AN ORGANIC SOLVENT-FLOCCULANT BASED SYSTEM FOR CHEMICAL DEWATERING OF ALGAE

A Thesis

by

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MASTER OF SCIENCE

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ABSTRACT

The concentration of algal water in a raceway pond is around 0.5 -1 gram of whole cell biomass per unit of volume (liter) of liquid. Because of its dilute nature, dewatering microalgae suspensions can account for a significant portion of downstream processing costs - and thus is a significant challenge for the sustainability of industrial scale algal processes. The Solvent Phase Algal Migration (SPAM) process presented here is a technique designed to separate suspended algal cells from their aqueous phase to a solvent while simultaneously displacing water. This investigation evaluates the dewatering performance of five factors pertinent to the SPAM process: algal surface modifier type, algal surface modifier concentration, solvent fraction, migration time, and initial algal broth concentration. The investigation revealed that the initial algal broth concentration, type of surface modifier and solvent fraction significantly affected the level of algal migration during the SPAM process.

DEDICATION

To my mother, Reverend Cora L. Rex-Carter (†), and mother-in-law, Margaret Snipes Spruill (†), for their hallmark brand of servant leadership, which has created a timeless legacy through life lessons that lucidly linked love to learning. I pray that their presence continue to illuminate every educational experience.

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A grand thank you to God, the Father, whose sovereignty will always reign supreme. My "pay it forward" prayer is that my walk through my pioneering unique educational journey will inspire others to pursue their own adventures. I acknowledge my own journey has been influenced by agriculturally-based trailblazers such as Norman Borlaug and George Washington Carver because of their relentless pursuit of diversity enhanced education and innovations for the benefit of society.

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May these acknowledgements serve as a testament to the contributions and sacrifices of my "cloud of witnesses" who can attest to my commitment to perseverance.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Professor Sergio Capareda serving as chair and Professor Sandun Fernando serving as co-chair, both from the Department of Biological and Agricultural Engineering. Additional committee members include Professor Ronald Lacey from the Department of Biological and Agricultural Engineering and Professor Timothy Devarenne from the Department of Biochemistry and Biophysics.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

1. INTRODUCTION

Even as a natural source of food in the biosphere, algae has played a significant role in the sustainability of basic life forms as well as advanced civilizations (Brune, et al., 2008; Spolaore, et al., 2006). Microalgae in particular have demonstrated their continued utility in today's society as a platform for generating a myriad of metabolite-based functionalized products such as polyunsaturated fatty acids (PUFAs), astaxanthin and bioactive compounds (Chiu, et al., 2009; A. Belay, 1997; Pulz and Gross 2004, M.A. Borowitzka,1988a,b; Zittelli, et al. 1999) and energy precursors (Schenk, et al., 2008). More specifically; in terms of future renewable biofuels, microalgae has emerged as a highly promising feedstock. A considerable amount of water is utilized to cultivate microalgae biomass on an industrial scale. After successfully reaching their desirable biomass accumulation, the cultivated microalgae are removed from their growth environment through an agriculturally-based process called harvesting. Nevertheless, algal biomass is typically accumulated at relatively low concentrations (Golueke and Oswald, 1965; Chen et al, 2011; F. Chen 1996 Chen et al. 1997). As a result, achieving high algal biomass concentrations necessary to perform downstream biorefinery processing remains a key challenge for biofuel production at industrial scale.

In biorefinery processing, the removal of the water from microalgae is interchangeably referred to as either harvesting or dewatering. Dewatering techniques are typically categorized by their mechanism of separation, i.e., physical and chemical (Molina Grima 2003, Danquah 2009, Uduman 2010). Physical dewatering involves

pressing, filtering, centrifugation, sonication and drying while chemical techniques primarily involve ion-exchange, flocculation and coagulation (Shelef et al. 1984, Sim et al. 1988, Danquah 2009, Yuan, et al. 2009). Regardless of the technique, concentration of dilute algal suspensions into higher concentration slurries is still energy intensive (Uduman et al., 2010) and techniques to reduce the energy penalty may hold the key to developing a sustainable algal biofuel industry (Sheehan et al. 1998; Wijffels et al., 2010). Accordingly, the overall goal of this work is to develop a chemical technique that allows the algal biomass to migrate from the aqueous phase to a solvent phase while making the dewatering processes potentially significantly less energy intensive.

1.1. Objectives

To achieve chemically enhanced--cell surface migration for dewatering algae, a process has been developed that uses a special class of surface active agents (surface modifiers) described as cationic polyelectrolytes. These positively-charged polymer molecules have an affinity for binding with negatively charged particles like algal surfaces that will result in charge neutralization of the combined complex. The polyelectrolytes that are responsive for SPAM are such that once neutralized (after attaching onto the algal cell walls), the ensembles become hydrophobic. The now hydrophobically enhanced algal cells, when exposed to a hydrophobic organic solvent, selectively migrate into the organic phase by removing itself from the presence of the hydrophilic environment. This novel technique for dewatering algae is known as the solvent phase algal migration (SPAM) process. The present study evaluates the dewatering performance of five factors pertinent to the SPAM process: algal surface

modifier type, algal surface modifier concentration, solvent fraction, migration time, and initial algal broth concentration with the algal species *Nannochloropsis oculata*.

