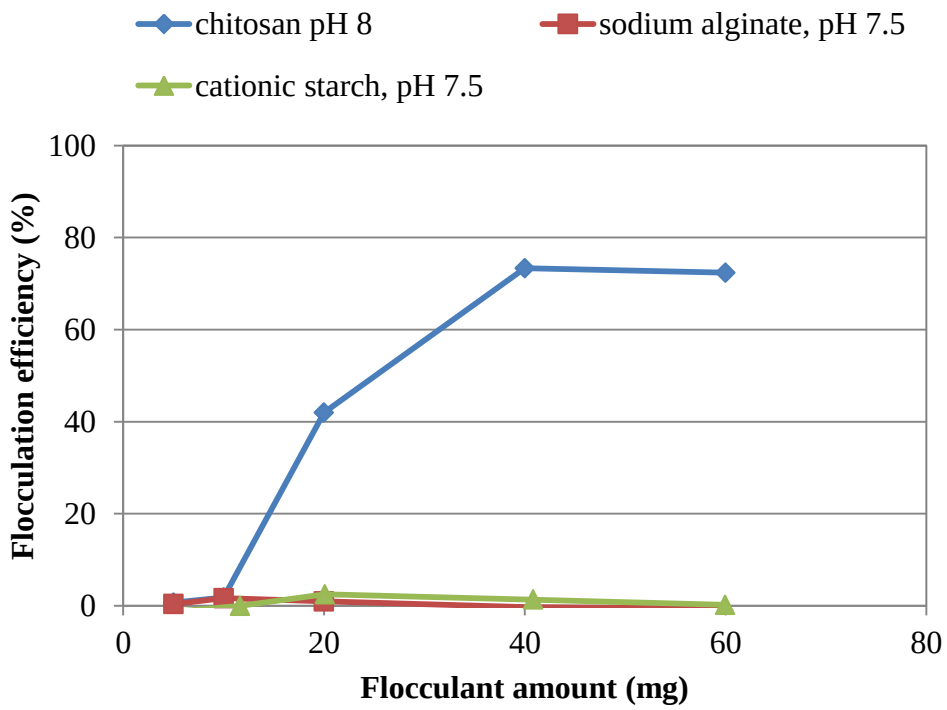


Figure 17: Flocculation of PO by biopolymers addition at different pH.

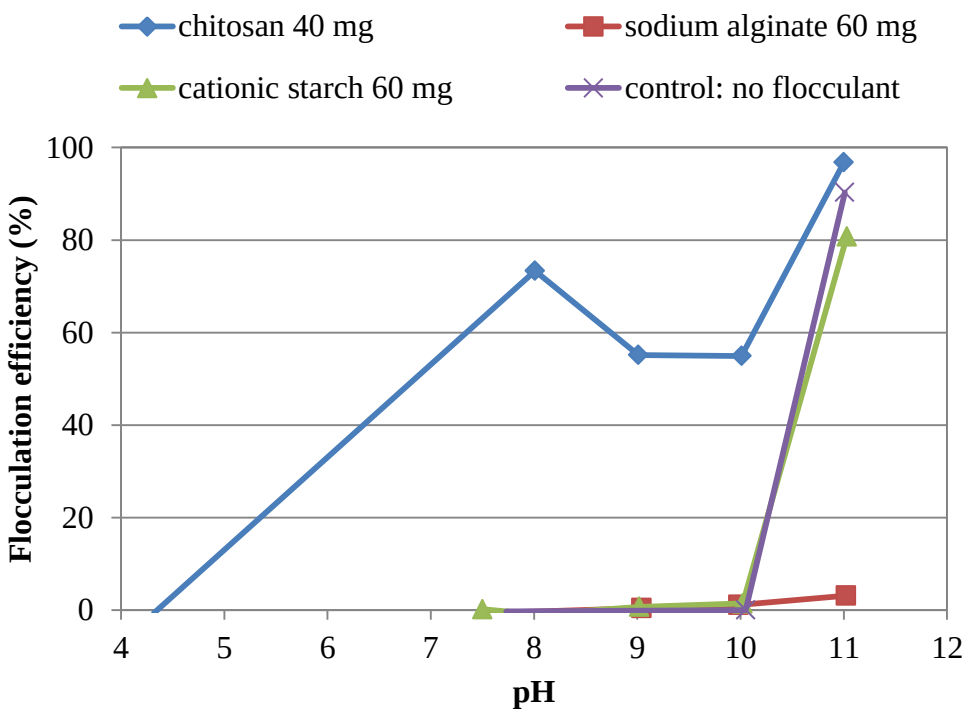


Figure 18: Flocculation efficiency for PO obtained from chitosan flocculation at settling time of 1 h.

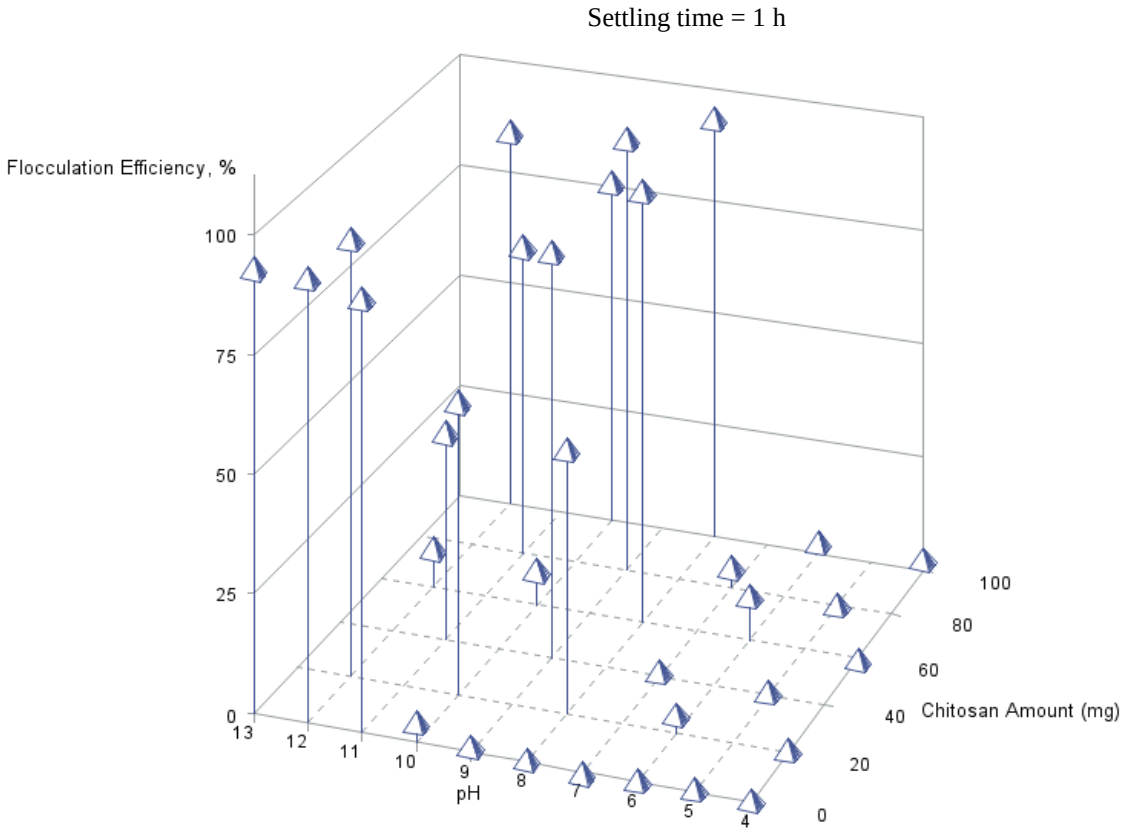


Figure 19: Flocculation efficiency for PO obtained from chitosan flocculation at settling time of 12 h.

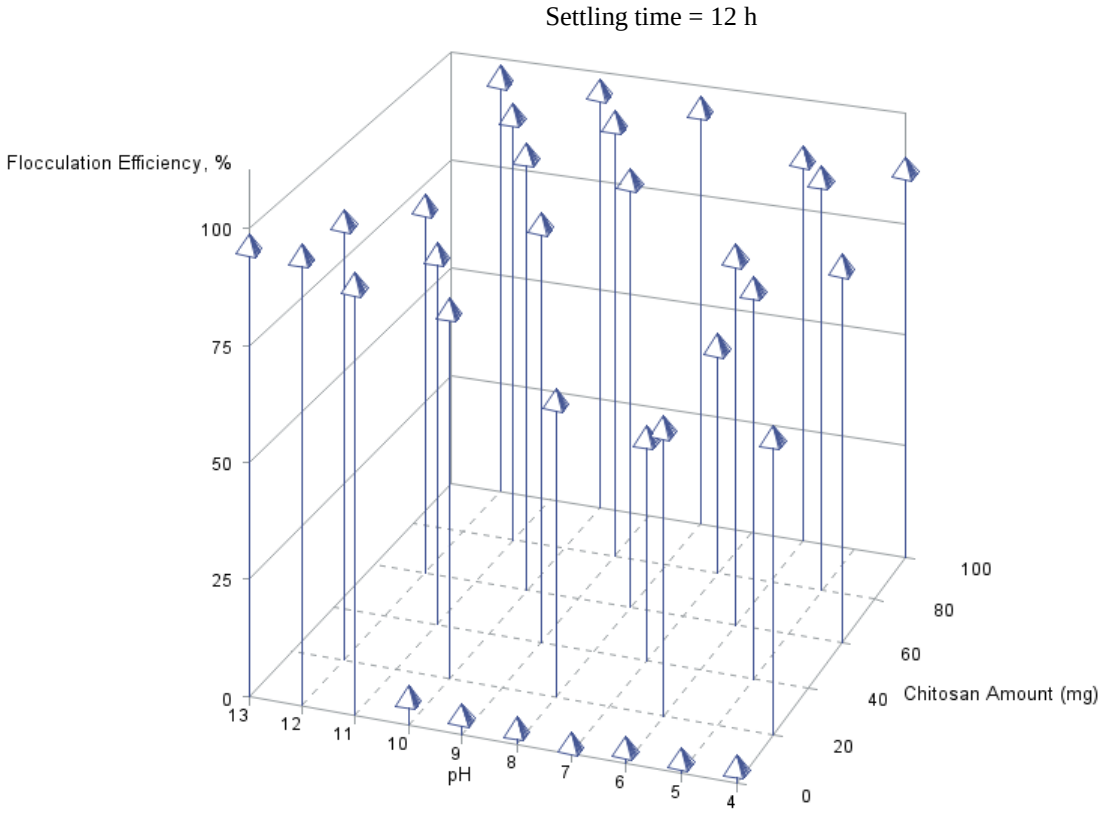


Figure 20: Flocculation efficiency of PO as a function of pH at different chitosan amount.

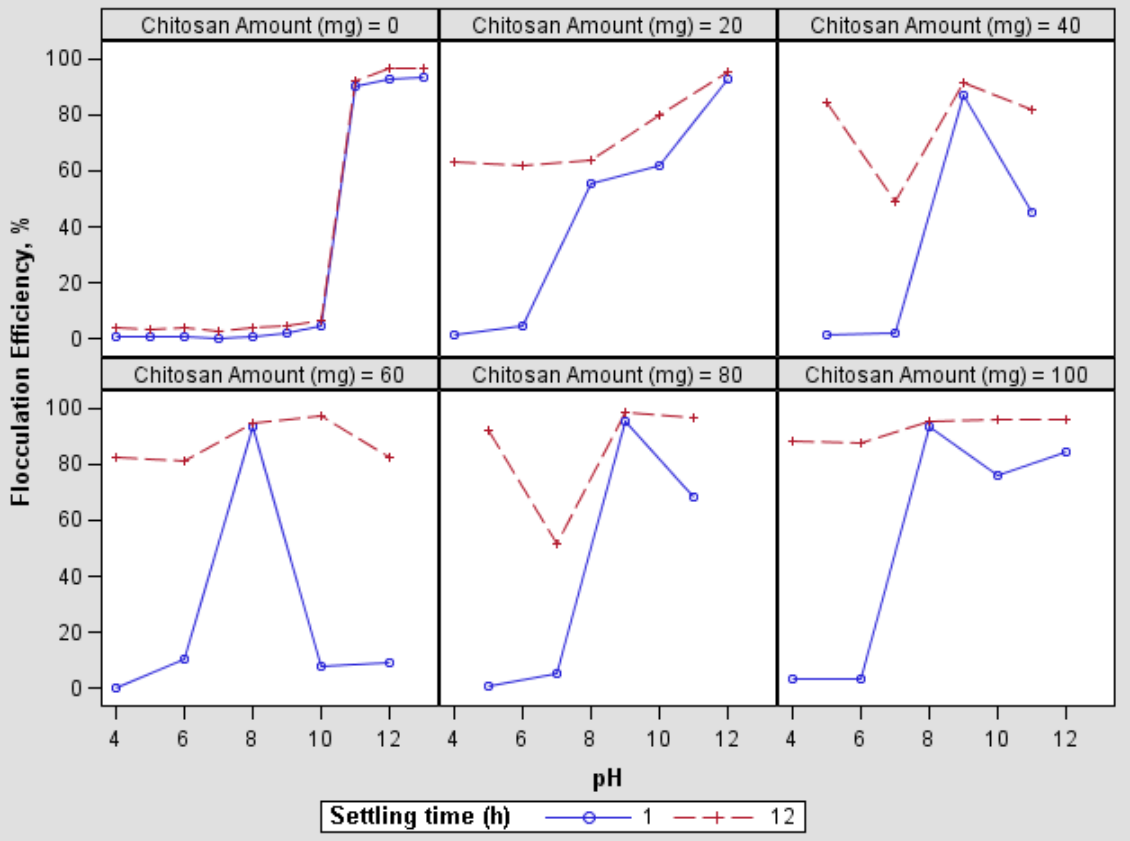


Figure 21: Flocculation efficiency of PO as a function of chitosan amount at different pH.

