A Thesis

entitled

Harvesting Microalgae-Development of a Short Residence Time Method Using Rapid-
response Temperature-sensitive Semi-IPN Hydrogels

by

Xiaofei Zhao

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in

Chemical Engineering

_________________________________________
Dr. Sridhar Vamajala, Committee Chair

_________________________________________
Dr. Sasidhar Varanasi, Committee Member

_________________________________________
Dr. Constance Schall, Committee Member

_________________________________________
Dr. Patricia R. Komuniecki, Dean
College of Graduate Studies

The University of Toledo

August 2015
An Abstract of

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Microalgae cultures obtained from cultivation in open ponds are usually at low concentrations (0.5-1g/L). Direct application of conventional solid-liquid separation processes (such as filtration, centrifugation or chemical flocculation) to such dilute cultures could be either prohibitively expensive for biofuel production or may compromise biomass quality and impact downstream conversion. During this study, the research team used fast-response and high-capacity temperature-sensitive hydrogels for dewatering dilute algal slurries. These gels had semi-interpenetrating network structures consisting of polyvinyl alcohol (PVA) intercalated into poly N-isopropylacrylamide chains (PNIPAAm-PVA semi-IPN). Further, these stimulus-sensitive gels collapse and release the absorbed medium at relatively low temperature (>32°C), which allows recovery (and reuse) of the hydrogels using low-quality waste heat.

In this study, we first compared the swelling/deswelling kinetics and mechanical properties of semi-IPN hydrogels with more conventional PNIPAAm hydrogels. Our results showed that semi-IPN10 gels (containing 10% PVA and 90% PNIPAAm) exhibited rapid swelling and deswelling kinetics with rates that were nearly two-fold
higher than PNIPAAm gels. Further, compression-stress measurements and rheological studies showed that the semi-IPN gels had greater elasticity and compression resistance than PNIPAAm. As such, the PNIPAAm-PVA semi-IPN hydrogels are expected to be less susceptible to breakage during the dewatering process. Subsequently, we demonstrated the feasibility of using semi-IPN hydrogels for harvesting microalgae by stagewise exposure of deswollen hydrogels to dilute microalgae cultures. We observed that the semi-IPN hydrogels rapidly uptake the aqueous medium and exclude microalgae cells, which results in an increase in the concentration of the residual culture. Through use of high gel loadings, we were able to increase the concentrations of cultures from nearly 1g/L to >10g/L in 3 stages over a net residence time less than 1.5h. Finally, our studies demonstrated that the concentrated cultures remained viable and photosynthetically-active; the recovered aqueous medium was also able to sustain re-growth of cultures.
Acknowledgements

I would like to express my appreciation to my advisory committee: Dr. Sridhar Viamajala, Dr. Sasidhar Varanasi, and Dr. Constance Schall. A special thanks to Dr. Viamajala for his guidance, support and understanding throughout this research study and my graduate education. Thanks for giving me the opportunity to be part of the algal biofuel research. Dr. Varanasi, thanks for sharing many ideas and knowledge regarding this project. Also, my gratitude goes to Dr. Schall for her kind guidance throughout my graduate study endeavors.

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The most special gratitude goes to my Lord, my family, and my friends, who give me consistent, unconditional support and love. Last, but certainly not least, I thank the members of the microalgal biofuel research group for their help and friendship. Agasteswar, thanks for sharing many experience and knowledge regarding the gel dewatering research.
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List of Abbreviations

APS .........................Ammonium persulfate
BIS ..........................N, N’-methylenebisacrylamide
Meta-BIS ....................Sodium meta-bisulfite
NIPA ..........................N-isopropylacrylamide
PNIPAAm ....................Poly (N-isopropylacrylamide) hydrogel
PVA ..........................Polyvinyl alcohol
Semi-IPN .....................Semi-interpenetrating network
TSS ..........................Total suspended solids
Chapter 1

Overview: Microalgal Biomass Harvesting and Temperature-Sensitive Hydrogels

1.1 Introduction

Petroleum, coal, and natural gas sourced fuels are now widely recognized as the dominant part of the world energy consumption, which is predicted to increase from $250 \times 10^{18}$ J/yr in 2000 to $950 \times 10^{18}$ J/yr by 2050 (Fridleifsson 2003). However, the depleting fossil fuel supplies and the environmental issues, such as the high carbon emissions caused by the consumption of fossil fuels, impel people to look for the substitutes of fossil fuels.

Unlike other alternative energy resources, biomass is considered advantageous since it can be converted directly to liquid fuels for helping to fulfill the transportation fuel demand. Biofuels produced from various biomass feedstock such as lignocellulose, starch-rich grains (e.g. corn), oil seeds (e.g. soybean and camelina), have become the major feedstock choices. However, the cultivation of terrestrial energy-containing crops may potentially change the land usage patterns, which can result in an increase of carbon emission and further shortage of arable land. In addition, it has been estimated that the lignocellulose-based biofuels, which are produced from agricultural waste and forest-
based sources, can only replace up to 30% of US fuel demand (USDOE 2005). Thus, in general, biofuels obtained from the terrestrial feedstock alone may not meet the growing fuel demand.

*Table 1.1* Comparison of oil yields from various oil crops and microalgae

<table>
<thead>
<tr>
<th>Crops</th>
<th>Oil Yield (Gallons/Acre/Year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td>48</td>
</tr>
<tr>
<td>Camelina</td>
<td>62</td>
</tr>
<tr>
<td>Sunflower</td>
<td>102</td>
</tr>
<tr>
<td>Jatropha</td>
<td>202</td>
</tr>
<tr>
<td>Oil Palm</td>
<td>636</td>
</tr>
<tr>
<td>Algae</td>
<td>1000-6500*</td>
</tr>
</tbody>
</table>

*Estimated yields

Microalgae-based biofuel could be additional renewable alternatives to fossils fuels due to several advantageous characteristics inherent to this feedstock (Chisti 2007, Davis, Aden et al. 2011). First, microalgae cells capture solar energy with higher efficiency than terrestrial plants, which allows the rapid growth. Second, year-round cultivation and harvesting are possible with microalgae which are aquatic phototrophs that do not depend on land quality. Additionally, microalgae can be cultivated in non-arable land with a variety of water sources, including waste water and brackish water, at a wide range of salinities and compositions (Barros, Gonçalves et al. 2015). Thirdly, some types of microalgae have high oil content (up to 40%) and can yield more oil per land acre (10 to 100 times greater) compared to other terrestrial oil crops (USDOE 2010). Table 1.1 lists the estimated oil yields of microalgae, compared with other oil crops.
Finally, residues of microalgal biomass, which contain carbohydrates and proteins, can be converted to multiple products by fermentation or thermochemical reactions (e.g. bioethanol, biomethane, acetone, biogas, bio-oil) or used as an additive in animal feed (Harun, Singh et al. 2010, Yanik, Stahl et al. 2013). Table 1.2 lists the opportunities and challenges for microalgae biorefineries.

**Table 1.2 Opportunities and challenges for algal biorefinery (USDOE 2010)**

<table>
<thead>
<tr>
<th>Opportunities</th>
<th>Challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>High area biomass productivity;</td>
<td>High water content in the microalgae culture;</td>
</tr>
<tr>
<td>Minimized competition with conventional agriculture;</td>
<td>Hurdles in biomass harvesting and other downstream processing.</td>
</tr>
<tr>
<td>Utilizes a wide variety of water sources;</td>
<td></td>
</tr>
<tr>
<td>Recycle carbon from CO₂-rich flue emissions from stationary sources, including power plants and other industrial emitters;</td>
<td></td>
</tr>
<tr>
<td>Compatible with integrated production of fuels and co-products within biorefineries.</td>
<td></td>
</tr>
</tbody>
</table>

Although several previous studies have illustrated that microalgae has definite advantages over conventional oil crops, cost-effective harvesting of microalgal biomass remains as a major barrier for commercial production of microalgae-based biofuels (Molina Grima, Belarbi et al. 2003, Greenwell, Laurens et al. 2010, Mata, Martins et al. 2010, Uduman, Qi et al. 2010, Amer, Adhikari et al. 2011). The difficulties of harvesting
microalgae are primarily brought about by the dilute nature of algal cultures (microalgal cultures in open ponds have relatively low concentrations of only around 0.5g/L to 1.0g/L), which results in high process volumes for a large scale production systems (Uduman, Qi et al. 2010, Vandamme, Foubert et al. 2013). In addition, microalgal cells form stable suspension with a low propensity for natural flocculation and sedimentation due to their small size (3-30µm), negatively charged surfaces and neutral buoyancy in growth medium (Shelef, Sukenik et al. 1984, Molina Grima, Belarbi et al. 2003).