2. MATERIALS AND METHODS

2.1. Microorganism and Culture Condition

Nannochloropsis is a genus of algae comprising 6 known species. The genus in the current taxonomic classification was first termed by Hibberd (1981). The species currently recognized as *Nannochloropsis oculata* was selected for the SPAM study because their typical size ranges from 1–2 μm in length and width, which provides a simplistic spherical morphology with uniform surface-to-volume ratio. *N. oculata* was kindly supplied by the TAMU Agrilife Algae facility located in Pecos, TX. The composition of the cultivation medium included a limited supply of nitrogen to provide growth stress for an increase in the production of oil in the algal cells. Standard cultures were grown in outdoor open pond raceway facilities for a period of 14 days. At the end of the growing phase, algal cells were transferred to a centrifuge for concentrating the suspension from approximately 0.1% (w/w, wet basis; 1g dry weight /liter) to 10.0% (w/w, wet basis; 100g dry weight /liter) final concentration (algae were pre-concentrated for transportation and diluted as appropriate for experimentation purposes). The 10.0% algal concentrate samples were transported to the Nanoscale Biological Engineering Laboratory located on the main campus of Texas A&M University. Upon arrival, the samples were stored at 4^{o}C until use.

2.2. Experimental Design

The design of experiments and data analysis were done using Design Expert® software. Data were categorized using a 2^5 mixed level factorial designed experiment

with two replicates. Each experimental unit was sampled randomly from the water phase and the organic solvent phase. The four response variables used in the data analysis of the SPAM system were: moisture content (MC), dry solids content (DCS), algal mass in phase (AMP), and distribution of dry solids content (DDSC). Table 2.2 lists the five design factors used to statistically measure and evaluate the experimental responses from algae subjected to SPAM.

Table 2.2 Design Factor Reference Table.

1	Surface Modifier Type (SMT)	Type A (Mel-Formaldehyde) Type B (PDADMAC), Type C (PADDAC)	
		Low	High
2	Surface Modifier Concentration (SMC)	1% wt	5% wt
3	Solvent Fraction (SF)	25%	75%
4	Migration Time (MT)	1 hr	24 hr
5	Initial Algal Broth Concentration (IABC)	0.1%	10.0%

The data used in the analysis represent the values obtained from the application of the SPAM process to *N. oculata* algal species. The statistical results of the designed experiments were used for the description of how the algae subjected to SPAM responds to the changes in the combinations of levels of the design factors.

2.3. SPAM Experiments

Characteristics of the SPAM process were studied by investigating combinations of the following parameters: Algal surface modifier type, algal surface modifier concentration, solvent phase, migration time, and initial algal broth concentration. All tests were conducted in 30ml glass vials (outer diameter = 25 mm and height = 95 mm, VWR Llc.) sealed with screw-thread twist top caps. Each tube received algae from the same batch of *N. oculata* culture at an initial algal broth concentration (IABC) of 0.1% or 10.0% algae suspension. Then a surface modifier concentration (SMC) of either 1% or 5% by weight was applied to the corresponding volume of algal mass in each tube. Algal cell surface modification was performed by mixing one of the three types of industry standard flocculants with algae. Characteristics of each reagent grade flocculant used in this study are provided in Table 2.3.

Table 2.3 List of Organic Polymers Examined for SPAM.

Polymer Commercial Name	***Chemical Character***	***Manufacturer***	***Reference Label***
FL-5228 Melamine-Formaldehyde	Cationic	SNF Inc. Riceboro, GA	A
PDADMAC Poly (Diallyl – Dimethyl-ammonium chloride)	Cationic polyelectrolyte	Sigma-Aldrich	B
ADDAC Poly (Acrylamide- Co-Diallyl dimethylammonium chloride)	Cationic polyacrylamide	Sigma-Aldrich	C

After sealing the vials with screw top caps, the contents were vortex mixed at 10,000 rpm for 30 seconds using an analog minivortexer (VWR Model No. 945300). Next, caps

were removed to add the specified volume of solvent. The amount of solvent added was determined by the fraction of solvent required at two levels 25% or 75%. More specifically, when given a solvent phase fraction (SPF) of 75%, the corresponding (X) ml of algae broth received (30 – X) ml of the solvent, chloroform, added to achieve a 75:25 level of solvent:broth ratio represented by SPF. For example, at *initial algal broth concentration of 0.1% the solvent:broth ratio for percentages 25:75 by volume is converted to (7.5 ml chloroform solvent: 22.5 ml of culture broth with a concentration of 0.1%).* Tubes were sealed by hand and vigorously shaken manually for 30 seconds, then mixed at 10,000 rpm for 120 seconds. The end of the combined 2.5 minutes of mixing marked the beginning of the migration time for each sample in the experiments. Experiments were terminated after 1 hour or 24 hours to investigate the effects of the two levels of migration time (MT) on algae subjected to SPAM (Figure 2.3).