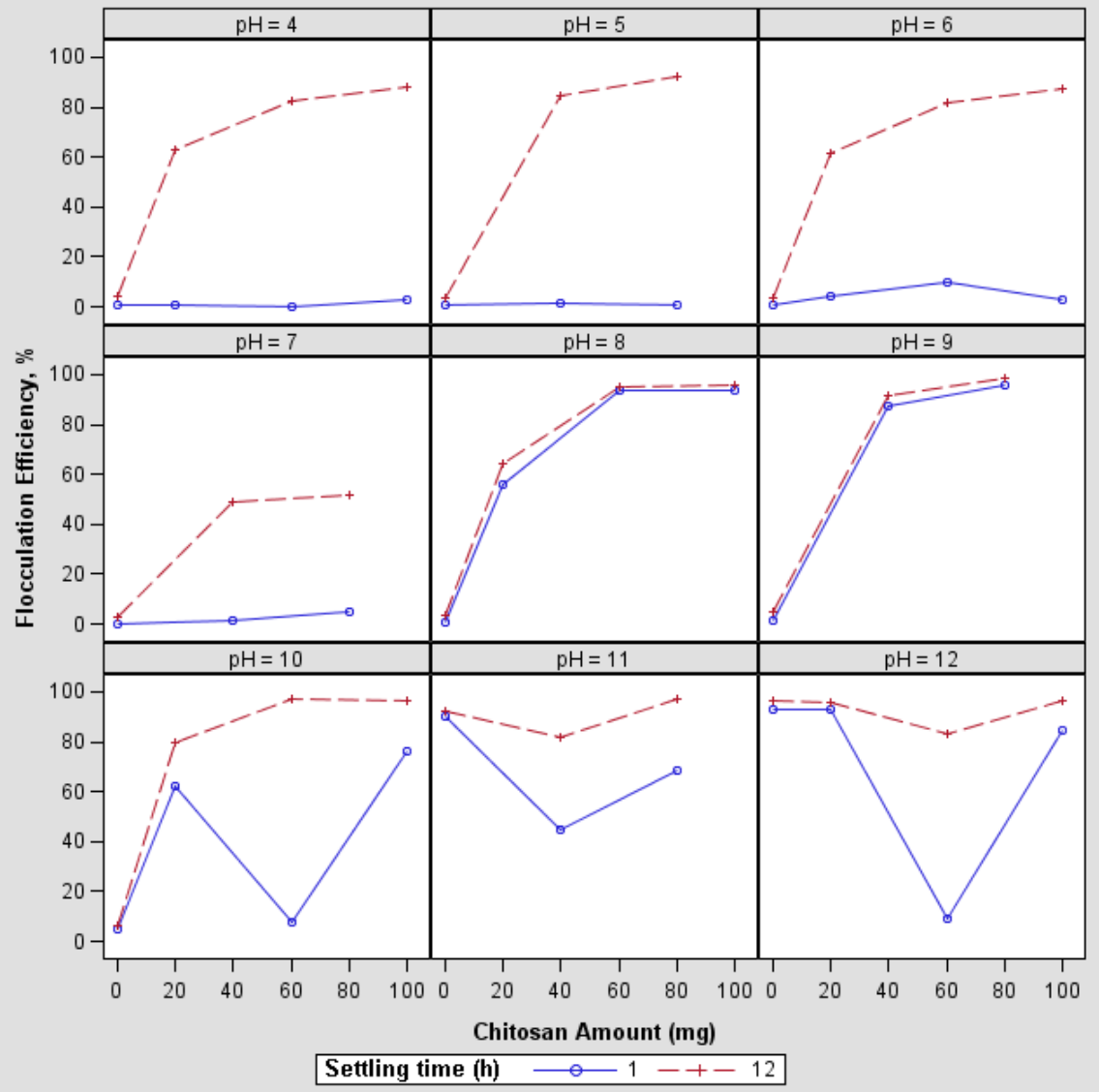
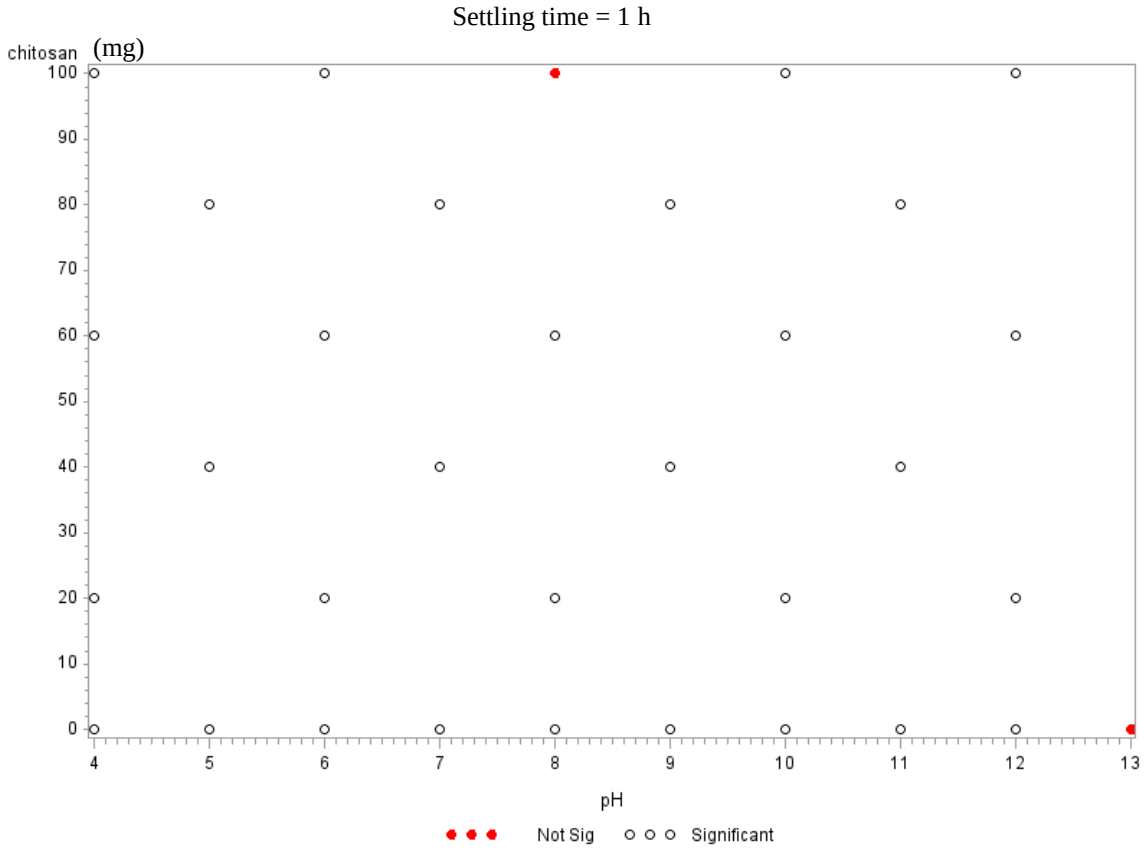
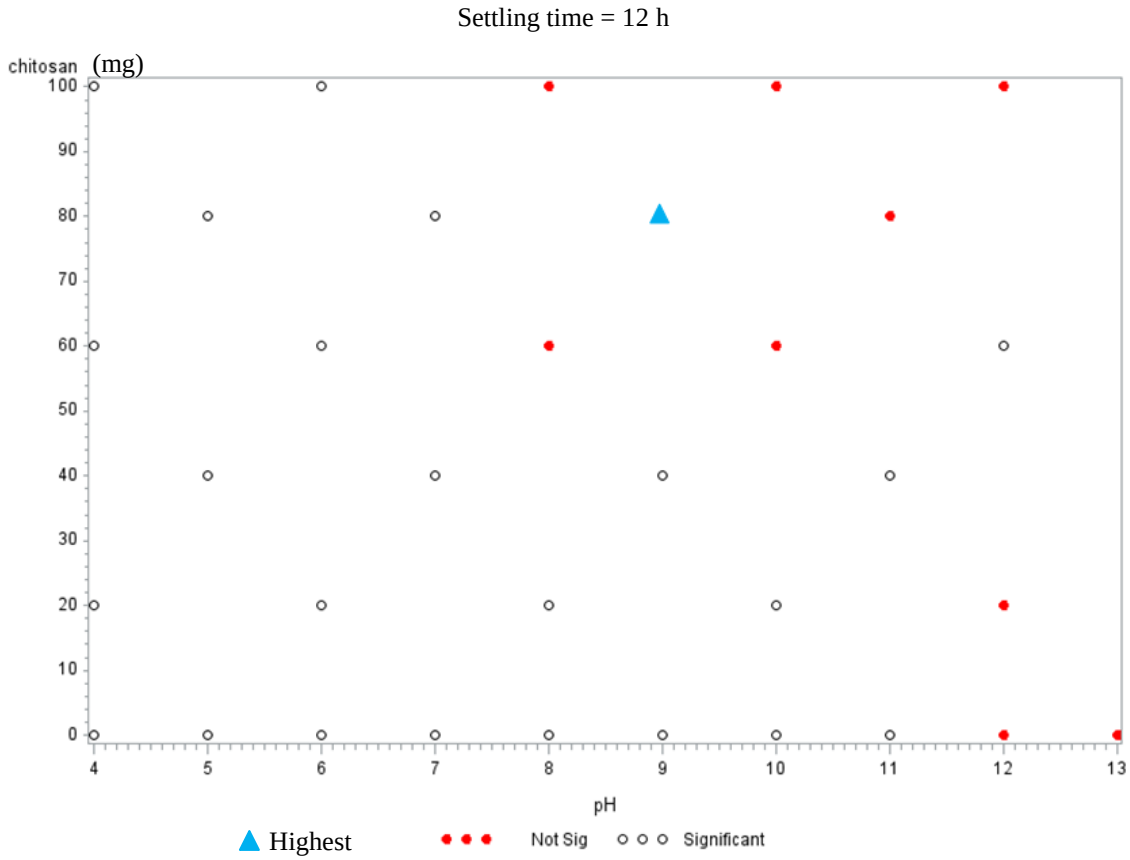


Figure 22: Combinations of variables at settling time of 1 h for PO chitosan flocculation after Dunnett's multiple comparisons with a "best" mean.



The "best" mean refers to the highest flocculation efficiency and the "Not Sig" represents "not significant".

Figure 23: Combinations of variables at settling time of 12 h for PO chitosan flocculation after Dunnett's multiple comparisons with a "best" mean.



The "best" mean refers to the highest flocculation efficiency and the "Not Sig" represents "not significant".

Figure 24: Flocculation efficiency for NO obtained from chitosan flocculation at settling time of 1 h.

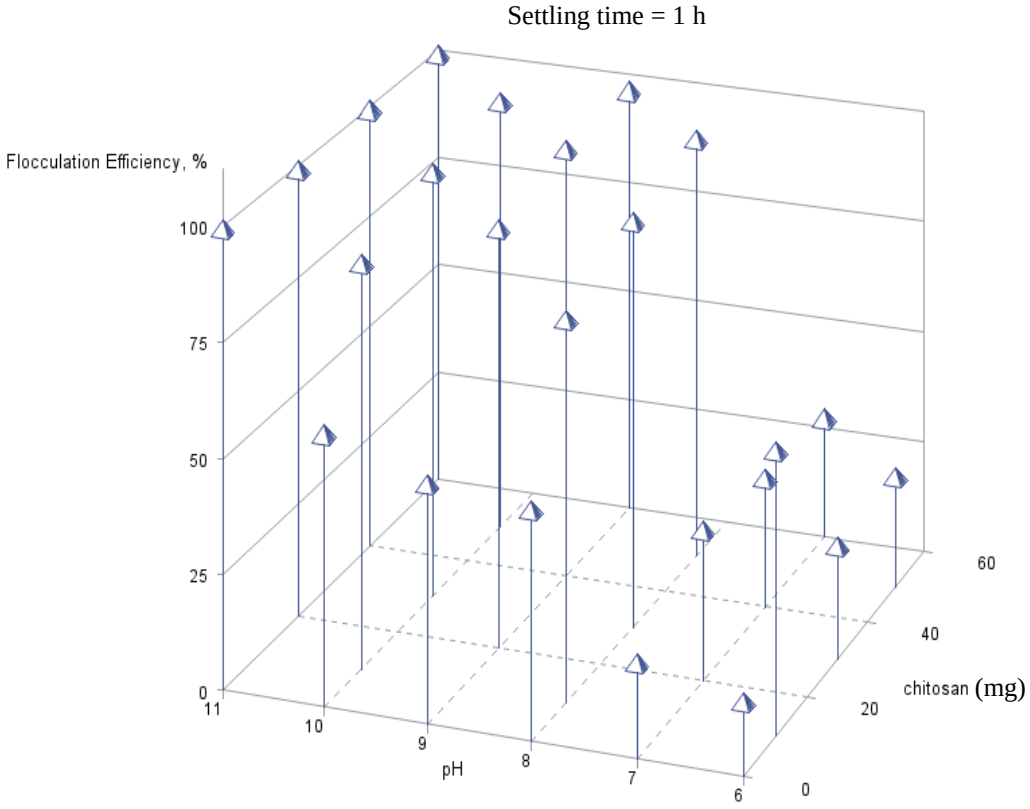


Figure 25: Flocculation efficiency for NO obtained from chitosan flocculation at settling time of 12 h.