Even though conventional techniques, including flocculation, centrifugation, filtration, sedimentation, and flotation can be applied to microalgae harvesting, they suffer from some economic or downstream processing-related drawbacks (Milledge and Heaven 2013). Innovative and cost effective harvesting methods are required for commercial viability of microalgae-based biofuels (Uduman, Qi et al. 2010).

In contrast to the conventional methods, we have recently developed an alternative harvesting method using temperature-sensitive poly-(N-isopropylacrylamide) hydrogels (PNIPAAm) (Vadlamani, Zhao et al. 2014). In this research, the hydrogel harvesting method was improved through use of poly (N-isopropylacrylamide)/polyvinyl alcohol (PNIPAAm/PVA) hydrogels with a semi-interpenetrating (semi-IPN) network. The semi-IPN gels showed improved swelling kinetics and better mechanical strength than PNIPAAm gels.

1.2 Microalgal Biofuel Refinery

Generally, a microalgal refinery contains three processing steps: (i) microalgae cultivation, (ii) harvesting and dewatering/drying, and (iii) biomass conversion. A block flow diagram of a microalgal biofuel refinery is shown in Figure 1-1.
Figure 1-1 Block flow diagram of an algal biorefinery

Microalgae can be cultivated under phototrophic, heterotrophic or mixotrophic growth conditions. Phototrophic cultivation requires light (e.g. sunlight), inorganic nutrient salts and CO₂ (e.g. from air or flue gases). Cultivation can be carried out in photobioreactors or raceway ponds that allow microalgal cells to grow through capture of light and fix CO₂ into sugars and lipids. In heterotrophic cultivation, no light is provided; instead cultures are grown using added carbon sources such as glucose (USDOE 2010). In this research, *Chlorella sp.* was cultivated under photoautotrophic conditions at alkaline pH (>8.5) and with air sparging provided. A photograph of our laboratory-scale cultivation reactor is shown in Figure 1-2.

Before oil extraction or biomass conversion processes, most of the water present in cultures must be removed. This process is named the biomass dewatering, which is basically a solid-liquid separation process. Algal cultures are usually dilute (0.5g/L – 1.5g/L), hence large volume of culture needs to be processed for biomass recovery. The small size of the algae cells (usually 3-30µm diameter) may further increase the difficulties for the dewatering process (Molina Grima, Belarbi et al. 2003).

Unfortunately, conventional harvesting methods, such as centrifugation, flocculation, and
filtration, are considered either cost-intensive or infeasible. A dehydration process, such as drying, is usually required after dewatering for those conversion processes that are sensitive to moisture in biomass.

![Microalgae growing in a photobioreactor](image)

**Figure 1-2** Microalgae growing in a photobioreactor

The dehydrated biomass can be converted to multiple products either by physical or chemical pathways after being harvested. For microalgae with high oil content, two unique approaches have recently been developed in our research group to recover the lipids from the cells - (a) reactive extraction (Viamajala, Nelson et al. 2011) and (b) pyrolytic fractionation (Maddi, Viamajala et al. 2015). In (a), microalgal lipid are recovered through in-situ transesterification as biodiesel or fatty acid methyl esters (FAME). Microalgal biomass residues collected after the oil extraction, rich in carbohydrates/polysaccharides and protein, could potentially be converted to other
products. They can also be converted to bio-oil, bio-gas, and char through thermochemical reactions.

1.3 Overview of Microalgal Biomass Harvesting

Microalgae harvesting is considered as one of the major hurdles for cost-effective microalgae-based biofuel production process. It is widely believed that harvesting would incur a large fraction of overall operating and equipment costs of microalgal biomass production (Molina Grima, Belarbi et al. 2003, Mata, Martins et al. 2010, Amer, Adhikari et al. 2011). The algal suspension cultivated in open ponds is usually very dilute (0.5g/L to 1.5g/L), which results in a very high volume of dilute culture that is required to be processed in a large scale production system. Furthermore, a stable suspension can be formed by microalgae cells because of their small size (3-30 µm), the negatively charged surfaces and the neutral buoyancy in growth medium (Shelef, Sukenik et al. 1984, Molina Grima, Belarbi et al. 2003). The stable suspension formed prevents microalgal cells from auto-flocculating and sedimentation. It is reported that harvesting the algal biomass from the dilute algal culture contributes 20-30% to the total cost in algal biomass production (Gudin C 1986). Currently, several techniques have already been applied in algal biomass harvesting such as filtration, centrifugation, and flocculation.

However, conventional harvesting methods have limitations. In a harvesting process, usually more than one dewatering method is required. Desired final products, growth medium, microalgae species and production cost benefit should be all considered in selecting the dewatering methods (Shelef, Sukenik et al. 1984, Uduman, Qi et al. 2010). For instance, the low-cost regular filtration may be only applicable to harvest fairly large microalgae species. For microalgae species with small cell size such as
*Chlorella sp.*, chemical flocculation should be introduced prior to further mechanical separation such as centrifugation, filtration or gravity sedimentation. Several conventional harvesting methods which have been currently used in microalgae harvesting are reviewed as following.

**Chemical flocculation**

Due to the small size of microalgal cells, chemical flocculation, which can induce and expedite the aggregation of microalgal particles, may be required prior to any mechanical separation step in microalgae harvesting. Flocculants, which are inorganic or organic salts or polymers carrying cations, should be added to neutralize or reduce the negative charges on algae cell surfaces. The negative charges on cell surface prevent cell aggregation and help algae cells maintain dispersed status in microalgal suspensions (Molina Grima, Belarbi et al. 2003). However, flocculants added into the algal culture may potentially contaminate the biomass product and make the post-harvesting recovered medium difficult to be reused.

**Centrifugation**

Centrifugation is currently regarded as a rapid and effective way to concentrate microalgal biomass, however, it is generally considered unviable due to excess capital and operating costs involved in processing large volume of cultures. In addition, cell structure may be disrupted under high shear forces in a centrifugation process (Mohn 1988, Harun, Singh et al. 2010).

**Filtration**

Filtration with pressure or vacuum provided is also a common method for water removal. It requires a permeable membrane and a relatively high pressure drop
maintained across the membrane. The permeable membrane retains the microalgal cells and allows the aqueous medium to pass through (Uduman, Qi et al. 2010). However, regular filtration method can only be utilized for harvesting the microalgae species with large cell size such as *Coelastrum proboscideum* and *Spirulina platensis*, but may be fail to concentrate small size microalgae species, which have bacterial dimensions such as *Chlorella* and *Dunaliella*, unless they have been flocculated in advance (Molina Grima, Belarbi et al. 2003). Even though tangential flow filtration, microfiltration and ultrafiltration may be used for harvesting microalgae species with smaller size, they are reported having a high capital and energy cost which is caused by (a) complex construction of the filtration device and affiliates; (b) extreme working conditions – high pressure or vacuum; (c) continuous membrane clogging and subsequent frequent membrane replacements. For an industrial-scale microalgae harvesting process, crossflow microfiltration is reported to be even more cost-intensive than centrifugation (MacKay and Salusbury 1988).