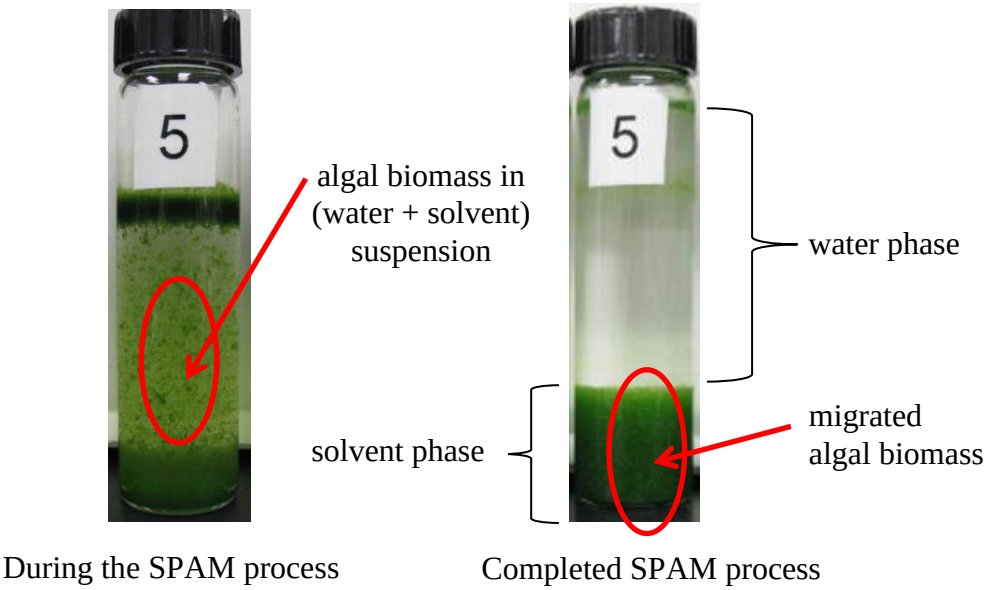


Figure 2.3 Experimental unit shows algal migration during and after the SPAM process.

2.4. Data Collection of Moisture Content and Dry Solids Content

After the completion of the migration time, up to 3 ml of sample from the water phase and up to 3 ml of sample from the organic solvent phase were placed in separate aluminum trays. Trays were placed in the oven at 70°C for one hour to remove residual solvent. To procure data for the moisture content analysis the same samples were then placed in the oven at 105°C until drying was complete. The oven dried samples were allowed to cool in a desiccator. Afterwards, desiccated samples were weighed to obtain per unit of volume of medium data for the calculations of moisture content and algal biomass dry solids content for both the aqueous phase and the solvent phase. Detailed information for calculation of moisture content on wet basis and solids content on mass

per volume basis is described by Hamilton and Zhang in the Oklahoma Cooperative Extension Service Brochure (BAE-1759).

3. DATA ANALYSIS

3.1. Moisture Content Analysis

The moisture content of algae subjected to SPAM refers to the amount of water present in each phase. Thus it is used as an indirect measure of the SPAM performance by relating the amount of algae remaining in the phase to the initial amount in the sample. There were significant differences among moisture content ranges (53.43% to 100.00%) of the aqueous phase compared to moisture content ranges of the solvent phase (0.00% to 89.52%). Figures 3.1.1 and 3.1.2 summarizes the statistical comparison for low levels of solvent used in the SPAM process. The differences in the dewatering characteristics were based on moisture content analysis at the end of one hour of migration time in both the aqueous and solvent phases. There was no difference ($\alpha <$ 0.05) detected between types of surface modifying agents for the solvent phase for low IABC. The data shows that for all surface modifiers at 1% concentration, 10% IABC moisture content was significantly higher than the moisture contents at 0.1% IABC samples (Figure 3.1.1). At 5% surface modifier concentration PolyDADMAC showed the lowest amount of moisture content in the solvent phase at 10% IABC (Figure 3.1.2). However, a moisture content analysis of the aqueous phase only showed one significant difference at 10% IABC between PADDAC and PolyDADMAC surface modifiers.