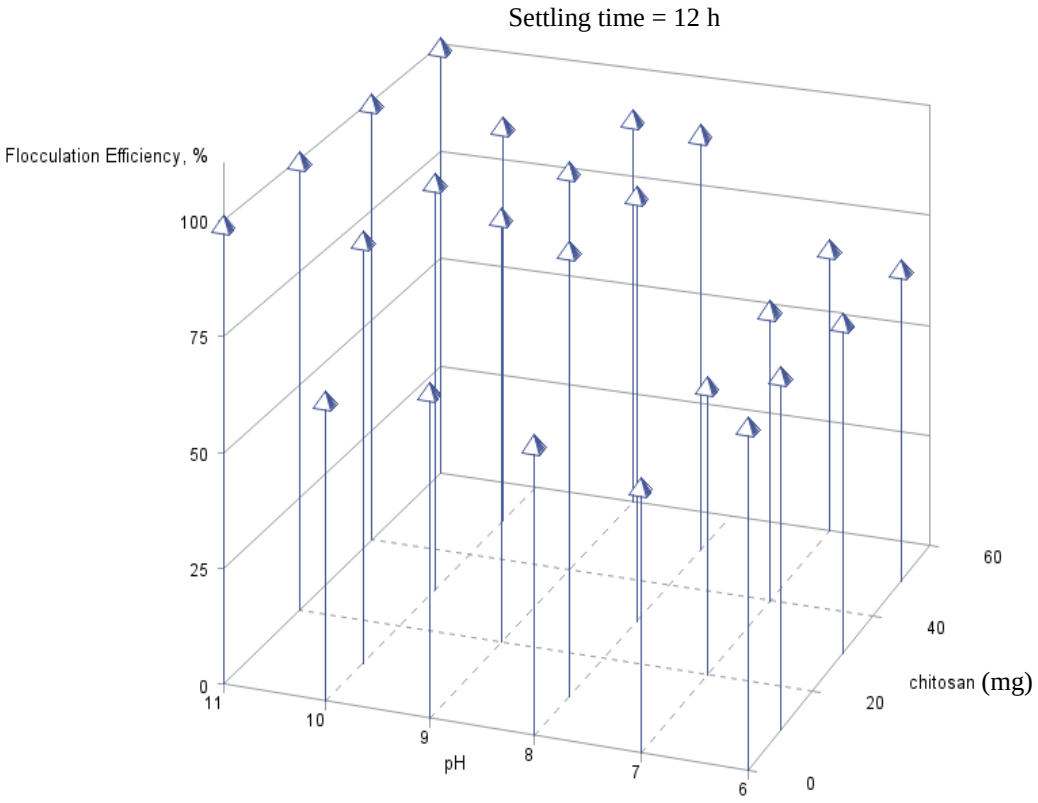


Figure 26: Flocculation efficiency of NO as a function of pH at different chitosan amount.

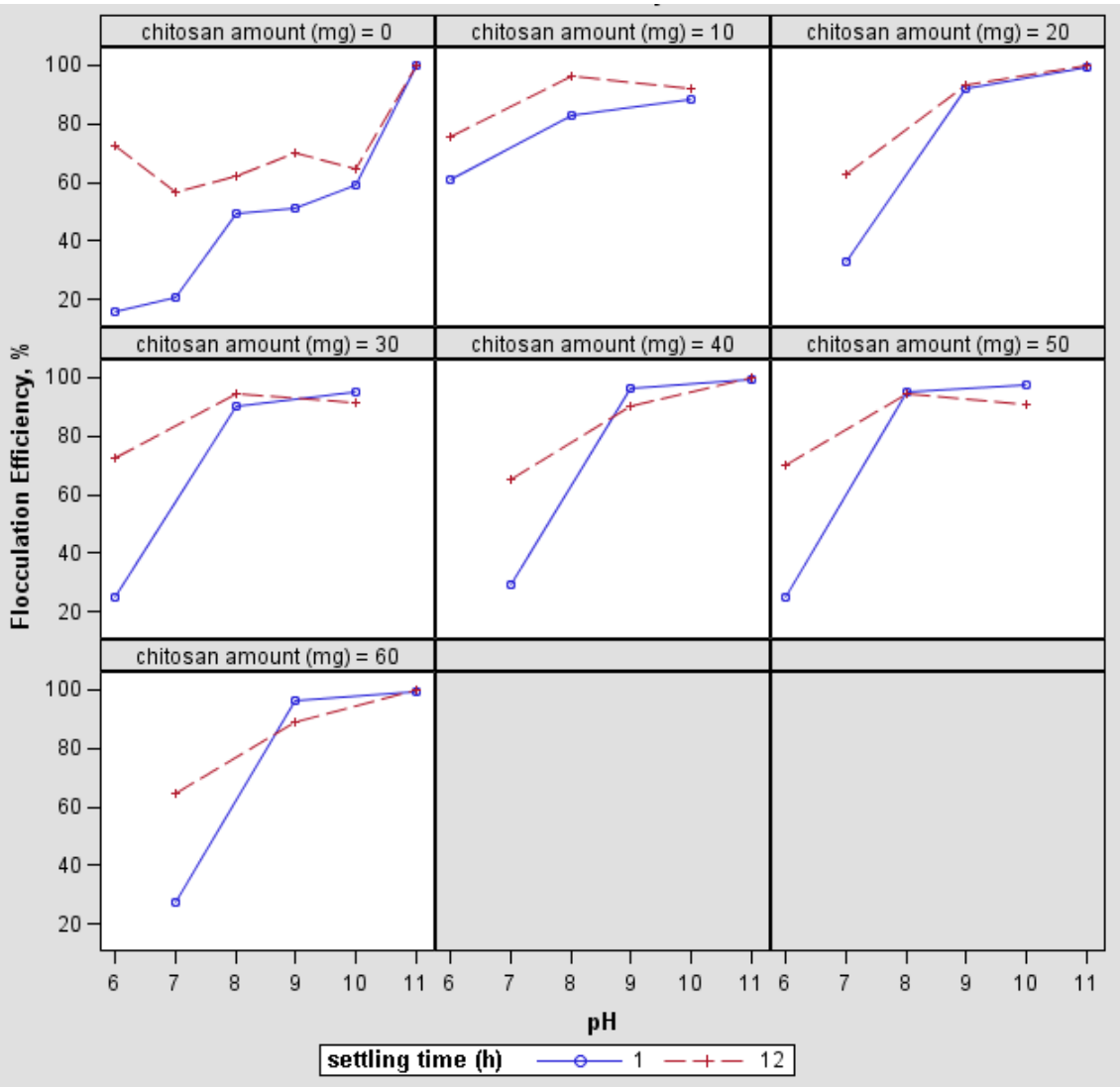


Figure 27: Flocculation efficiency of NO as a function of chitosan amount at different pH.

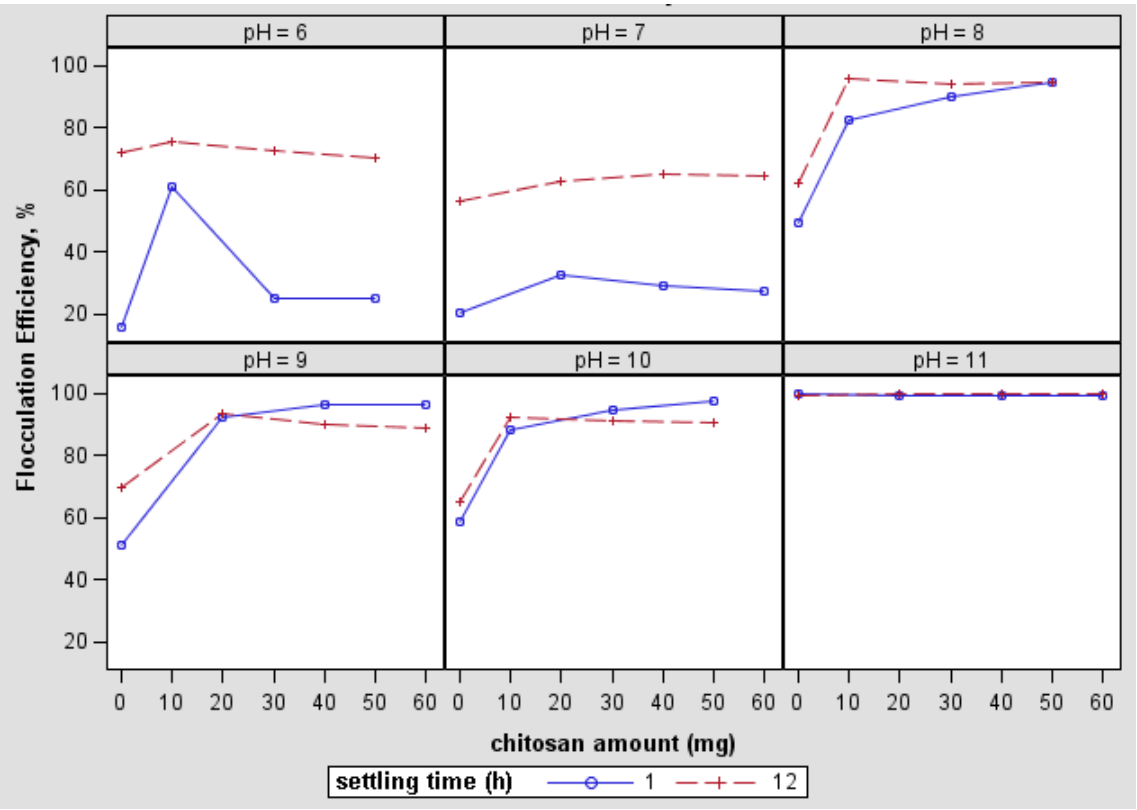
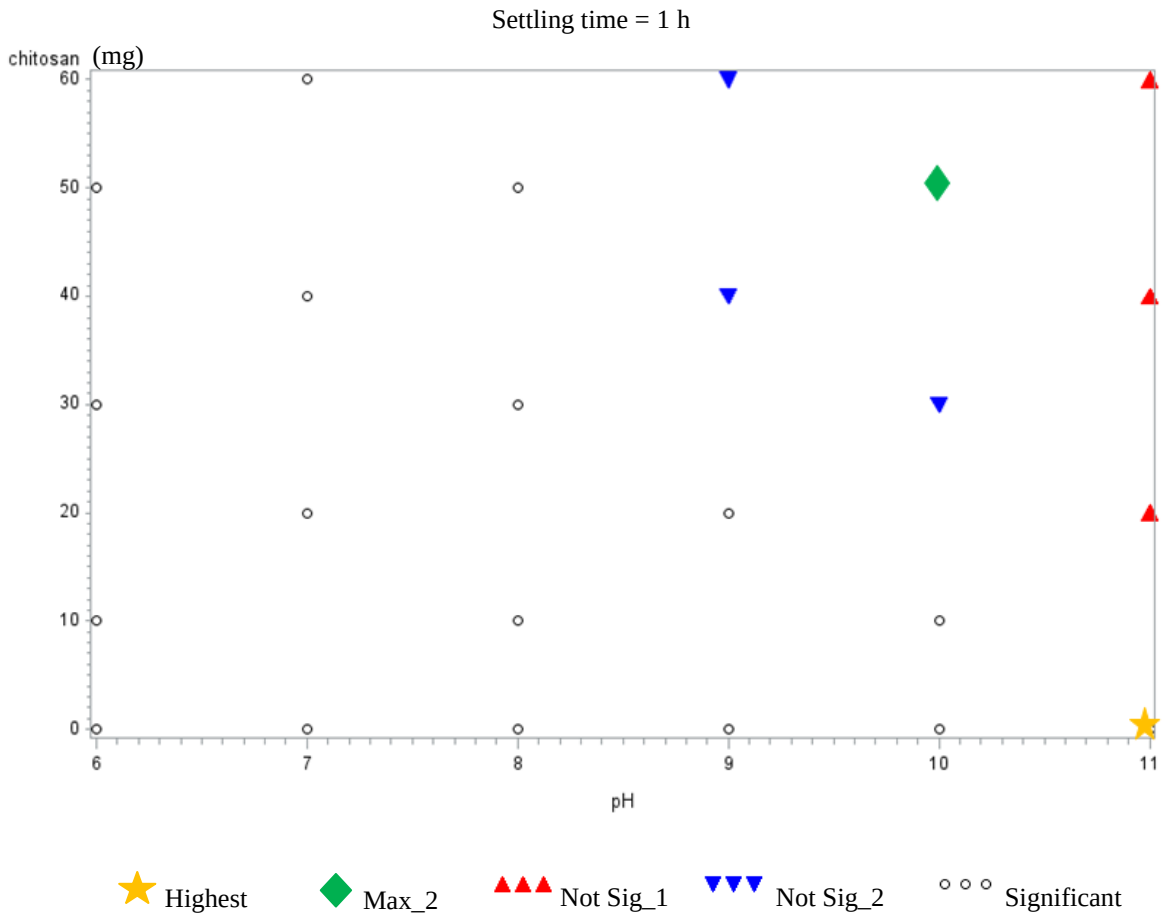
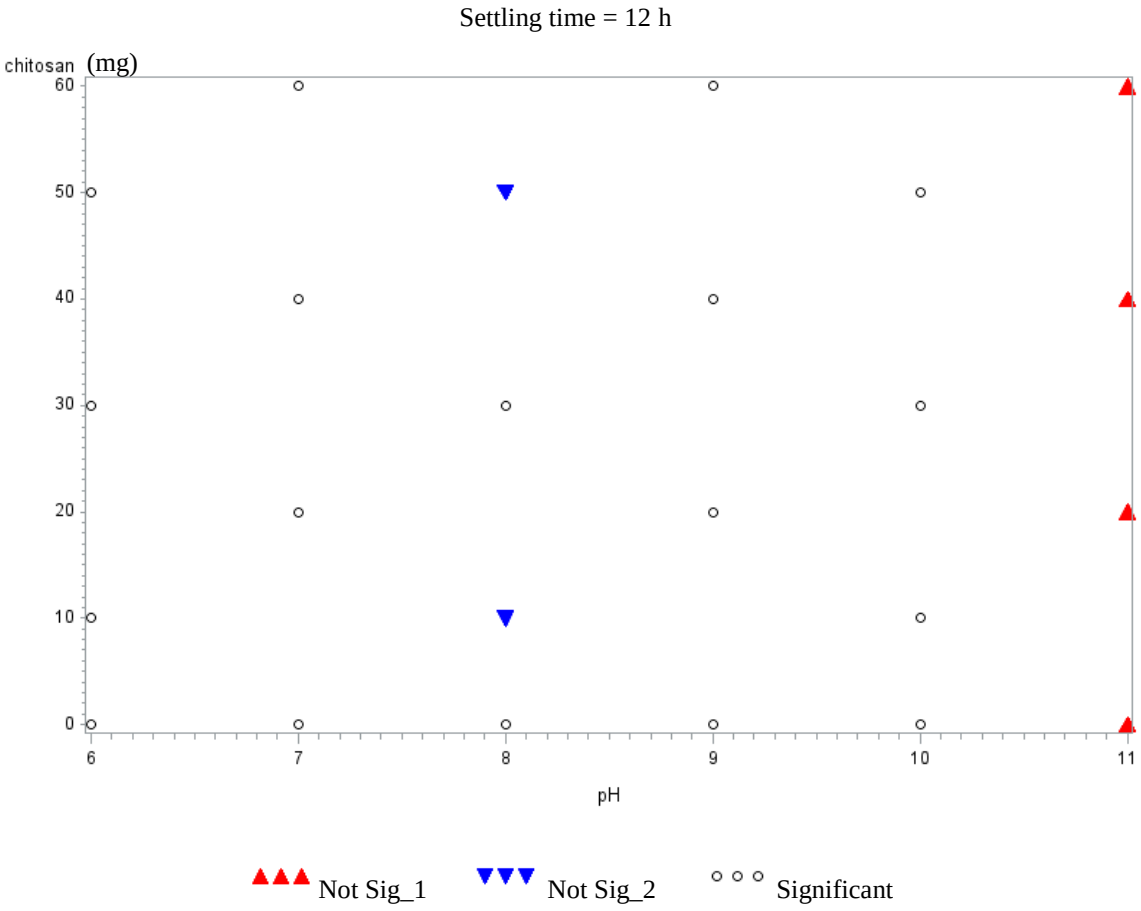


Figure 28: Combinations of variables at settling time of 1 h for NO chitosan flocculation after Dunnett's multiple comparisons with a "best" mean.