We have recently developed an alternative harvesting method using temperature-sensitive hydrogels (Vadlamani, Zhao et al. 2014). Temperature-sensitive hydrogels such as cross-linked poly-(N-isopropylacrylamide) hydrogels (PNIPAAm) exhibit a sharp temperature-dependent volume change. Thus, these polymers are able to absorb water/medium at ambient conditions and release it when temperature is increased above lower critical solution temperature (LCST) (32-34°C) (Freitas and Cussler 1987, Zhang, Bhat et al. 2009). When microalgal culture are incubated in the presence of temperature-sensitive gels at a temperature below LCST, culture medium is absorbed while cells are excluded resulting in an increase in algal cell concentration. Subsequently, the swollen
gels could be recovered and reused for several cycles. The relatively low cost of the hydrogels and energy required to recover the hydrogels could make the gel harvesting process more cost-competitive relative to other conventional harvesting methods.

1.4 Temperature-sensitive Hydrogels

1.4.1 Hydrogels – Water Absorbent Polymers

Hydrogel is a hydrophilic two-phase polymer network that can absorb and hold a large volume of water (Kim and Park 2002). Hydrogel network can be stably formed because cross-linking reactions happen under the influence of covalent bonds, hydrogen bonding, van der Waals interaction, physical entanglements or hydrophobic interactions, after the “crosslinker” is introduced into linear monomer chains (Kamath and Park 1993). When dehydrated hydrogels (simplified as “gels”) contact with aqueous solution, water molecules diffuse into the gel network because hydrophilic polymer chains of the hydrogel try to “dissolve” in aqueous phase (Kim and Park 2002). After absorbing water, hydrogels swell and become enlarged. An equilibrium swelling status can be achieved when the gel is unable to absorb any additional water. At equilibrium, the rates of diffusion of water molecules into the network equals the rate of diffusion out.

Hydrogels with relatively fast swelling rate and large water retention capacity are called super water-absorbent polymers (SAPs). SAPs usually have the ability to absorb large amount of water at 200-700 times their original weight or volume (Omidian and Park 2010). Swelling time of hydrogels can vary from a fraction of a minute to several hours depends on the gel components, gel size, surface area and environmental conditions such as temperature and pH value.
Hydrogels have a large variety of applications in agriculture, waste water treatment, pharmacy and biomedical/biotechnological areas, such as soil water retention, low rate irrigation for plants, drug delivery, biopolymer, and cell separation.

1.4.2 Environment-responsive Hydrogels

In some types of hydrogels, water may be released upon being stimulated by changes in some environmental parameters such as temperature, aqueous solution pH, light intensity, electric field or pressure change (Kim and Park 2002). Such hydrogels are termed “environment-responsive” or “stimulus-responsive” hydrogels. Compared to environmental parameters such as solution pH, electrical field and light intensity, temperature is the most common and easily-controlling parameter, which has been widely used to stimulate hydrogels. In addition, temperature can be easily monitored and controlled by straightforward industrial heating and cooling techniques without adding impurities into the culture. If the swelling kinetics can be changed or controlled by temperature, this type of hydrogel is called a temperature-sensitive hydrogel. For example, poly (N-isopropylacrylamide) hydrogels (PNIPAAm) can be switched from being hydrophilic to hydrophobic when the environment temperature is raised above 34°C. The change of swelling kinetics is usually rapid and occurs over a few minutes.

In contrast to the environment-responsive gels, a few types of hydrogels used for agriculture can also release water due to evaporation. However, those gels are not considered as environment-sensitive gels since water release takes several days. Table 1.3 lists some of the environmental factors which may stimulate the sharp response of hydrogels. From Table 1.3, we can see that most of environment-responsive hydrogels
are utilized in biomedical or bio-separation areas, such as drug delivery and bio-particles selective separation.

Table 1.3 Environmental factors that can control the response of hydrogels (Kim and Park 2002)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Applications</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperatures</td>
<td>Drug delivery</td>
<td>(Bae et al., 1991; Chun and Kim, 1996; Dong and Hoffman, 1990)</td>
</tr>
<tr>
<td></td>
<td>Separation</td>
<td>(Huang et al, 1988; Freitas and Cussler, 1987)</td>
</tr>
<tr>
<td></td>
<td>Bioreaction</td>
<td>(Park and Hoffman, 1993)</td>
</tr>
<tr>
<td></td>
<td>Shape memory</td>
<td>(Hu et al., 1995)</td>
</tr>
<tr>
<td></td>
<td>Enzyme immobilization</td>
<td>(Shiroya et al., 1995)</td>
</tr>
<tr>
<td>pH</td>
<td>Drug delivery</td>
<td>(Bala and Vasudevan, 1982)</td>
</tr>
<tr>
<td>Electric field</td>
<td>Drug delivery</td>
<td>(Kwon et al., 1991; Sawahata, 1990)</td>
</tr>
<tr>
<td>Ions</td>
<td></td>
<td>(Park and Hoffman, 1993)</td>
</tr>
<tr>
<td>Solvents</td>
<td></td>
<td>(Tanaka, 1981)</td>
</tr>
<tr>
<td>Light</td>
<td></td>
<td>(Mamada et al., 1990; Suzuki et al., 1996)</td>
</tr>
<tr>
<td>Pressure</td>
<td></td>
<td>(Lee et al., 1990; Zhong et al., 1996)</td>
</tr>
<tr>
<td>Specific Molecules</td>
<td>Drug delivery</td>
<td>(Kokufata et al., 1991)</td>
</tr>
</tbody>
</table>

1.4.3 PNIPAAm and PNIPAAm-PVA Temperature-sensitive Hydrogels

Temperature-sensitive hydrogels such as cross-linked poly-(N-isopropylacrylamide) hydrogels (PNIPAAm) exhibit a sharp temperature-dependent volume change. Thus, these polymers are able to absorb water/medium at ambient
conditions and release it when temperature is increased above a certain temperature, which is defined as the lower critical solution temperature (LCST) (Zhang, Bhat et al. 2009). LCST is an inherent property of a temperature-sensitive hydrogel. For the gel dewatering process, PNIPAAm gels were chosen because their LCST is only around 32-34°C, which is close to ambient temperature. First, it is easy to raise the gel temperature above the LCST. Waste heat sources such as flue gas could be used. Then, relatively low LCST close to ambient temperature would not affect the quality of the growth medium. However, PNIPAAm gels showed slow swelling rates due to their limited hydrophilicity. In addition, the deswelling rate was also restricted because the PNIPAAm gel can form a dense, thick skin layer during deswelling, which prevents water molecules migrating from the polymer (Zhang, Cheng et al. 2003). Improvements in swelling/deswelling kinetics would reduce the harvest-process residence time and also lower holdup volumes in the process.

In this research, poly (N-isopropylacrylamide)-polyvinyl alcohol hydrogel (PNIPAAm-PVA) with a semi-interpenetrating network structure (semi-IPN) was developed as the replacement of PNIPAAm gels. PNIPAAm-PVA semi-IPN hydrogels maintain the good thermal sensitive property of PNIPAAm gels, which means they have the same low LCST that may result in a low recovery cost (Zhang, Wu et al. 2004). Moreover, polyvinyl alcohol (PVA) interlaced in the semi-IPN can increase the hydrophilicity of polymer network which results in the enhancement of swelling kinetics. In addition, the interpenetration of two polymer networks can also increase the mechanical strength of the polymer networks. It is reported that the semi-IPN gels may have better malleability and toughness compared to PNIPAAm hydrogels (Zhang, Cheng
et al. 2003). The loss of hydrogels can be reduced due to the higher mechanical strength, which may keep the gels have a long lifespan. Thus, the cost of hydrogels can be reduced since the gels used do not need frequent replacements. Finally, the microalgal cells will not move into the semi-IPN structure during the dewatering process, which makes the hydrogels capable of being recovered and reused.

*Figure 1-3* The synthesized swollen PNIPAAm-PVA semi-IPN hydrogel

*Figure 1-4* Dehydrated PNIPAAm-PVA semi-IPN gel pieces
Deswelling started when transferring the swollen gel pieces into warm water (40°C).