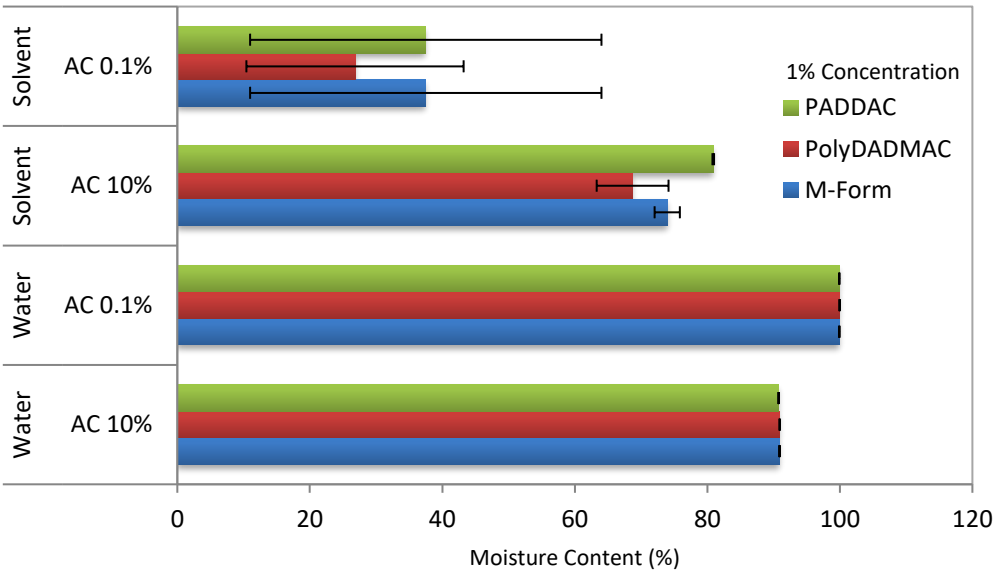


Figure 3.1.1 Moisture content analysis at 25% solvent fraction at 1-hour migration with 1% surface modifier concentration note: AC = (initial) algal (broth) concentration.

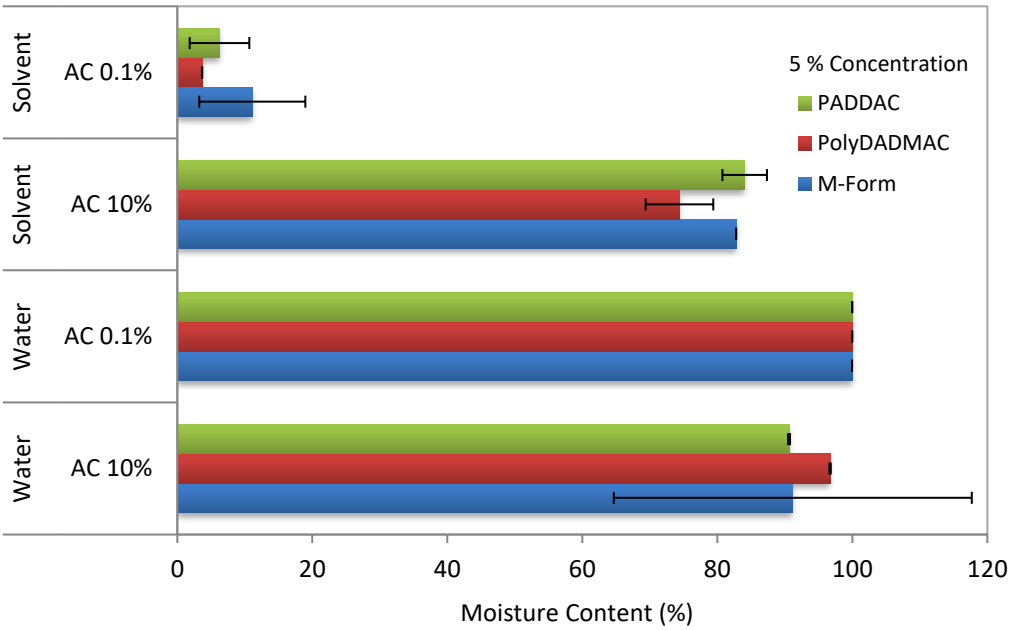


Figure 3.1.2. Moisture content analysis at 25% solvent fraction at 1-hour migration with 5% surface modifier concentration.

In contrast, all three surface modifiers demonstrated significantly lower moisture content at the initial algal broth concentration 0.1% for the two levels of solvent fractions. The highest moisture contents were observed for the 24 hour migration at an initial algal broth concentration of 0.1%. In the solvent phase analysis, the largest difference ($\alpha < 0.05$) in moisture content occurred for the PolyDADMAC surface modifying agent (Figure 3.1.3).