The "best" mean refers to the highest flocculation efficiency and the "Not Sig" represents "not significant".

Figure 29: Combinations of variables at settling time of 12 h for NO chitosan flocculation after Dunnett's multiple comparisons with a "best" mean.



The "best" mean refers to the highest flocculation efficiency and the "Not Sig" represents "not significant".

Figure 30: Flocculation efficiency for PO obtained from electro-flocculation at settling time of 0.5 h.

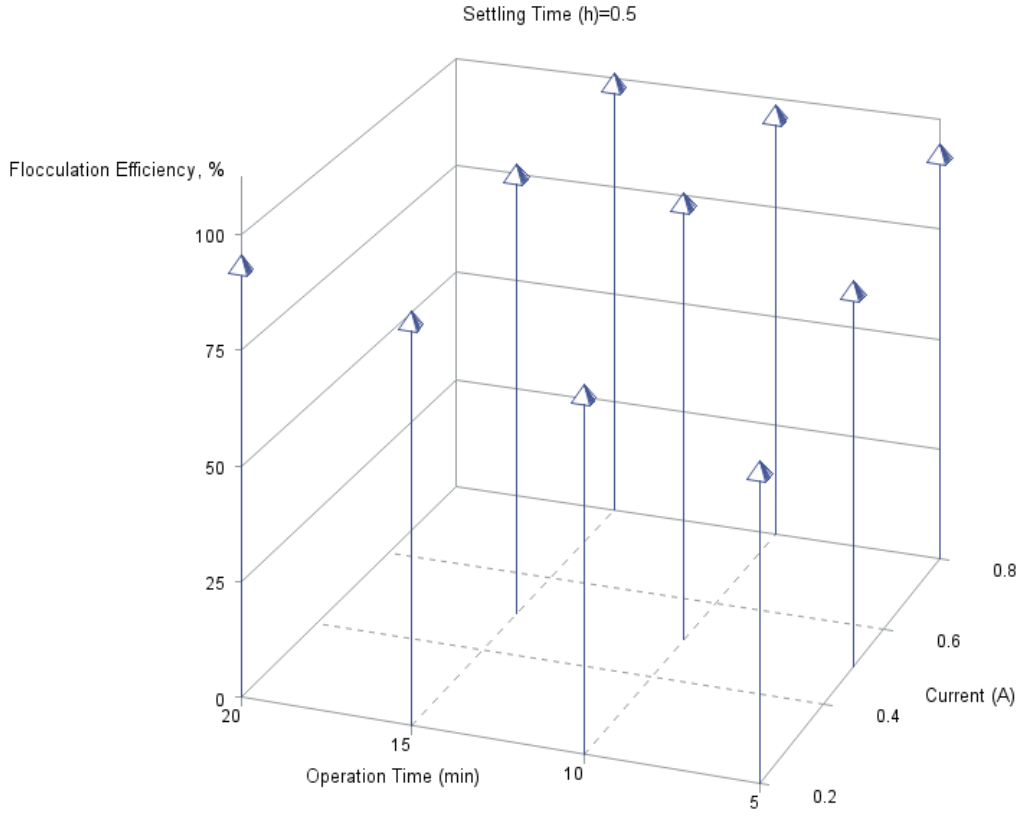


Figure 31: Flocculation efficiency for PO obtained from electro-flocculation at settling time of 1 h.

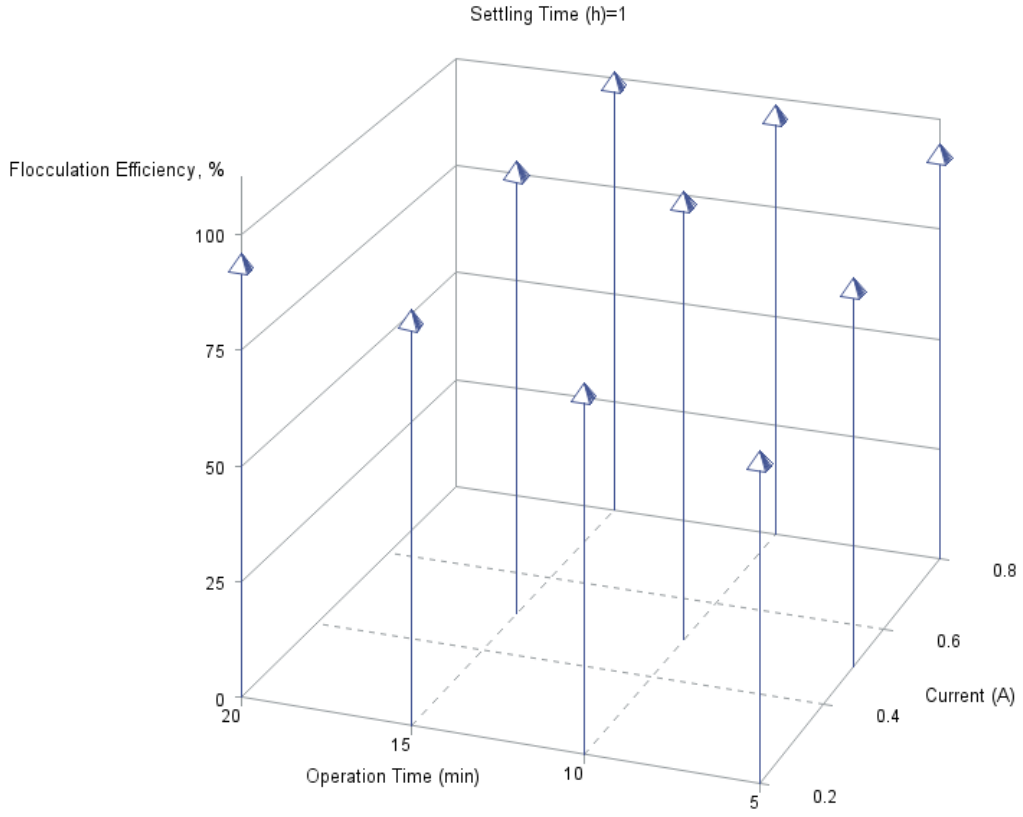


Figure 32: Flocculation efficiency for PO obtained from electro-flocculation at settling time of 12 h.

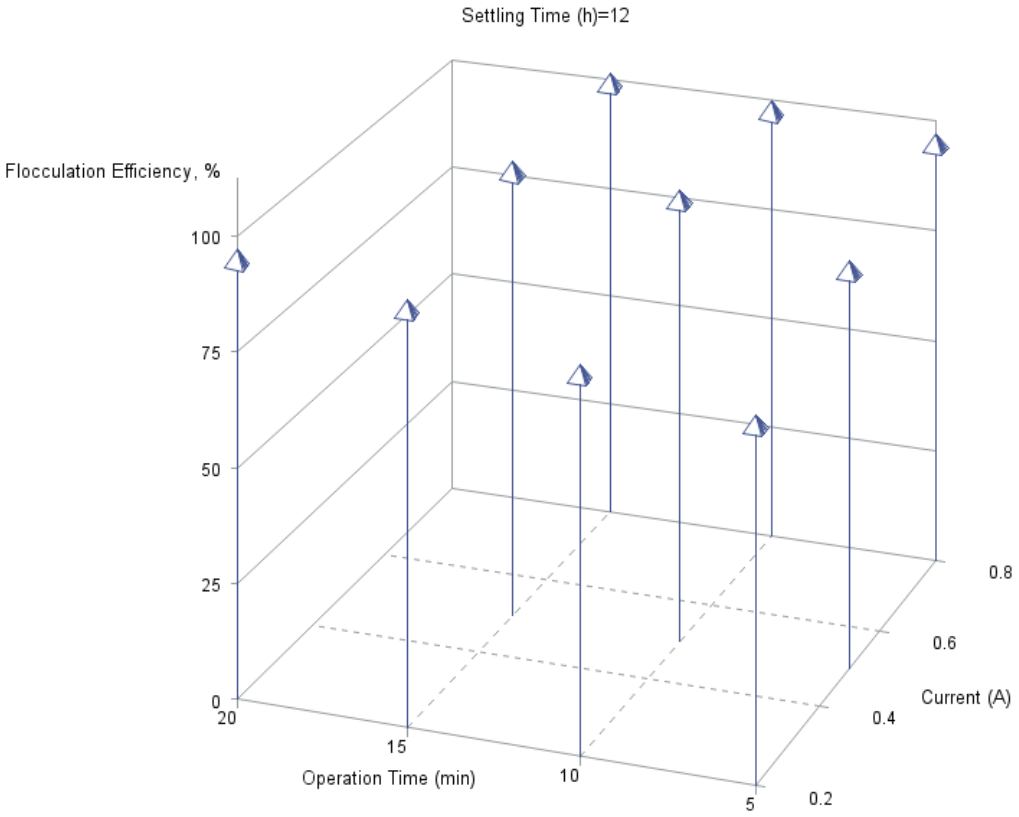


Figure 33: Electro-flocculation efficiency of PO as a function of current value at different settling time.

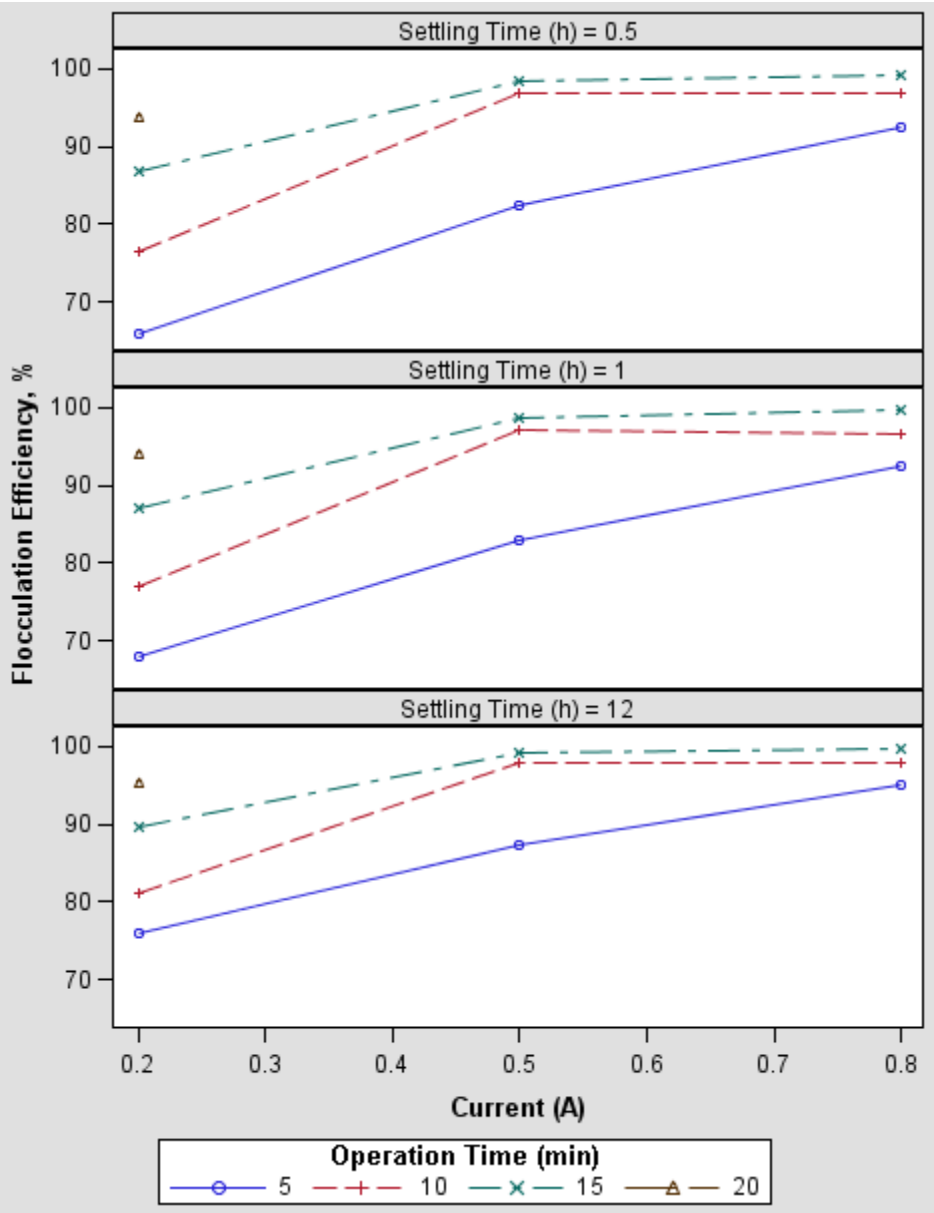


Figure 34: Electro-flocculation efficiency of PO as a function of operation time at different settling time.