Compared to conventional PNIPAAm hydrogel, PNIPAAm/PVA hydrogel has a unique semi-interpenetrating polymer network (semi-IPN) structure, which is formed by PNIPAAm network and linear PVA molecules. A semi-interpenetrating polymer network (semi-IPN) is composed of one cross-linked polymer and one polymer in linear form,
which are interlaced on a molecular scale, however, there is no chemical bond between them and they cannot be separated under the influence of chemical bonds (Zhang, Bhat et al. 2009).

1.5 Design of a Gel Dewatering Process

In this research, deswelled hydrogels were first incubated with dilute algae solutions. Algae concentration in the slurry increased due to preferential uptake of water by the gels (marked as “swelling conditions”). The biomass harvesting was achieved by letting the concentrated algae culture pass through a coarse sieve. Swollen gels were then heated to 40°C. This resulted in gel collapse and release of absorbed growth media (marked as “collapsing conditions”). The shrunken gels were recovered on a sieve and recovered growth medium was recycled back to the algal growth reactors. Semi-IPN hydrogel can be recovered and reused for many cycles. Figure 1-7 shows the conceptual process of gel dewatering.

1.6 Summary

In this research, PNIPAAm-PVA hydrogel with semi-interpenetrating network was successfully synthesized and utilized in biomass dewatering process. In contrast to the conventional harvesting methods, the gel dewatering process can potentially become a cost-effective pathway to harvest the large volume of microalgal culture. Compared to conventional PNIPAAm, PNIPAAm/PVA hydrogels showed better swelling kinetics and higher mechanical strength. In sum, PNIPAAm-PVA semi-IPN gels can be selected as the absorbent in the gel harvesting process, which may potentially make the harvesting process more economic and efficient.
Figure 1-7 Design of a gel dewatering process
Chapter 2

Hydrogel Characterization and Harvesting Process

2.1 Introduction

Microalgae-based biofuels could be a renewable alternative to fossils fuels due to several advantages of this feedstock (Davis et al. 2011; Chisti 2007). First, microalgae cells capture solar energy with higher efficiency than terrestrial plants that allows rapid growth of these phototrophs. Second, microalgae can be cultivated in non-arable land with a variety of water sources, including wastewater and brackish water, at a wide range of salinities and compositions (Barros et al. 2015). Third, some species of microalgae cultivation can yield significantly more oil per land acre (10 to 100 times greater) compared to other terrestrial oil crops (USDOE, 2010). Finally, residues of microalgal biomass (after oil extraction) which contain carbohydrates and proteins, can be converted to other fuels or co-products by fermentation or thermochemical reactions (e.g. bioethanol, biomethane, acetone, biogas, bio-oil) or used as an additive in animal feed (Harun et al. 2010; Yanik et al. 2013).

Although several previous studies have illustrated that microalgae has definite advantages over conventional oil crops, cost-effective harvesting of microalgal biomass
from dilute cultures remains as a major barrier towards commercialization of microalgae-based biofuels (Uduman et al. 2010; Mata et al, 2010; Molina Grima et al. 2003; Amer et al. 2011; Greenwell et al. 2010). The difficulties of harvesting microalgae are primarily due to the dilute nature of microalgal cultures (microalgal cultures in open ponds have relatively low concentration only around 0.5-1.0g/L), which results in a very high throughput volume for harvesting systems (Uduman et al. 2010; Vandamme et al. 2013). Straightforward natural sedimentation methods for recovery are generally not possible since microalgal cells are small (3-30µm) and form stable suspensions due to their negatively charged surfaces and neutral buoyancy (Reynolds et al. 1984; Molina Grima et al. 2003). Other conventional solid-liquid separation methods including chemical flocculation (with settling or flotation), centrifugation and filtration are technically feasible but unviable due to high costs and/or negative impacts on biomass quality (Milledge et al. 2013). Centrifugation is generally considered unviable due to excess costs (capital and operating) involved in processing large volume of cultures and potential for cell disruption under high shear (Mohn et al. 1988; Harun et al. 2010). Tangential flow microfiltration is also likely to suffer from high costs due to frequent membrane clogging and subsequent membrane replacements (Uduman et al. 2010; Barros et al. 2015). Chemical flocculation can be used for improving separation efficiency by gravity sedimentation or dissolved air flotation. However, the excess cationic flocculants needed to induce and expedite cell aggregation in dilute cultures could significantly contaminate the biomass product and negatively impact downstream catalytic conversion methods. Further, presence of soluble flocculants in the post-harvest recovered medium could preclude the reuse of water and unutilized nutrients (Harith et
Therefore, in order to increase the competitiveness of microalgal biofuels, economically viable harvesting methods that also preserve the quality of biomass as well as recovered media, must be developed.

We have recently developed a microalgae harvesting technique that uses temperature-sensitive hydrogels that has the potential to circumvent limitations posed by conventional solid-liquid separation methods (Vadlamani et al. 2014). In general, hydrogels are hydrophilic two-phase polymer networks that absorb large amounts of water (Kim and Park 2002). Specifically, temperature-sensitive hydrogels, such as cross-linked poly-(N-isopropylacrylamide) hydrogels (PNIPAAm), absorb aqueous solutions at ambient conditions, but are able to release the absorbed liquids when their temperature is increased above a “lower critical solution temperature” (LCST; 32-34°C for PNIPAAm) (Zhang et al. 2009). We have observed that when microalgal cultures are incubated with PNIPAAm at a temperature below LCST, culture medium is absorbed into the gels while cells are unable to penetrate the small-sized surface pore of these materials and remain excluded (Vadlamani et al. 2014). As a result of the preferential uptake of the aqueous phase, algal cell concentration increases in the un-absorbed medium. Subsequently, the swollen gels can be recovered and “deswelled” by increasing the temperature above LCST to release the media. The deswelled hydrogels can then be reused to absorb more medium. Thus, a cyclic process can be implemented that allows repeated use of gel over multiple cycles. Due to recovery and reuse of the hydrogels, a relatively small amount of gel material is ultimately needed to absorb (or process) large amounts of media.

In our previous study, stagewise implementation of the hydrogel dewatering method with PNIPAAm hydrogels allowed an increase in concentration from
approximately 1g/L to 100 g/L over 8 stages with a 2h duration per stage (Vadlamani et al. 2014). While high final concentrations were achieved, the process time remained lengthy due to (1) the long residence time per stage due to slow swelling kinetics of the hydrogels and (2) the large number of stages due to the low mass of absorbent hydrogels used in each stage. The slow swelling rates of PNIPAAm gels could be due to the limited hydrophilicity of these polymer networks.

In this research, the more hydrophilic poly (N-isopropylacrylamide)-polyvinyl alcohol hydrogel (PNIPAAm-PVA) with a semi-interpenetrating network (semi-IPN) structure was used to improve upon the swelling kinetics of the PNIPAAm hydrogel. While the presence of PVA does not change the LCST and thermal responsive properties of the underlying PNIPAAm, the semi-IPN is expected to display faster swelling kinetics relative to PNIPAAm due to increased hydrophilicity from the presence of PVA interlaced in the hydrogel structure (Zhang et al. 2004, Zhang et al. 2003). In addition, the interpenetration of two polymer networks can also increase the mechanical strength of the polymer networks and improve the longevity of hydrogels during prolonged reuse. Previous studies have measured that semi-IPN gels have better malleability and toughness compared to PNIPAAm hydrogels (Zhang et al. 2002).

Several other methods have also been proposed in previous research for improving hydrogel response rates such as copolymerization of PNIPAAm with comonomers (Zhang et al. 2004), preparing macroporous gel by phase-separation technique (Wu et al. 1992), and introducing mobile grafted hydrophilic chains (Kaneko et al. 1998). However, hydrogels prepared by these other techniques were deemed less suitable for hydrogel-based algae harvesting due to potential for higher transition
temperatures (and higher energy requirements for deswelling), large pore sizes that may entrap microalgal cells or less favorable mechanical properties. For example, copolymerization technique may weaken, even eliminate the thermal sensitivity of the PNIPAAm-based hydrogels due to the introduction of the non-thermosensitive moiety into the backbone of the hydrogel polymer.