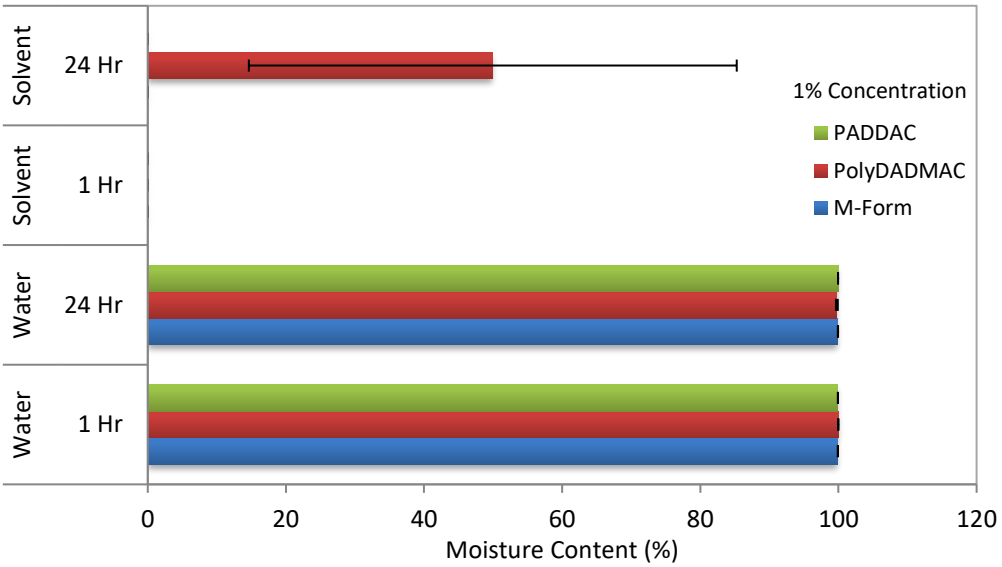


Figure 3.1.3 Moisture content analysis for 75% Solvent Phase Fraction and 0.1% Initial Algal Broth Concentration (IABC) with 1% Concentration.

Moisture content values for initial algal broth concentration (IABC) at 75% solvent to broth ratio and 5% surface modifier concentration showed some variations at 24 hour in the solvent phase (Figure 3.1.4).

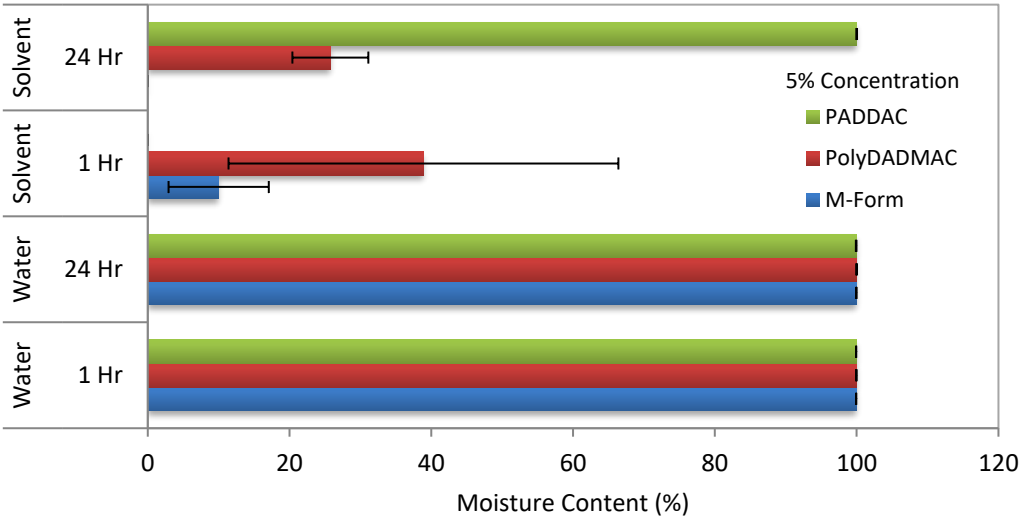


Figure 3.1.4 Moisture content analysis for 75% Solvent Phase Fraction and 0.1% IABC with 5% Concentration

However, under conditions of 10% initial algal broth concentration and 1% surface modifier concentration only the PolyDADMAC did not exhibit significant difference in moisture content between the two migration times (Figure 3.1.5).

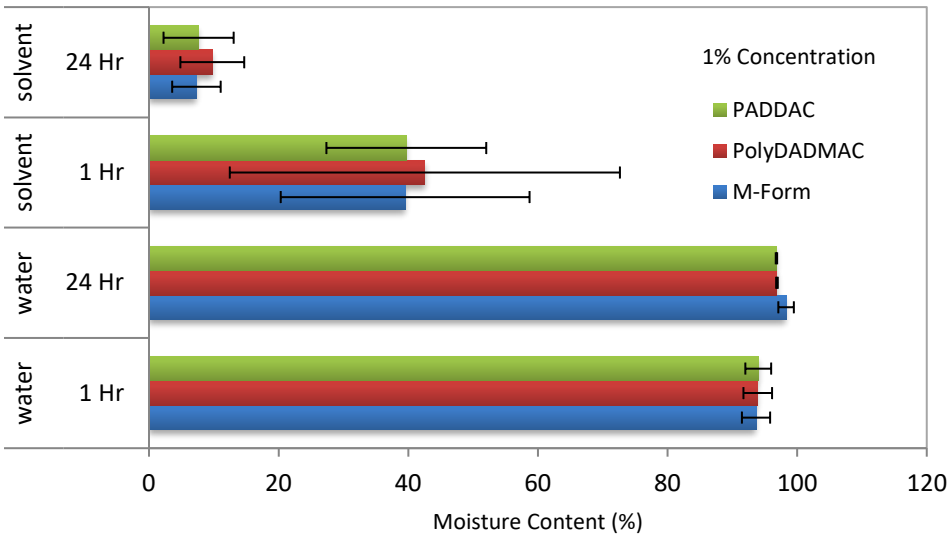


Figure 3.1.5 Moisture content analysis for 75% Solvent Phase Fraction and 10.0% IABC with 1% Concentration

Two significant differences were observed for the treatment combination of 75% solvent fraction, 10% initial algal broth concentration, and 5% surface modifier concentration. M-Form was significantly difference in both water and solvent phases over 1 hour and 24 hour migration times. PolyDADMAC showed a greater amount of moisture content in the water phase at 24 hours compared to 1 hour migration time (Figure 3.1.6).