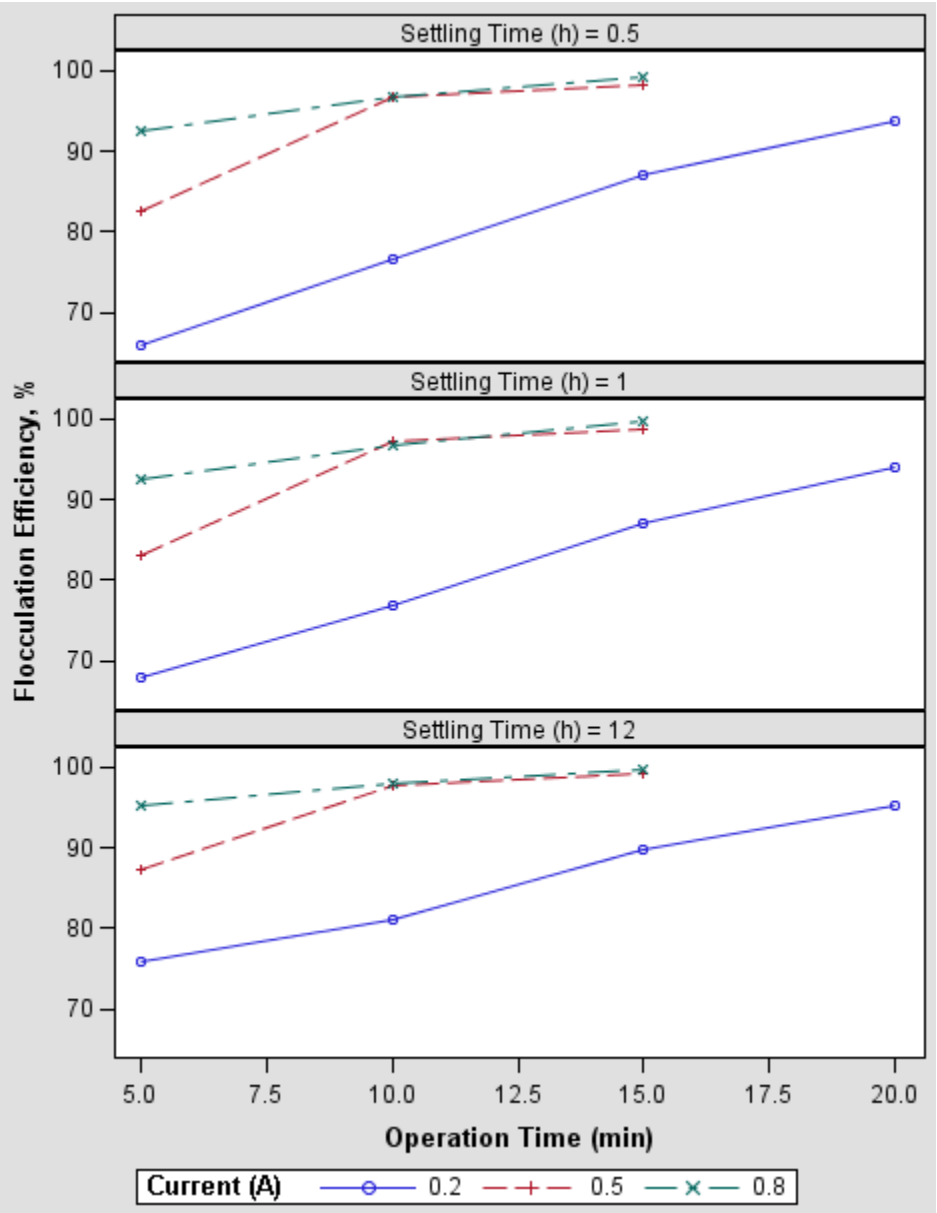


Figure 35: Electro-flocculation efficiency of PO as a function of operation time at different current value.

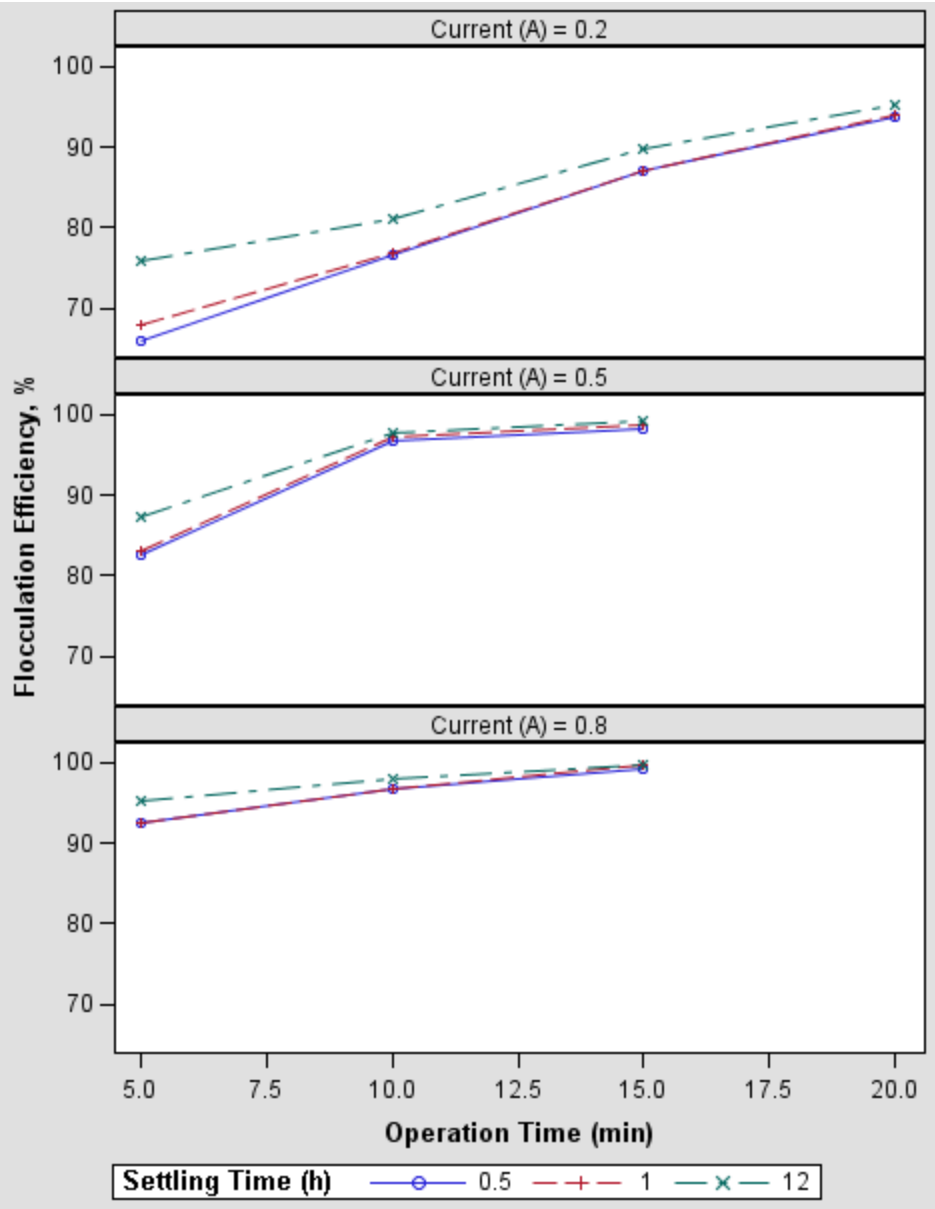
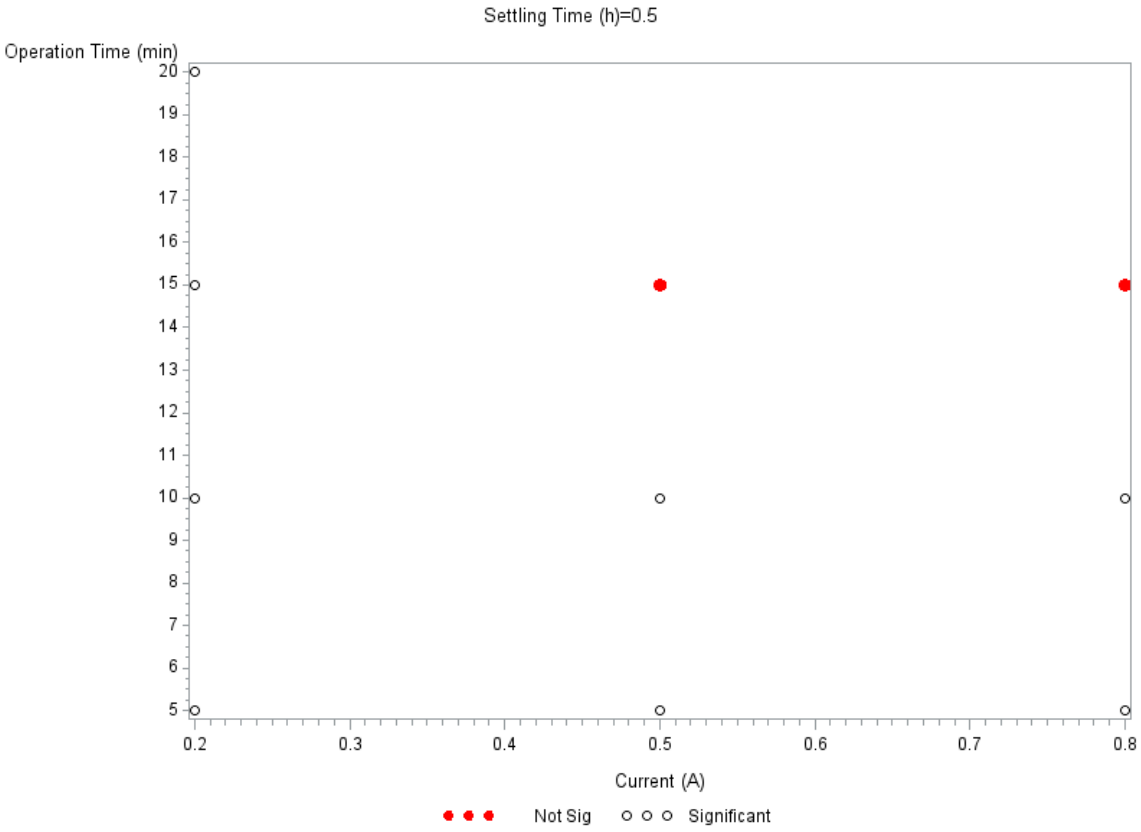
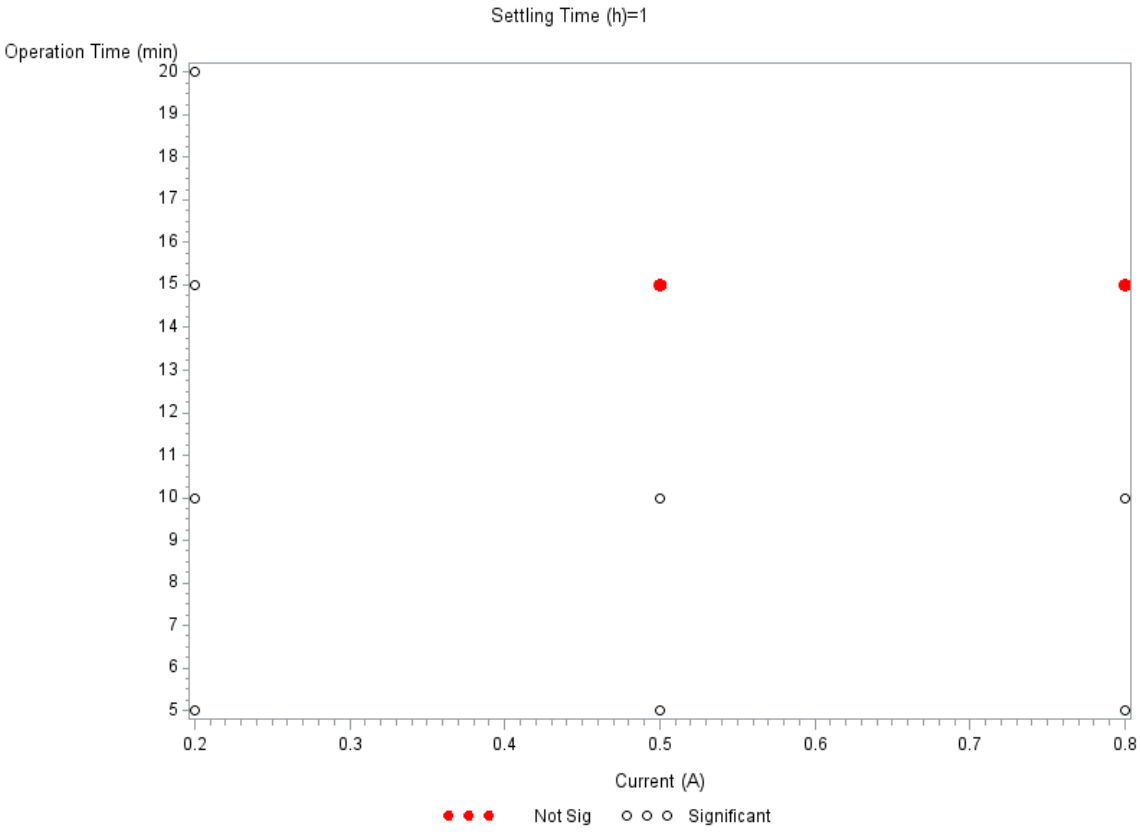


Figure 36: Combinations of variables at settling time of 0.5 h for PO electro-flocculation after Dunnett's multiple comparisons with a "best" mean.