In this study, we attempted to reduce overall processing time for microalgae harvesting through use of faster-response PNIPAAm-PVA semi-IPN hydrogels. In addition, we attempted to reduce the total number of stages by employing a higher ratio of “gel to culture volume”. Finally, we characterized the mechanical properties of the semi-IPN hydrogels that could be relevant to the process conditions encountered during the hydrogel-based harvesting process.

2.2 Experiments and Materials

2.2.1 Materials

Polyvinyl alcohol with degree of polymerization (Dp) of 1750±50 was purchased from TCI.CO (Japan). Reagent grade N-isopropylacrylamide (NIPA) and N, N’-methylene-bis-acrylamide (BIS) were purchased from Polysciences, Inc. (Warrington, PA, USA). Reagent grade ammonium persulfate (APS) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Reagent grade sodium meta-bisulfite (Meta-BIS) was obtained from Fisher Scientific (Fair Lawn, NJ, USA). All chemicals were used as purchased.
2.2.2 Hydrogel Synthesis and Characterization

2.2.2.1 Synthesis of Semi-IPN Hydrogels

The synthesis procedures for the PNIAPPm and the PNIPAAm-PVA semi-IPN hydrogels were similar to protocols of Frietas and Cussler (Freitas and Cussler 1987) and Zhang (Zhang, Cheng et al. 2003), with minor modifications. Polyvinyl alcohol (PVA) was first dissolved in distilled water at 70°C before use. Then, the monomer NIPA, crosslinker BIS, and prepared PVA solution were mixed and dissolved well in distilled water to prepare hydrogels with specific compositions (see Table 2.1 for detailed composition). Thereafter, pure N\textsubscript{2} was bubbled through the mixture solution for 10 min to remove dissolved O\textsubscript{2}. APS and Meta-BIS, as the initiator and the accelerator respectively, were added last to induce and expedite the crosslinking and polymerization reactions. A sealed glass cylinder was used as the polymerization reactor. All the chemicals were weighed by an analytical balance (Shimadzu AUW120D, Kyoto, Japan; max: 120g, min: 1mg; accuracy: d=0.1mg).

Polymerization reactions were carried out at room temperature for 10h. Subsequently, formed hydrogels were removed from the cylinder and washed in DI water for at least 3d, with the wash water being changed frequently to remove the unreacted chemical residues. The bulk of hydrogel was cut into small pieces with dimensions of approximately 3mm×3mm×3mm using an onion chopper (Chop Magic, Allstar Products Group, Hawthorne, NY). PNIPAAm gels were synthesized by following the similar protocol, however, without the PVA added. The composition of the hydrogels is reported based on the mass of components added during the hydrogel synthesis.
Table 2.1 Chemical components for hydrogels synthesis

<table>
<thead>
<tr>
<th>Component</th>
<th>PNIPAAm</th>
<th>Semi-IPN10 (High CD*)</th>
<th>Semi-IPN10</th>
<th>Semi-IPN20</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIPA</td>
<td>7.92</td>
<td>7.128</td>
<td>7.128</td>
<td>6.336</td>
</tr>
<tr>
<td>PVA</td>
<td>0</td>
<td>0.792</td>
<td>0.792</td>
<td>1.584</td>
</tr>
<tr>
<td>BIS</td>
<td>0.08</td>
<td>0.072</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>APS</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>Meta-BIS</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>Total volume (mL)</td>
<td>118</td>
<td>118</td>
<td>118</td>
<td>118</td>
</tr>
</tbody>
</table>

* Cross-linker density (CD).

2.2.2.2 Hydrogel Characterization

Swelling Kinetics

Water/medium uptake by hydrogels was quantified by gravimetrically monitoring swollen gel weights. Before starting the experiment, prepared hydrogel pieces were dehydrated in an oven at 40°C. 0.5g of dry gel pieces were weighed at room temperature, placed on a sheet of nylon mesh (approximately 6 cm in diameter) with a known weight and incubated in fresh growth medium (composition of the growth medium is described later). As the hydrogels swelled, the nylon mesh along with the enclosed hydrogels was periodically removed and weighed. Before weighing, the free liquid (around the hydrogel pieces) was allowed to drain by gravity and the outside of the mesh was dabbed with moist paper towels for removing any additional external liquid. Swollen gel weights were measured using an analytical balance (Shimadzu AUW120D, Kyoto, Japan; max: 120g, min: 1mg; accuracy: d=0.1mg). Hydrogels were allowed to swell until nearly-
constant gel weight was achieved. Thereafter, swollen hydrogel pieces were transferred into warm growth media or an oven maintained at 40°C for deswelling above the LCST. During the deswelling process, gels were periodically weighed at short time intervals (5-10min) until constant weight was achieved.

Mechanical Characterization

The mechanical properties of hydrogels were characterized through compression tests and rheological measurements.

To characterize the compressive resistance and deformation of hydrogels, a confined compression ramp stress protocol was employed. All compressive tests were conducted at room temperature using a bench-top mechanical testing device (Electroforce 3200, BOSE, Eden Prairie, MN, USA) carrying a 100N load cell. Both fully-swollen and partially-swollen gels were tested with this method. Fully swollen hydrogels were cut into thin cylindrical pellets (thickness d: 3-4mm; diameter Ø: 17-18mm). To prepare partially swollen gels, pre-dehydrated gels were swelled in water for 6h. Diameter and thickness of a sample pellets were exactly measured and recorded with a digital caliper before starting the compressive test. To perform the tests, one flat end of each gel disk was placed on the sample plate, which had been covered with fine sand paper to prevent gels from slipping. Once the zero was set for the sample, a linear ramp compression displacement was applied to the sample at a strain rate of 0.1%/s until reaching 80% strain of the sample thickness. The stress was measured via the built-in force sensor of the load cell. A stress-strain profile (σ vs. ε) could be generated from the data collected.
For rheological measurements, a parallel-plate rotational rheometer (Rheometric Dynamic Analyzer Model 3, Rheometric Scientific, Piscataway, NJ, USA) was used for frequency-sweep tests. Tests were performed on thin cylindrical-shaped hydrogel samples (thickness d: approx. 3mm; diameter Ø: 17-18mm). Both the fully- and partially-swollen gels were characterized. After gels were placed on the lower plate, the gap between the plates was set to either 3mm (low vertical compression on gel surface) or 2mm (with nearly 33% compression of gel). Thereafter, dynamic frequency-sweep tests at a constant 20% strain were recorded over the frequency range of 1.0 - 300.0rad/s with 1.0rad/s increment.

2.2.3 Microalgal Culture Dewatering

2.2.3.1 Microalgal Culture Preparation

*Chlorella sp.* cultures for dewatering experiments were cultivated under continuous illumination, stirring, and air sparging in 3L Cytostir® spinner flask reactors (Kimble/Kontes, Vineland, NJ, USA). The growth medium contained sodium nitrate NaNO₃ (0.25g/L), ammonium chloride NH₄Cl (0.05g/L), magnesium sulfate heptahydrate MgSO₄ • 7H₂O (0.075g/L), calcium chloride dihydrate CaCl₂ • 2H₂O (0.025g/L), sodium chloride NaCl (0.025g/L), ferric ammonium citrate C₆H₅+4yFe₅N₃O₇ (0.01g/L), potassium phosphate dibasic K₂HPO₄ (0.25g/L), sodium carbonate Na₂CO₃ (0.25g/L), and trace element solution (1mL/1 Lmedium). Biomass concentration during culture growth was determined by measuring total suspended solids (TSS) concentration (described later).
2.2.3.2 Microalgal Culture Dewatering with Semi-IPN Hydrogels

A stagewise hydrogel swelling method was carried out to increase microalgae concentrations. During each stage, cultures at room temperature were placed in a beaker and exposed to shrunken hydrogels that had been previously deswelled in warm growth medium (40°C). The amount of hydrogel to be used in a stage was determined based on the volume of culture feed to stage. In general, the ratio of the feed culture volume to the expected weight of the swollen gels was kept at approximately 1.5. During each stage, deswelled gel pieces were added to the microalgal culture in a beaker and incubated for 20-30 min. A stir bar was used for keeping the suspension well mixed. At the end of the incubation period, the suspension was passed through a nylon mesh to separate the swollen gel pieces from the concentrated culture. The swollen gels collected on the mesh were deswelled in an oven to recover the absorbed growth medium (henceforth referred to as “recovered medium”). During deswelling, the oven temperature was maintained at T<40°C to prevent microalgal cells from losing viability. The volume of concentrated culture collected was measured with a measuring cylinder. A part of the concentrated culture was set aside for TSS measurements and the remaining volume was transferred to the next dewatering stage.