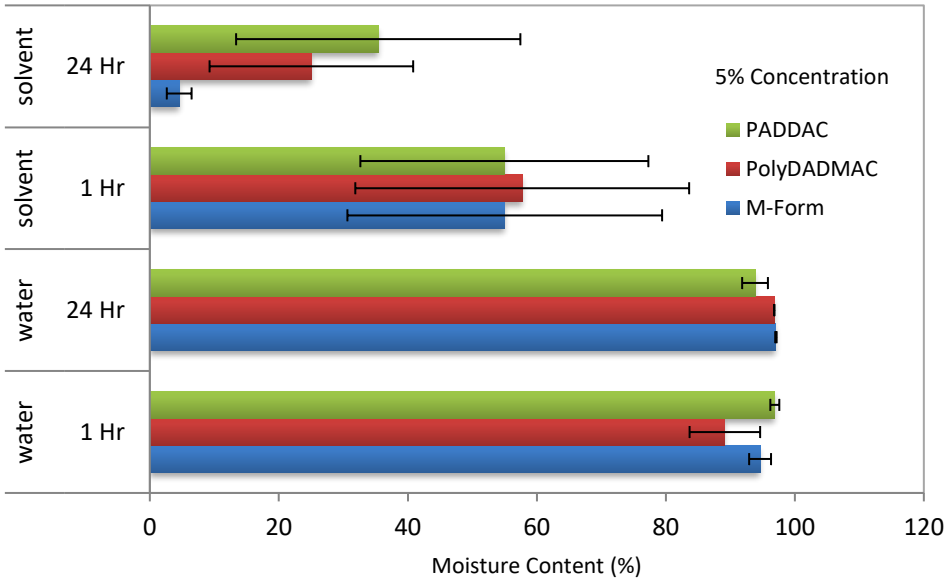
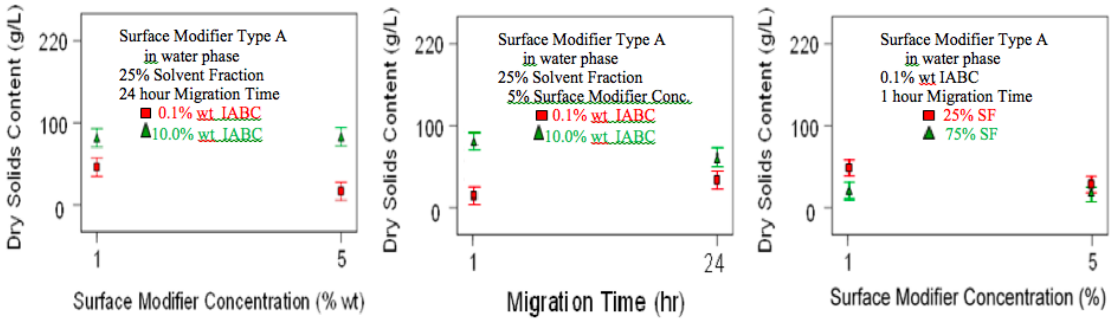


Figure 3.1.6: Moisture content analysis for 75% Solvent Phase Fraction and 10.0% IABC with 5% Concentration

3.2. Dry Solids Content Analysis

To account for all biomass in the system, an analysis of the algae biomass reported in grams per liter of dry solids content in both the water phase and solvent phase was recorded. Dry solids content (DSC) refers to the quantity of soluble and insoluble algal biomass measured after drying at 105^{o}C per unit volume of medium. The dry solids content levels for all samples from the aqueous phase ranged from 0.0 to 94.0 g/L. While the dry solids content levels of the solvent phase spanned from 0.0 to 452.0 g/L for all solvent fraction samples. The affect of using 1% (w/w) of FL-5228 surface modifier type A in the SPAM process resulted in a significantly higher quantity of dry solids content for initial algal broth concentration 10.0% compared to the 0.1% measurements of the water phase (Figure 3.2.1).