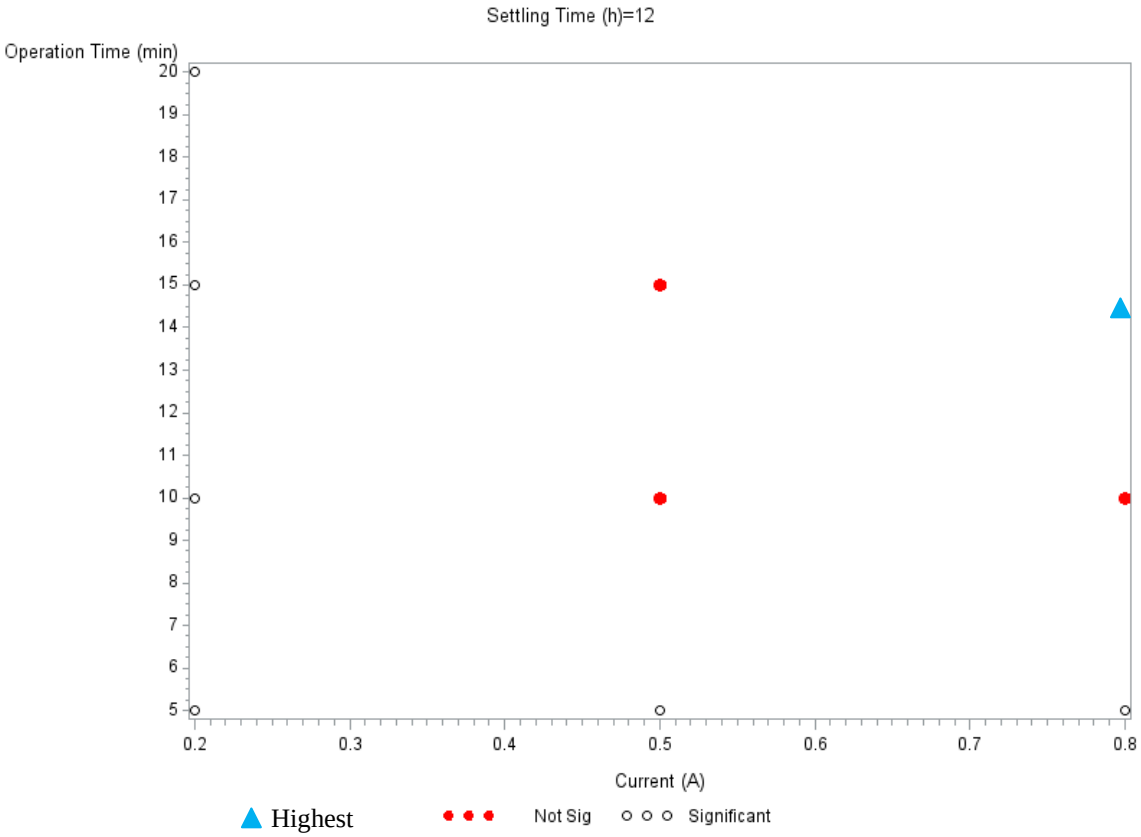
The "best" mean refers to the highest flocculation efficiency and the "Not Sig" represents "not significant".

Figure 37: Combinations of variables at settling time of 1 h for PO electro-flocculation after Dunnett's multiple comparisons with a "best" mean.



The "best" mean refers to the highest flocculation efficiency and the "Not Sig" represents "not significant".

Figure 38: Combinations of variables at settling time of 12 h for PO electro-flocculation after Dunnett's multiple comparisons with a "best" mean.



The "best" mean refers to the highest flocculation efficiency and the "Not Sig" represents "not significant".

Figure 39: Flocculation efficiency for NO obtained from electro-flocculation at settling time of 0.5 h.

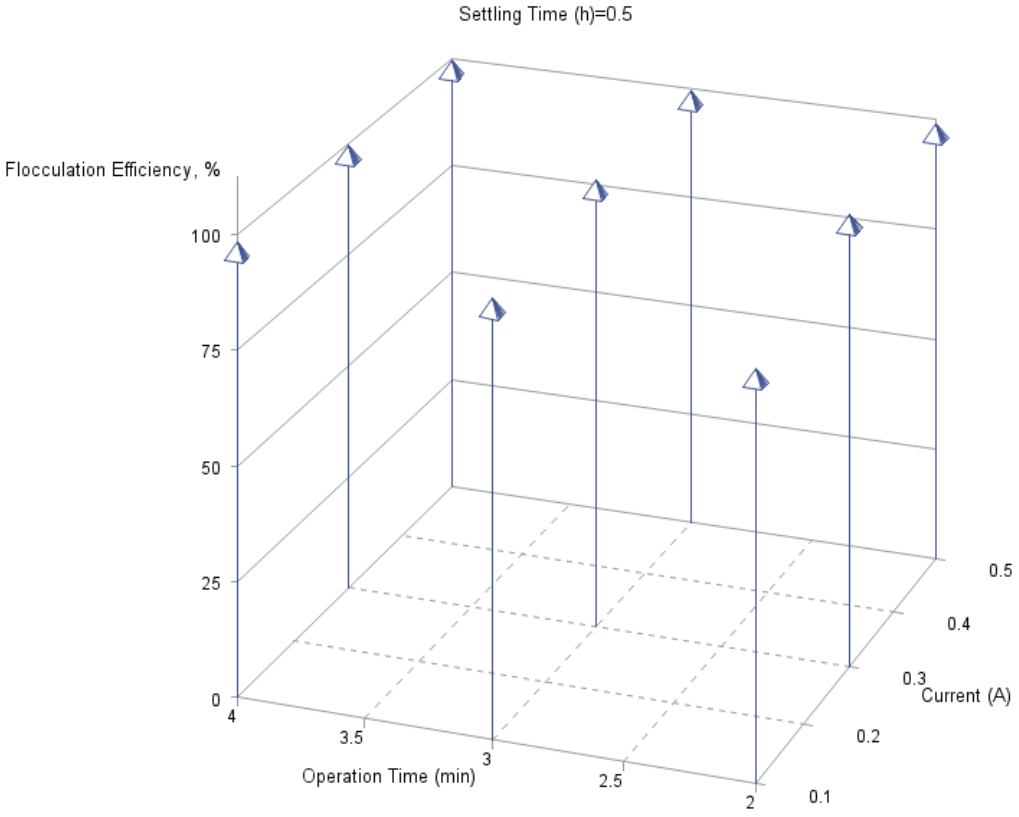


Figure 40: Flocculation efficiency for NO obtained from electro-flocculation at settling time of 1 h.

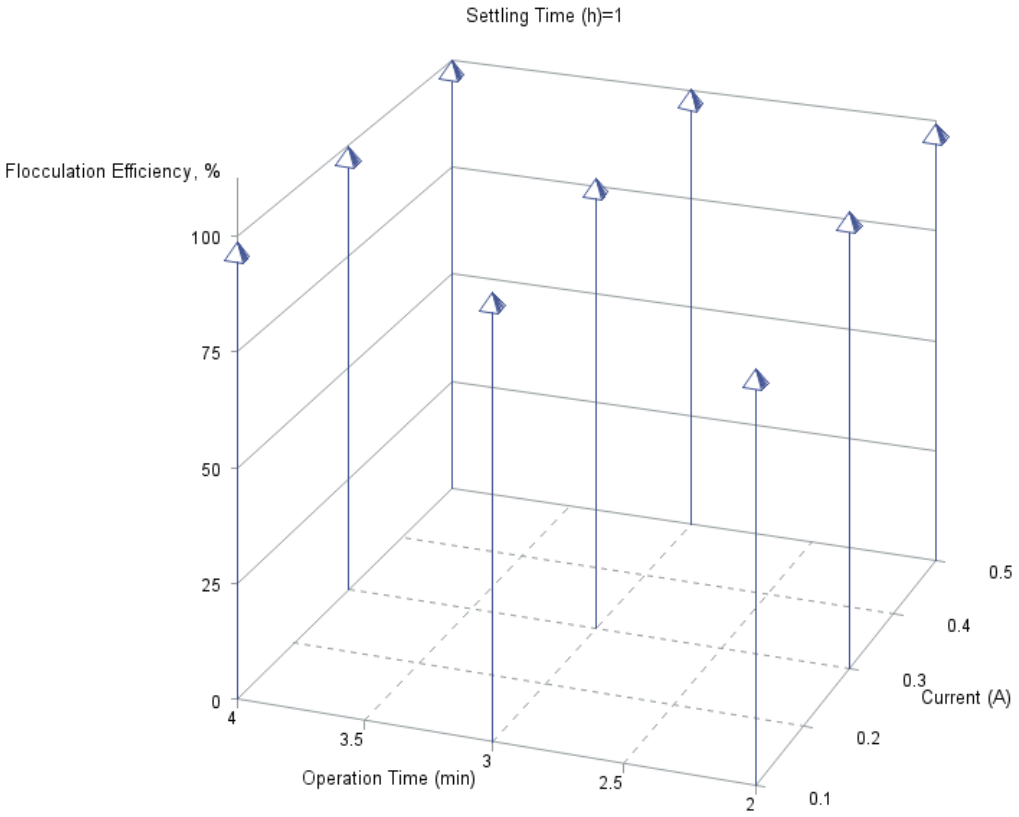


Figure 41: Flocculation efficiency for NO obtained from electro-flocculation at settling time of 12 h.

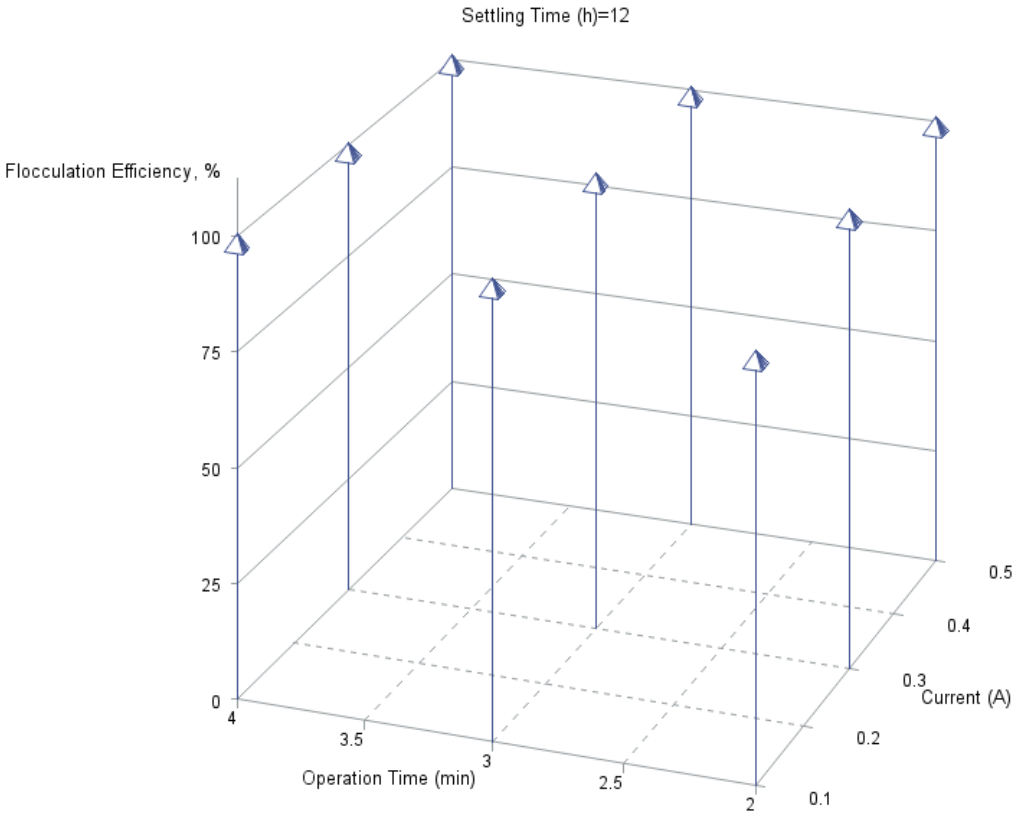


Figure 42: Electro-flocculation efficiency of NO as a function of current value at different operation time.