2.2.3.3 Assessment of Post-harvest Culture Viability

Photosynthetic activity in the concentrated cultures was measured by Pulse Amplitude Modulated (PAM) fluorometry (described below). In addition, the viability of microalgae cells recovered from hydrogel dewatering were assessed by direct re-cultivation. The concentrated cultures were from dewatering were diluted in fresh medium to approximately 0.5g/L before being re-cultivated. Dilute cells in the recovered
medium were directly incubated without any further processing. As controls, microalgae cultures that hadn’t been exposed to hydrogels were inoculated into fresh media and cultivated alongside the cultures recovered from the hydrogel dewatering process. Initial cell concentrations were kept similar between the cultures obtained from hydrogel dewatering (recovered medium and concentrate) and the controls to allow side-by-side comparison of growth performance. The recultivation experiments were performed in 400mL photobioreactor (PBR101 Algae Photo Reactor, Phenometrics, Inc., Lansing, Michigan, USA) in the presence of light (14h/d) and with constant agitation. The pH was controlled at 8.5 by periodic sparging with a 5% CO₂-air mixture. Growth was monitored by measuring changes in cell numbers.

2.2.4 Analytical Methods

2.2.4.1 Total Suspended Solids (TSS)

For TSS estimates, culture samples were filtered through a pre-weighed glass fiber filter. Subsequently, the filter paper containing the algae cake was washed with pure water to dissolve inorganic precipitates, if any. Thereafter, the filter paper was carefully removed and dried in an oven at 50°C until a constant weight was obtained. Dry weight of algae biomass collected on the filter paper was calculated as the difference in weight of the filter paper before and after filtration. The concentration was calculated by dividing the measured algae mass by the filtered culture volume.

2.2.4.2 Pulse Amplitude Modulated (PAM) Fluorometry

Pulse Amplitude Modulated (PAM) fluorometry was performed using a DUAL-PAM-100 Chlorophyll Fluorescence & P700 Photosynthesis Analyzer (Heinz Walz
GmbH, Effeltrich, Germany). 5mL sample was placed in the instrument vial. A stir bar was used to mix the sample. Then, the sample was kept in dark in the chamber for at least 5 min before starting the measurements of chlorophyll fluorescence parameters. Saturation pulse light intensity was set to 10000 µE.

2.2.4.3 Cell counting by FlowCAM

Cell concentrations in microalgal cultures were measured by direct counts using a flow cytometer (FLOWCAM, Fluid Imaging Technologies, Inc., Yarmouth, Maine, USA). Samples from the ePBRs were first appropriately diluted to concentrations expected to measure 10,000 cells/mL or lower. Camera focus was adjusted before taking measurements. 1ml sample was analyzed by FlowCAM using the auto-image mode at an auto-image rate of 20 frames/s, 100× magnification (10× objective and 100-µm flow cell). The flow rate was set to 0.83mL/min.

2.3 Results and Discussion

2.3.1 Hydrogel Characterization

*Swelling Kinetics*

The absorption/desorption characteristics of hydrogels were first assessed by performing experiments to determine swelling and deswelling kinetics in growth media. Absorption (at ambient temperature) and release (at T>LCST) of medium by the hydrogels was quantitatively described in terms of gel swelling ratio (SR) which represents the mass of water contained per unit mass dry gel. Swelling ratios were
calculated as $SR = (W_S - W_D)/W_D$, where $W_S$ is the weight of the swollen hydrogels and $W_D$ is the weight of the dehydrated hydrogels.

Changes in swelling ratios over time during swelling and deswelling in the growth medium are shown in Figure 2.1. Consistent with previous reports of similar experiments in water (Freitas and Cussler 1987, Zhang, Cheng et al. 2003), it can be seen that the swelling rate remained high only during the initial incubation period (0-4h) and gradually decreased later. After 10h, swelling rates were significantly diminished indicating that the gels were close to their equilibrium swelling capacity. These results suggest that a short residence time during the swelling stage would be preferred in a harvesting process that seeks to minimize overall process time. Longer residence times would allow lower use of gel per stage, but savings in gel cost may not be significant in this approach if gel recycle is effective (Vadlamani et al. 2014). Additionally, compared to the swelling rates, the deswelling rates of all hydrogels were much higher and the deswelling periods are not likely to contribute as significantly to overall process times as the residence time during the swelling stages.

From Figure 2.1, it can also be seen that the PNIPAAm-PVA semi-IPN hydrogels with 10% PVA content (semi-IPN10) have an equilibrium swelling ratio of 40g-media/g-gel after 10h, and significantly faster overall water absorption rates than the other gels. The PVA chains, which were incorporated into the cross-linked PNIPAAm network, likely contributed to favorable swelling kinetics of semi-IPN10 gels. The hydrophilic PVA chains, which are reported independent from the PNIPAAm backbone network (Zhang, Cheng et al. 2003), possibly facilitated the increase in water uptake rates due to the favorable molecular interactions between polymer chains and water. However, too
much PVA added in the network could also occlude the pores (i.e. reduce the open channel area of pores), especially in de-swollen gels and impede water uptake. Our results show that the semi-IPN20 gels, which have a higher (20%) PVA content (based on PVA monomer added during polymerization), did not perform as well as the semi-IPN10 gels. Moreover, semi-IPN10 hydrogels with higher crosslinker density, synthesized in order to provide higher mechanical strength to the gels, also showed unfavorable gel swelling kinetics possibly due to reduction in effective pore size. Compared to the semi-IPN gels synthesized with higher crosslinker density, the structure of semi-IPN gels with the same PVA content and lower crosslinker density is expected to be more open with larger-sized pores since the polymer chains may be not as tightly linked with each other.

PVA-containing semi-IPN gels also showed higher deswelling rates compared to PNIPAAm. PVA chains are reported to act as water-releasing channels in the networks, which may have contributed to the fast deswelling of semi-IPN hydrogels (Zhang et al. 2002). Both the semi-IPN 10 and 20 gels were able to release more than 95% of absorbed media in 15 min (Figure 2.1). In summary, the swelling and deswelling rates could be greatly improved because of the introduced PVA chains and semi-IPN10 gels had the best performance.
Swelling and deswelling kinetics of PNIPAAm and PNIPAAm-PVA semi-IPN hydrogels. Error bars represent one standard deviation from mean values of triplicate experiments.

Hydrogel particle size also has an influence on the performance of gel swelling kinetics. Vadlamani et al. reported that hydrogels with smaller dimensions have faster swelling rates, likely due to the increase in surface area available for water diffusion (Vadlamani et al. 2012). However, excessively small size of gel particles may potentially cause difficulties in recovering swollen gels from concentrated cultures. The 3mm gels used in this study were recovered without difficulty.

One concern with semi-IPN hydrogels is that gels could lose performance over time due to loss of the intercalated PVA chains from the hydrogel network during the swelling and deswelling process. Although Zhang et al. stated that, for PNIPAAm-PVA semi-IPN gels, the PVA chains do not dissolve out from semi-IPN structure (Zhang et al.
2002), we tested for prolonged performance of our gels. In these tests, semi-IPN10 hydrogels were repeatedly swelled (1h) and deswelled (0.5h) and no obvious change in kinetics could be observed over multiple successive uses of the gels (Figure 2.2). We also assessed swelling kinetics after the semi-IPN10 hydrogels had been used for several preliminary experiments to develop protocols for dewatering algae slurries (over 30 swelling and deswelling cycles). From Figure 2.3, it can be seen that hydrogels that had been used repeatedly, in fact, exhibited slightly higher swelling and deswelling rates than freshly-prepared hydrogels. The consistent swelling/deswelling performance observed from these studies suggests that the semi-IPN10 hydrogels are likely suitable for prolonged use and may not need frequent replacements.