After 24 hours migration time, the 1% concentration of surface modifier type A performed about the same as 5% concentration of surface modifier type A in its ability to remove algae biomass from the water phase for high initial biomass (10.0% IABC) experiments at 25% solvent fraction. However, for the same migration time, the 4% increase in concentration of surface modifier type A achieved significantly better results for removing algae biomass in terms of dry solids content (DSC) in the water phase than the 1% surface modifier concentration with surface modifier type A for low initial biomass (0.1% IABC) experiments at 25% solvent fraction (Figure 3.2.1.A). This result suggests that SPAM technology performs more efficiently with a lower initial biomass concentration per unit of surface modifier type A. Significant differences in dry solids content between the low and high levels of initial algal broth concentrations occurred at 25% solvent fraction in combination with the 5% surface modifier concentration for both the one hour migration time and 24 hour migration time (Figure 3.2.1.B). Another significant difference in dry solids content was detected between the 25% and 75% solvent fractions for the 1% surface modifier concentration experiments. No change was detected for 5% surface modifier concentration between the two solvent fractions for 1 hour migration time and low IABC (Figure 3.2.1.C).



(A) (B) (C)

Figure 3.2.1. Dry solids content of the aqueous phase: (A)-surface modifier concentration comparison for IABC after 1 hour migration time, (B)-migration time comparison for IABC, (C)-surface modifier concentration comparison for solvent fraction.

There was no significant difference in algal mass levels of the water phase (0.00 to 1949.25 mg) compared to dry solid content levels of the solvent phase (0.00 to 10170.0 mg) overall. Both levels of the migration time factor in combination with 10.0% initial algal concentration and 1% surface modification exhibited the highest retention of algal mass in the water phase for 25% solvent fraction. The lowest level of algal mass in the aqueous phase was achieved by 5% concentration of PDADMAC surface modifier type B occurring at 0.1% initial algal broth concentration (Figure 3.2.2).

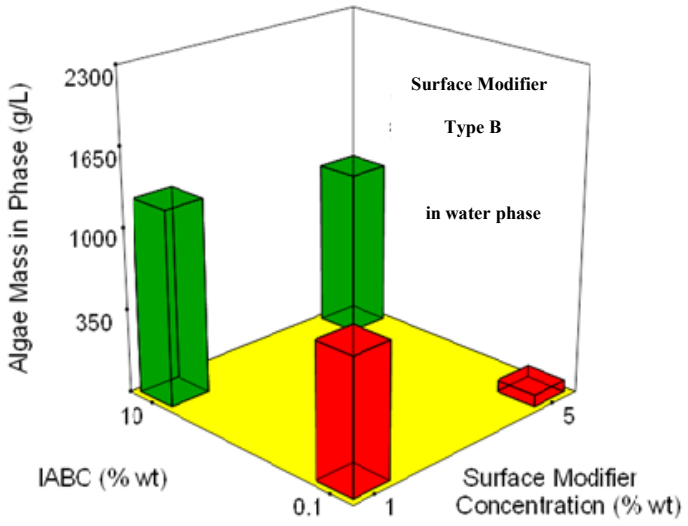


Figure 3.2.2 Aqueous phase quantity of algal mass (initial concentration vs. surface modifier concentration).

After 24 hours of migration time, 25% solvent fraction exhibited significantly higher quantities of algal mass than 75% solvent fraction using 1% surface modifier in the water phase in the environment of initial algal broth concentration at 0.1% (Figure 3.2.3).

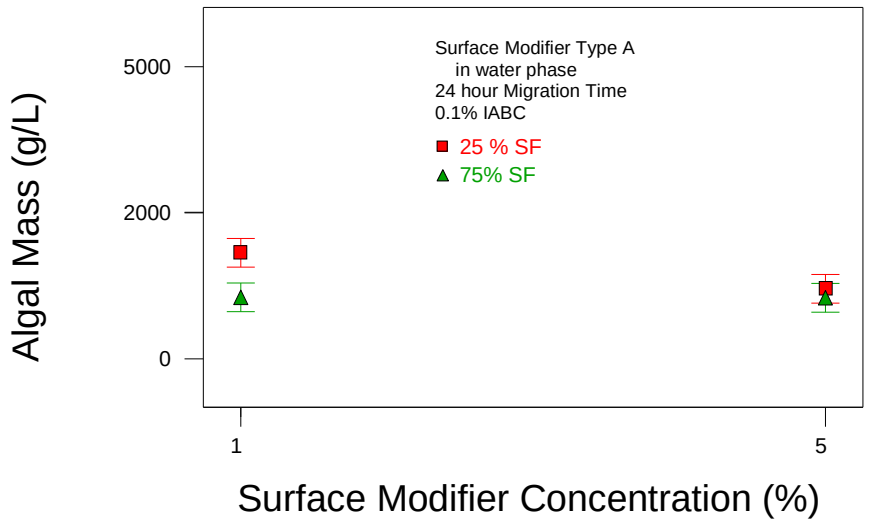


Figure 3.2.3. Aqueous phase quantity of algal mass for 24 hour migration time and 0.1% initial ABC (solvent fraction vs. surface modifier concentration).