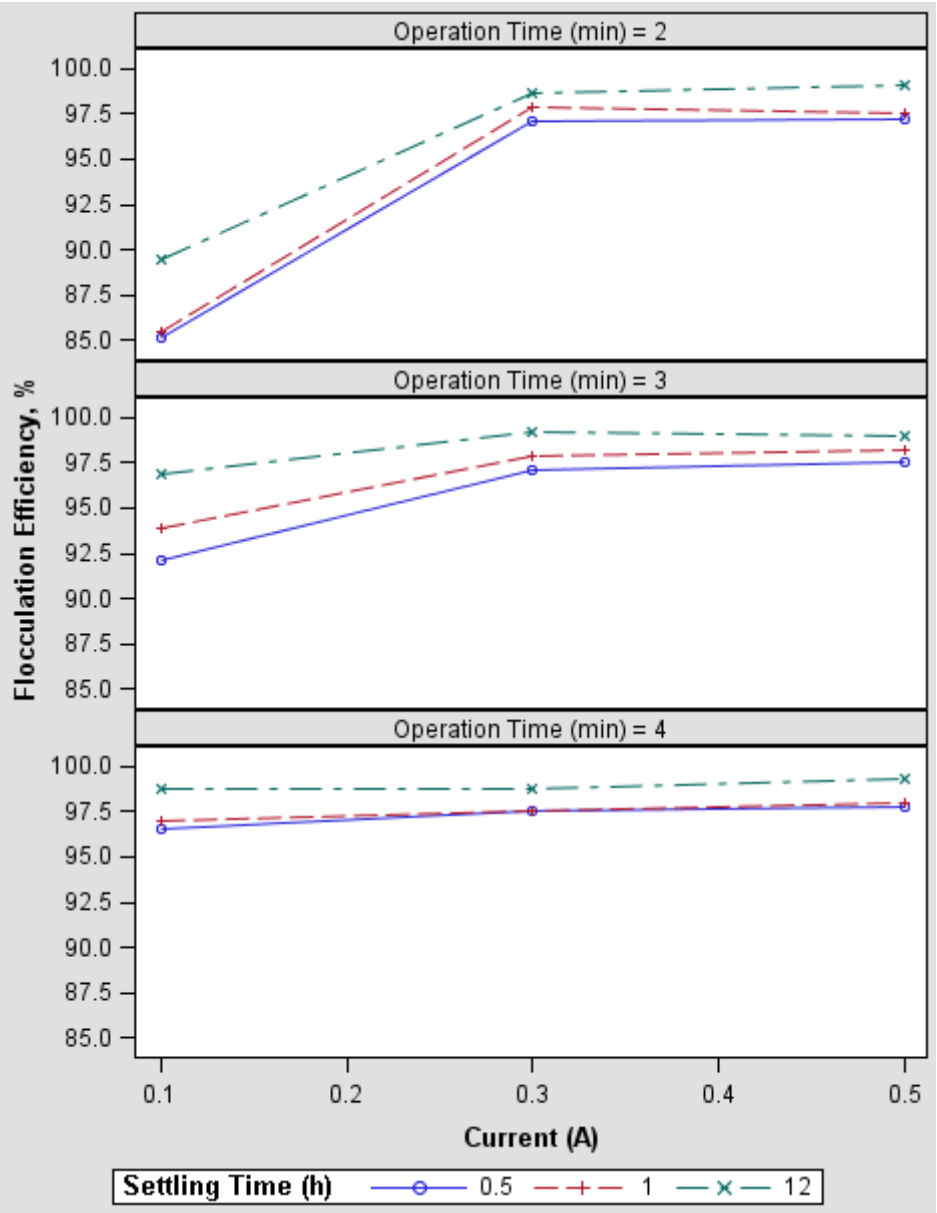


Figure 43: Electro-flocculation efficiency of NO as a function of current value at different settling time.

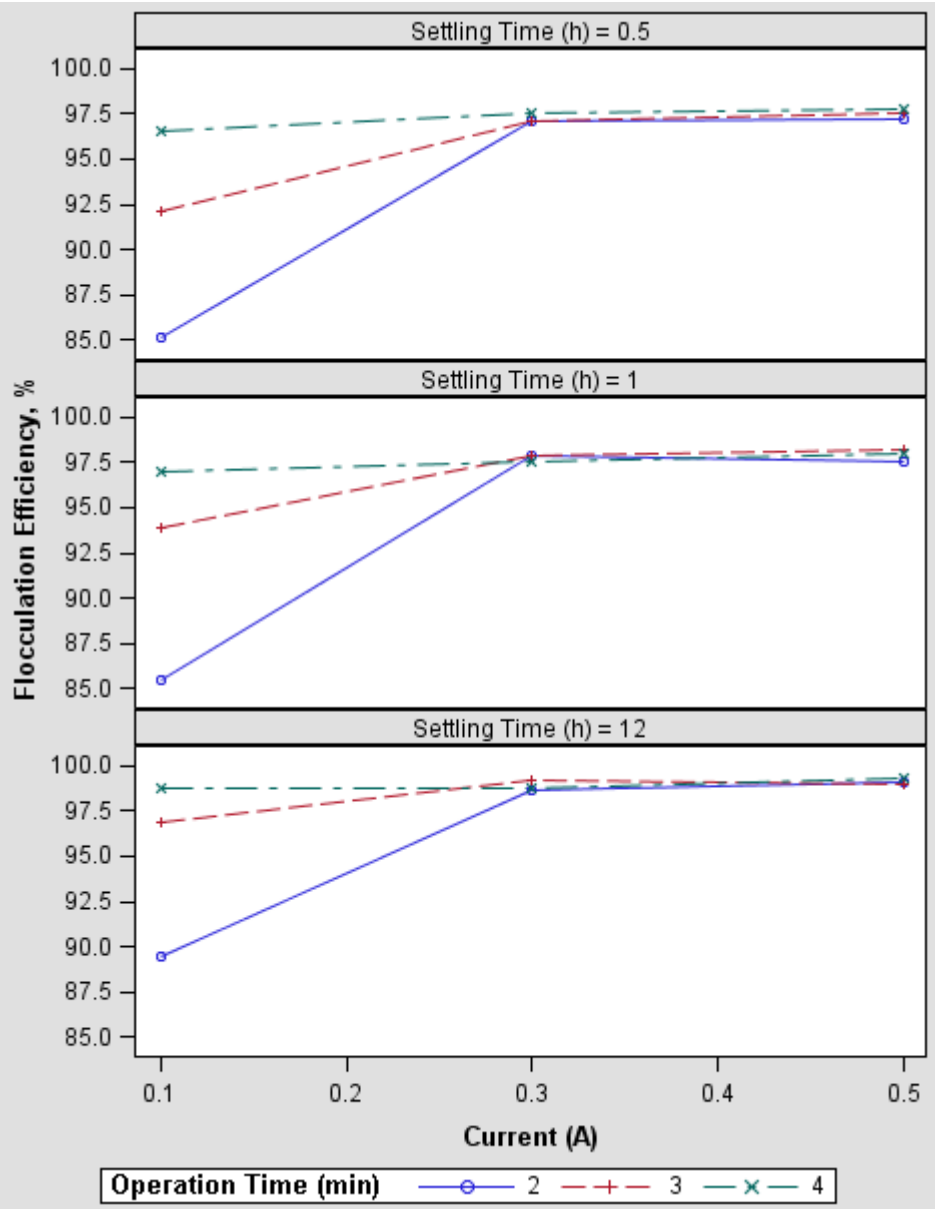


Figure 44: Electro-flocculation efficiency of NO as a function of operation time at different settling time.

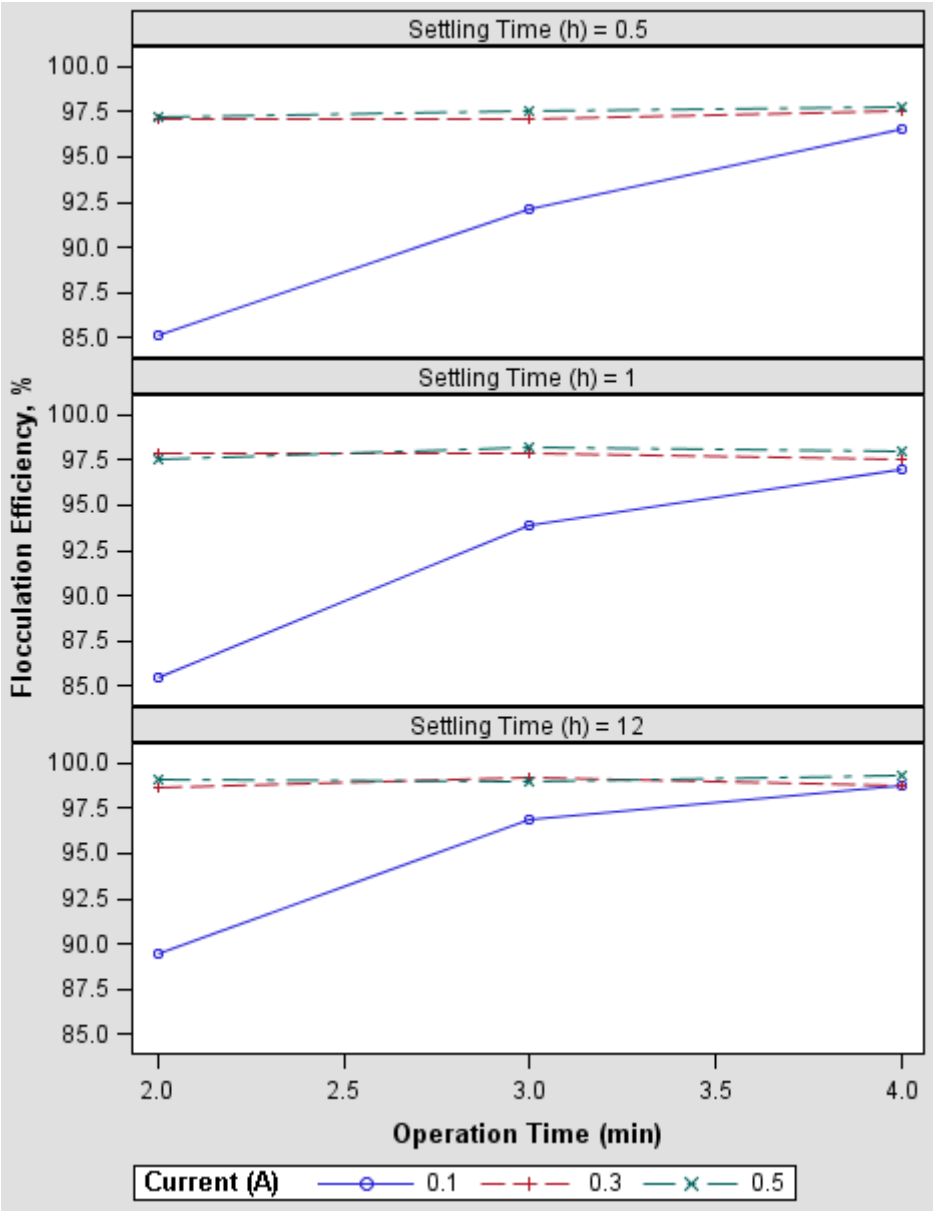
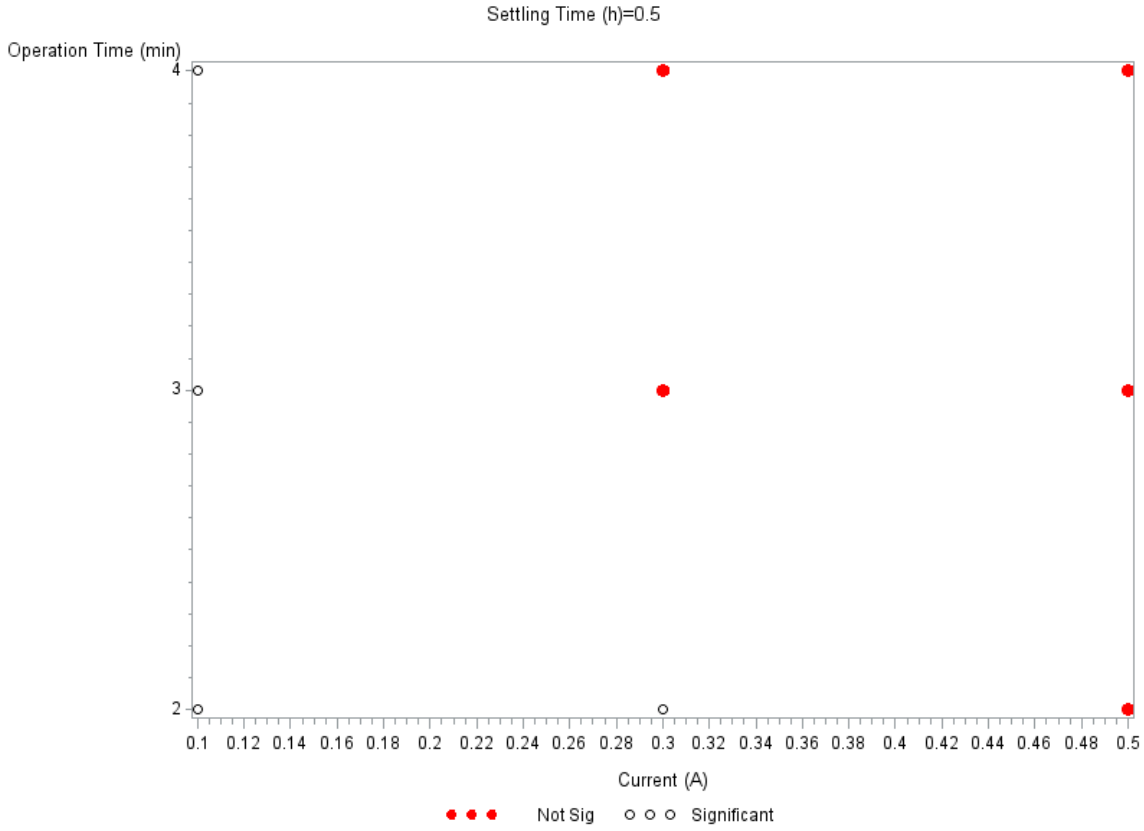
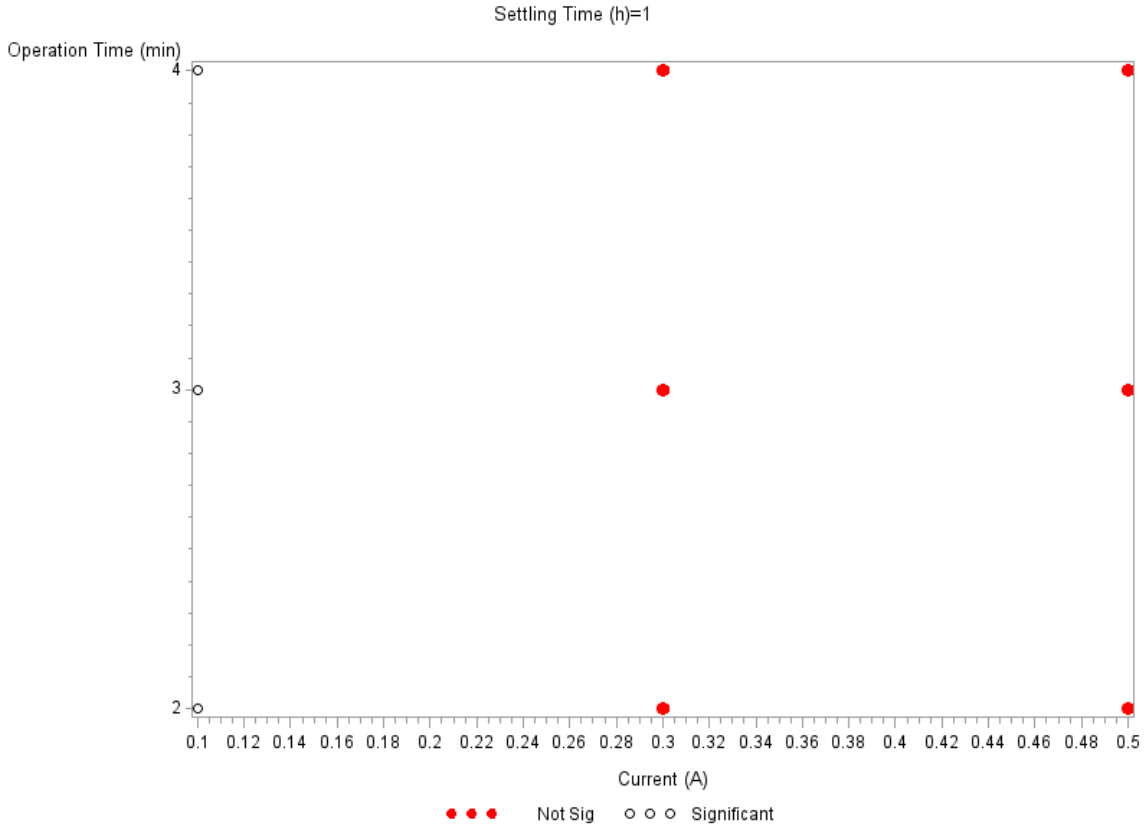


Figure 45: Combinations of variables at settling time of 0.5 h for NO electro-flocculation after Dunnett's multiple comparisons with a "best" mean.