![Swelling Ratio vs Time Graph](image)

**Figure 2-2** Gel absorption and release performance for five successive swelling/deswelling cycles with semi-IPN10 gels.
Figure 2-3 A comparison of swelling kinetics between freshly-prepared semi-IPN10 hydrogels and gels that had undergone multiple (>30) swelling/deswelling cycles.

**Mechanical Properties**

Based on previous studies with other IPN gels such as poly(methacrylic acid)/PNIPAAm that have shown improvements in elastic modulus compared to PNIPAAm, it has been hypothesized in the literature that PNIPAAm-PVA semi-IPN gels would also have similar superior mechanical properties (Zhang, Bhat et al 2008); however, the mechanical properties of PNIPAAm-PVA semi-IPN hydrogels have not been measured.

In the hydrogel harvesting process, the mechanical properties of hydrogels play an important role in determining the durability and thereby the cost of replacement of hydrogels. Hydrogels are soft materials that would likely be subjected to compression (due to hydrostatic forces) as well as shear (from the motion of aqueous media around the
hydrogels) in large-scale harvesting equipment. Consequently, we tested the mechanical performance of the most promising semi-IPN10 hydrogels when exposed to both kinds of stresses. Further, we tested the performance of fully- as well as partially-swollen gels since repeated use of gels in a “fast kinetics region” (Figure 2.1) would result in only partial swelling of the hydrogels. For reference and comparison, mechanical properties of PNIPAAm gels were also tested.

The stress-strain plots from the compression tests are shown in Figure 2.4. The arrows in the Figure mark the onset of gel rupture as evidenced by a transition from smooth to jagged or irregular stress-strain curves. For equilibrium-swollen hydrogels, the rupture of the PNIPAAm gel sample occurred after 58% strain (corresponding to a stress of nearly 35.5kN/m$^2$), while the semi-IPN10 gel sample was breached at a much higher strain of 75% (corresponding stress was nearly 54.5kN/m$^2$). These results indicate that the PNIPAAm-PVA semi-IPN10 gels show greater tolerance to compressive stress than the PNIPAAm gels. Moreover, rupture of the partially swollen gels (if any) occurred only at or beyond the maximum strains (80%) tested suggesting that partially swollen gels are significantly more resilient to breakage due to compressive stresses and are likely to be more durable in large scale dewatering operations. Further, for both of the equilibrium-swollen- and partially-swollen- gels, the strain-stress curves of the PNIPAAm gel samples showed generally larger slopes than the semi-IPN10 gels, suggesting that PNIPAAm gels are more rigid compared to the semi-IPN10 gels.
Rheological measurements were performed to assess hydrogel response to shear stresses alone or a combination of mechanical compression and shear. Figure 2.5 to 2.8 show the results of frequency-sweep tests performed using a rotational rheometer. The elastic modulus $G'$ and viscous modulus $G''$ were recorded as a function of frequency, increasing from 0 rad/s to 300 rad/s at a fixed amplitude $\gamma^o = 0.2$. It has been reported that a characteristic feature of cross-linked hydrogels is that the values of $G'$ remain largely unchanged over wide frequency ranges and are much larger than values of $G''$ (Zhang 2009). Our results are consistent with these previous observations (Figures 2.5 and 2.6). Of greater interest, however, was the observation that the elastic modulus ($G'$) of semi-IPN10 gel samples were always lower than $G'$ values of PNIPAAm gels (Figures 2.5 and 2.6). Thus, the semi-IPN10 gels are more flexible (or less rigid) than PNIPAAm.
under shear and therefore would be less susceptible to damage and attrition when exposed to fluid motion in the dewatering process. In addition, for hydrogel samples which were at partially swollen status, the elastic modulus \( G' \) was always lower than \( G' \) of equilibrium swollen gels (compare \( G' \) values between Figures 2.5 and 2.6) suggesting that the partially swollen gels had greater elasticity than fully swollen gels and would be more suitable in the dewatering process.

Rheological measurements were also made on gels that were compressed by setting the plate gap at 2mm (approximately 33% compression of the gel, Figures 2.7 and 2.8). As a result of the compression, the \( G' \) of both PNIPAAm gel and semi-IPN10 gel samples showed a decrease relative to uncompressed gels likely due to loss in elasticity from the additional normal force. However, semi-IPN10 gels retained greater flexibility than PNIPAAm even under compression and the partially-swollen semi-IPN10 gels showed the lowest \( G' \) values (Figure 2.7). Finally, since water occupied the most of the gel sample volume, \( G'' \), which reflects the gel viscous properties, did not show any big difference among these gel samples (Figure 2.8).
Figure 2-5 Frequency-sweep characterization of equilibrium-swollen PNIPAAm and semi-IPN10 gels (3mm gap without compression applied).

Figure 2-6 Frequency-sweep characterization of partially-swollen PNIPAAm and semi-IPN10 gels (3mm gap without compression applied).
Figure 2-7 Elastic modulus $G'$ from frequency-sweep tests of equilibrium and partially-swollen PNIPAAm and semi-IPN10 gels in the presence of compression (plate gap adjusted to 2mm to provide compression).

Figure 2-8 Viscous modulus $G''$ from frequency-sweep tests of equilibrium and partially-swollen PNIPAAm and semi-IPN10 gels in the presence of compression (plate gap adjusted to 2mm to provide compression).
In conclusion, the mechanical studies of PNIPAAM and semi-IPN gels show that partially-swollen semi-IPN gels exhibit good resistance to compressive- and shear-damage and therefore would be suitable for the dewatering process.

2.3.2 Hydrogel Dewatering Process

As discussed in the previous sections, the semi-IPN10 gels showed highest initial swelling rates (Figure 2.1), retained their performance over multiple cycles (Figure 2.2) and also had suitable mechanical properties for microalgae harvesting (Figures 2.4 to 2.8). Consequently, we tested the feasibility of using the semi-IPN gels for concentrating microalgae cultures by implementing a stagewise dewatering approach. In each stage, the added hydrogels absorbed a fraction of the growth media to cause a partial increase in culture concentrations. In order to keep the absorption rate high during the harvesting process and maintain good hydrogel mechanical properties, the gels were allowed to partially swell (and absorb the aqueous growth media) for approximately 30min. A swelling ratio of nearly 10 g-media/g-dry-gel was achieved over this incubation period.

Next, the mass of gel to be loaded per stage was determined through some preliminary trial and error experiments. When excessive gel mass was used, we observed that the aqueous phase was rapidly depleted and the resulting decrease in the level of the free liquid surface exposed a portion of the gels to air by the end of the 30min incubation period. Under these conditions, only the gels that remained submerged in aqueous phase were able to absorb media throughout the incubation period, while the absorption capacity of gels that became exposed to air remained underutilized. Further, as the liquid level decreased, the algae cells in contact with the gels became stuck to the gel surfaces due to the lack of free liquid to keep the cells suspended. When low amounts of gel was
used, all gels remained completely immersed, but only a small portion of the aqueous phase was absorbed by the end of the incubation period. Through several preliminary experiments, we determined that when the ratio of feed aqueous volume to the final swollen gel volume (at the end of 30 min of incubation) was kept at or lower than 2:1, gels remained completely immersed in solution throughout the swelling period. Under these conditions, there was sufficient free liquid to keep cells in suspension without significant adhesion to gel surfaces. To demonstrate dewatering of microalgal cultures, the initial mass of gels to be added per cycle was calculated using this ratio as the basis.