3.3. Distribution of Dry Solids Content Analysis

The calculation for the distribution of dry solids content was performed on data generated from the measurements of dried algal biomass. These distributions ranged in value from 0.0% to 100%. There were significant differences in distribution of dry solids content remaining in the water phase compared to the distribution of dry solids content detected in the solvent phase for several combinations of factors. The largest differences in distribution of DSC for the SPAM system occurred between the 25% solvent fraction and the 75% solvent fraction under the conditions of 24 hour migration with surface modifier type B using IABC of 10.0%. Within this combination of factors, an additional difference was detected between 1% and 5 % concentration of surface modifier B. There were significant differences for IABC after 1 hour migration and even greater differences after 24 hours of migration time for the treatment combination

of 75% solvent fraction and 5% of surface modifier concentration for both surface modifier type A and type C as determined from ANOVA analysis ($p < 0.05$).

A specific case analyzing the difference in the distribution of dry solids content for different types of surface modifiers is graphically summarized in Figure 3.3.

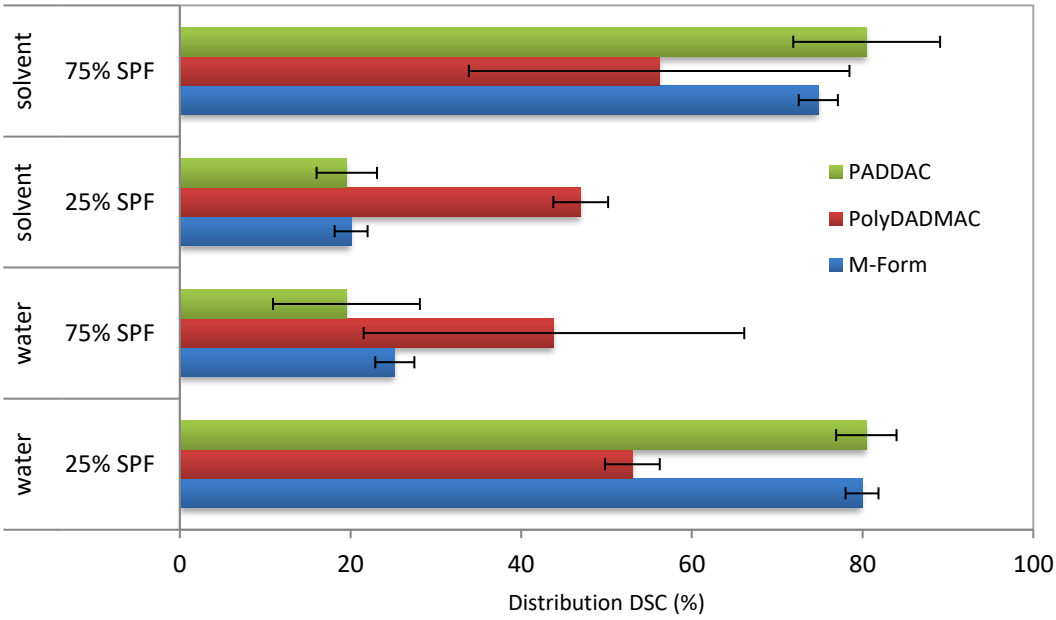


Figure 3.3. Affect of 5% surface modifier concentration for 10% IABC after 24 hours MT.

4. CONCLUSIONS

Five parameters associated with the solvent phase algal migration (SPAM) dewatering/harvesting process were investigated in this research for their ability to influence algal migration from aquous phase to solvent phase. It was clear from the studies that all the surface modifiers positively contributed to SPAM. However, from the assessment of this data, algal cell migration does not have a unique set of combined parameters that produce the best results across all response categories. The combination of settings to achieve the best SPAM results is a function of the specific type of response desired and do not necessarily correlate with each other. For instance, a given surface modifier type and concentration producing a low algal mass level or moisture content in the water phase may not necessarily correspond to high dry solids concentration levels in the solvent phase. In further study, it may be useful to develop optimization models for each response variable to describe the interaction of the system in more detail. The conclusions of this study are categorized in terms of the four response variables.

The type of surface modifier had the most significant influence on moisture content in the water phase followed by IABC. Surface modifier PolyDADMAC demonstrated the best SPAM performance.

Dry solids content is mainly influenced by the initial algal broth concentration. More specifically, the 10.0% initial algal broth concentration provides a higher quantity of dry solids content per unit volume of sample than the 0.1% initial algal broth concentration. The higher quantity of dry solids at 10.0% may correspond to an

increased algal cell to cell proximity and thus interaction with the polymer(s) resulting in greater SPAM performance.

The solvent fraction proved to be the most dominant factor in determining the amount of algal biomass in each phase. However, it is important to note that even with combination of the other significant factor, initial algal broth concentration, these factors only contribute less than 17% of the total variability in determining values for algal mass in both aqueous and solvent phases.

This analysis of the distribution of dry solids content proposes that the individual factors: surface modifier concentration, solvent phase, migration time, and initial algal broth concentration all do not significantly play a role on the dry solids content. The influence of surface modifier type combined the interaction effects of surface modifier concentration work together to represent 47% of the variability of dry solids content. The results of the four responses provide evidence that SPAM is effective as an alternative microalgae recovery process suitable for biofuel based production systems.

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