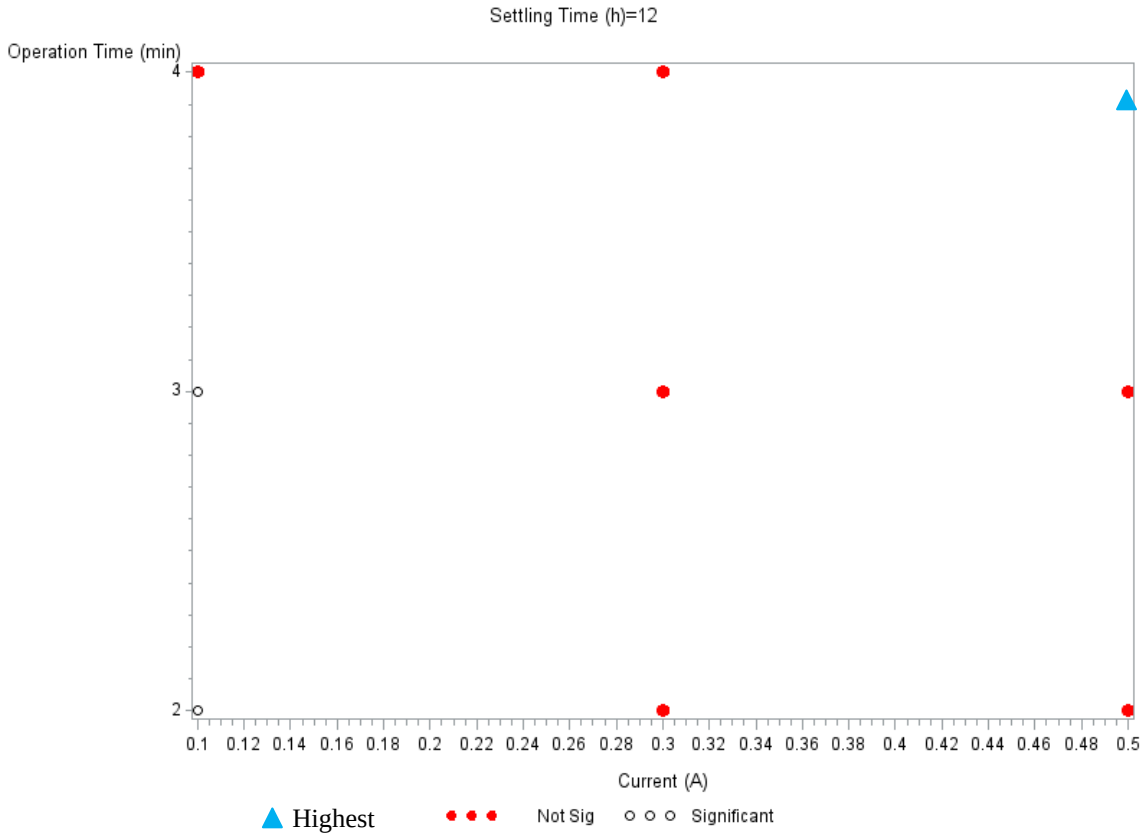
The "best" mean refers to the highest flocculation efficiency and the "Not Sig" represents "not significant".

Figure 46: Combinations of variables at settling time of 1 h for NO electro-flocculation after Dunnett's multiple comparisons with a "best" mean.



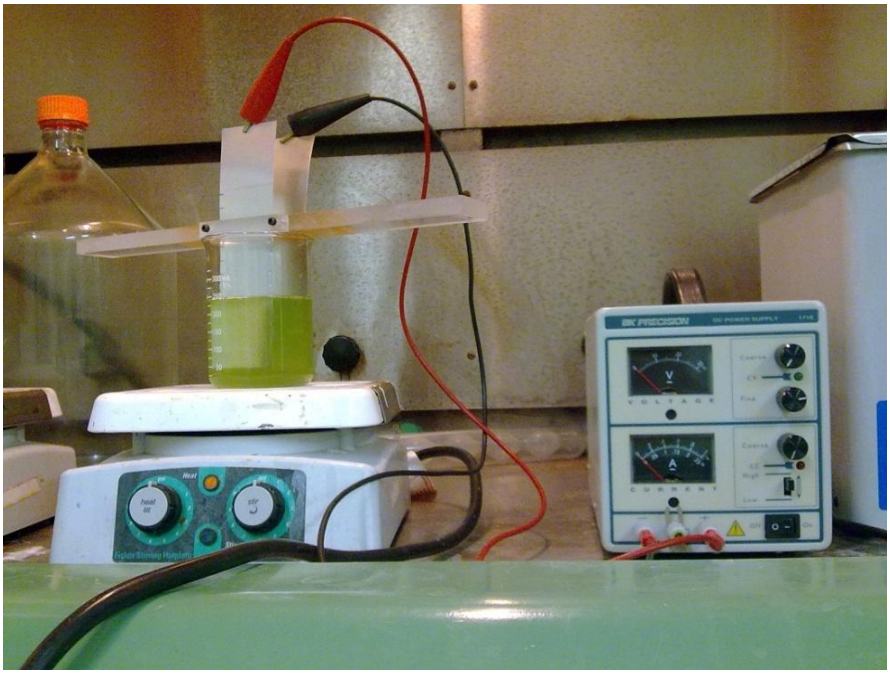
The "best" mean refers to the highest flocculation efficiency and the "Not Sig" represents "not significant".

Figure 47: Combinations of variables at settling time of 12 h for NO electro-flocculation after Dunnett's multiple comparisons with a "best" mean.

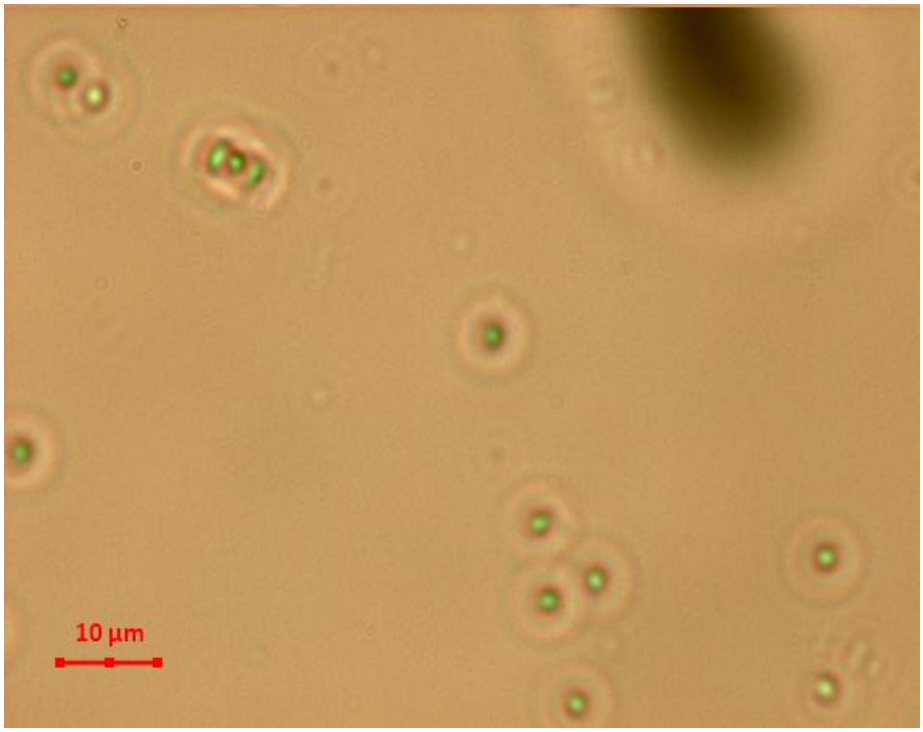


The "best" mean refers to the highest flocculation efficiency and the "Not Sig" represents "not significant".

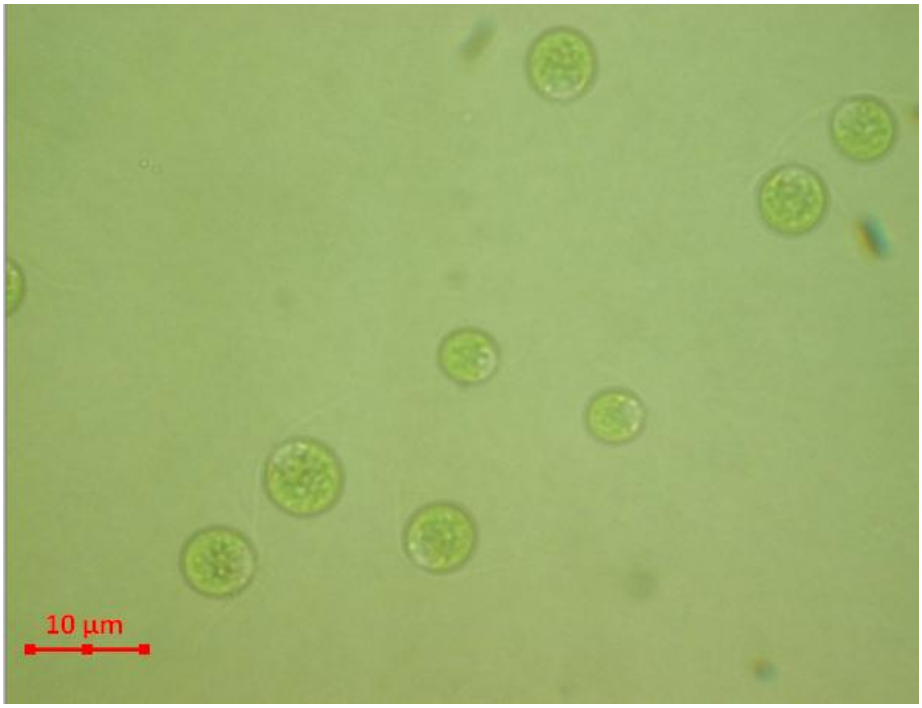
Picture 1: Picture of an electro-flocculation device for harvesting microalgae.



Picture 2: Micrograph of PO.



Picture 3: Micrograph of NO.



VITA

Yan Zhu

Candidate for the Degree of

Master of Science

Thesis: BIOMASS AND FLOCCULATION CHARACTERISTICS OF *PICOCHLORUM OKLAHOMENSIS* AND *NANNOCHLOROPSIS OCULATA*

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Title of Study: BIOMASS AND FLOCCULATION CHARACTERISTICS OF *PICOCHLORUM OKLAHOMENSIS* AND *NANNOCHLOROPSIS OCULATA*

Pages in Study: 118 Candidate for the Degree of Master of Science

Major Field: Biosystems and Agricultural Engineering

Scope and Method of Study: In this study, two microalgae strains, *Picochlorum oklahomensis* (PO) and *Nannochloropsis oculata* **(**NO), were examined for their biomass properties and harvesting characteristics. Three flocculation methods, pH adjustment, biopolymer (chitosan) addition and electro-flocculation, were investigated for effective harvesting of algal biomass. A generalized linear mixed model using a beta distribution for response was utilized to optimize the processing variables.

Findings and Conclusions: The results obtained from characterization of microalgae indicated that PO and NO can be potential feedstock for biofuel production. Adjusting the pH of algae suspensions to 11-13 resulted in effective biomass flocculation efficiency, 90.5-97.0 % for PO and above 99 % for NO. The highest flocculation efficiency obtained from the flocculation by chitsoan addition was 98.4 % for PO, and 99.8 % for NO. The highest electro-flocculation efficiency was 99.7 % for PO, and 99.4 % for NO. In conclusion, pH adjustment, chitosan addition and electro-flocculation were effective methods to flocculate PO and NO cells under the conditions examined in this study. The scalability of these flocculation techniques for commercial algal biomass production needs to be further examined. The selection of the most suitable flocculation technique and the optimum treatment conditions would be based not only on the application of algal biomass, but also on a feasibility study.

ADVISER'S APPROVAL: Nurhan T. Dunford