Figure 2.9 Concentrations achieved during the stagewise dewatering using PNIPAAm-PVA Semi-IPN10 hydrogels. The mass of deswelled hydrogels added to each stage for absorption of media were such that the ratio of feed aqueous volume to swollen hydrogel volume was between 2:1 and 1.7:1. Values indicate average of duplicate 3-stage runs. Error bars represent one standard deviation from mean values.
A three-stage dewatering process was implemented to demonstrate the feasibility of rapidly increasing culture concentrations. Two experimental runs were performed. Concentration changes of the microalgae culture with each process stage are shown in Figure 2.9 where the horizontal axis shows the cumulative residence time through the process. These results show that more than 8-fold increase in concentrations were achieved through this stage-wise approach over a net residence time of 1.5h. Biomass concentrations of nearly 11g/L were obtained over this period with removal of nearly 90% of the aqueous media. In previous studies with PNIPAAm and low gel loadings, only a 2-fold increase in concentrations was obtained over a 2h period. Further, starting from a feed concentration of nearly 1g/L, a process residence time of approximately 7h would be needed to obtain concentrations >10g/L with PNIPAAm and low gel loadings (Vadlamani et al. 2014). Our results with semi-IPN hydrogels and high gel loadings represent a significant improvement in hydrogel-based dewatering of microalgal cultures.

Stagewise mass balances and for each of the duplicate 3-stage runs are shown in Biomass mass and concentrations for two of the dewatering processes are summarized in Figures 2-10 and 2-11. From mass balances, 97% and 91% of the feed mass in Runs 1 and 2, respectively, could be accounted for through the process in the concentrate, sampling and dewatering streams. Visually, no algae cells were observed to be present within the gel and mass balance results also confirm no accumulation of biomass inside the gels consistent with our previous observations made during harvesting experiments with PNIPAAm (Vadlamani et al. 2014). The small fraction of the feed mass (10-15%) recovered during the deswelling step was likely from cells adsorbed to gel surfaces.
To assess the viability and photosynthetic activity of recovered cells in deswelled media, Pulse Amplitude Modulation fluorescence measurements were performed and these results are shown in Figure 2.12. Cells in the recovered media showed good photosynthetic activity (as represented by the $F_v/F_m$ values close to 0.5 which suggests that the cells remained viable after incubation with the hydrogels and the subsequent deswelling operation. The small decrease in photosynthetic activity relative to fresh feed cultures could however have been due to the slight thermal shock during the deswelling process which occurred at temperatures above ambient conditions (~38°C). PAM measurements were made immediately after media and cells were recovered by hydrogel deswelling.

To more directly assess the viability of cells in the concentrate and in the media recovered after deswelling, direct cultivation experiments were performed. Fresh cultures that had never been exposed to hydrogels were used as positive controls. As shown in Figure 2.13, cells in the deswelled media as well in the concentrate had growth similar to control cultures and positively confirmed that the hydrogel dewatering process did not result in any measurable loss in long-term cell viability. As a result, it should be possible to recycle the recovered media and accompanying cells back into cultivation ponds or photobioreactors to reuse water and soluble nutrients. In cases where cell viability may be an important parameter in the value of the harvested biomass (e.g. nutraceutical products), hydrogel-based harvesting would also provide a superior product.
**Figure 2-10** Stages and mass flow in a gel dewatering process (Run No.1).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Liquid feed input to stages</th>
<th>Deswelled gel added</th>
<th>Concentrated culture output from stages</th>
<th>Post-deswelling recovered media</th>
<th>TSS sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass (mg)</td>
<td>Volume (mL)</td>
<td>Concentration (g/L)</td>
<td>Biomass (mg)</td>
<td>Volume (mL)</td>
</tr>
<tr>
<td>1</td>
<td>258</td>
<td>200</td>
<td>1.29</td>
<td>15</td>
<td>244.5</td>
</tr>
<tr>
<td>2</td>
<td>219</td>
<td>86</td>
<td>2.55</td>
<td>7.5</td>
<td>199.7</td>
</tr>
<tr>
<td>3</td>
<td>172</td>
<td>31</td>
<td>5.54</td>
<td>1.84</td>
<td>167</td>
</tr>
</tbody>
</table>

**Feed**
1.29g/L
258mg biomass
200mL

**Stage 1**
$\tau = 30\text{min}$
Deswelled gel 15g

- **Aqueous phase**: 13.8mg biomass
- **104mL media**

**Stage 2**
$\tau = 30\text{min}$

- **Deswelled gel 7.5g**
- **Hydrogel and media recovery**

**Stage 3**
$\tau = 25\text{min}$

- **Deswelled gel 1.84g**
- **Hydrogel and media recovery**

**Recovered growth media**
30.6mg biomass
170mL media

**Recovered concentrate**
11.13 g/L
167mg biomass
15mL

**TSS sampling**
53.2mg biomass
Figure 2-11 Stages and mass flow in a gel dewatering process (Run No.2).
**Figure 2.12** Photosynthetic efficiency changes for recovered medium and dilute culture.

**Figure 2.13** Cell number changes in recultivated samples.
2.3.3 Economic Evaluation of Gel Harvesting Process

Compared to traditional harvesting methods, such as centrifugation, filtration, and flocculation, hydrogel dewatering process could have a much lower cost. The cost could be mainly contributed by three parts: capital cost of dewatering device, hydrogel expense, and energy cost.

First, the capital cost may be low because of the mechanical simplicity of the hydrogel harvesting equipment (primarily tanks) compared to the more complex machinery involved in operations such as centrifugation. Second, semi-IPN hydrogels, which are the major consumable in the harvesting process, are relatively cheap and easily available in bulk quantities. In addition, the of semi-IPN gels could be expected to have a long lifespan based on our results which show consistent swelling kinetics over even after multiple reuse of the gels, relative high mechanical strength, and successful recovery after use. Without frequent replacements, the cost associated with gel procurement would be low. Finally, the energy cost of gel dewatering process in an industrial scale process could be contributed mainly by two parts: electric energy input for pumping systems and thermo-energy input for deswelling and recovering the swollen hydrogels. Compared to the energy-consuming conventional dewatering methods, the electric energy consumption in gel dewatering process could be very low due to the ambient pressure process. Moreover, the cost spent on the heating supplied for recovering the gels may be limited because semi-IPN gels have low LCST 32°C which is much close to the environmental temperature. Also, this part of heat for gel deswelling could be supplied by flue gas or waste heat from the downstream biorefinery processes.
2.4 Conclusion

A hydrogel-based dewatering process that uses poly (N-isopropylacrylamide)/polyvinyl alcohol (PNIPAAm-PVA) with a semi-interpenetrating network structure has been developed for efficient harvesting of dilute microalgal cultures. The PNIPAAm-PVA semi-IPN gels showed enhanced swelling kinetics and mechanical properties, compared to conventional PNIPAAm gels. Results from this study also show that the hydrogels can be reused over multiple dewatering cycles without measurable loss in swelling/deswelling performance. In addition, a stagewise hydrogel-based harvesting process that uses high gel loadings and results in a low process residence time has been demonstrated. The cells associated with the growth medium recovered after deswelling were shown to be viable and could be recycled back to growth reactors. The reuse of water and soluble nutrients recovered from hydrogel-based harvesting would improve overall sustainability and economics of microalgae-based biofuels.
References


Appendix A

Morphology Study of Hydrogels

A.1 Experiment and Method

First, the equilibrium-swollen gel pieces with dimensions of 3mm×3mm×3mm were frozen using liquid N2 in a freeze flask. After all the liquid N2 evaporated, the frozen gel pieces were freeze dried (Labconco FreeZone 2.5 Freeze Dryer, Labconco, Kansas City, Missouri) for 20h. After dehydration, the gel pieces were carefully taped on a substrate holder sputter coated with gold. Then, a Quanta 3D FEG Dual Beam Electron Microscope (ESEM) (FEI, Hillsboro, Oregon) was used to visually inspect the morphology of PNIPAAm and PNIPAAm-PVA semi-IPN 10 hydrogels. The gold coated samples on the substrate holder were inserted into the electron microscope chamber. Next, the chamber was pumped to vacuum before the beam was turned on. The beam were adjusted and navigated to the sample surface. The focus and magnification were also adjusted to ensure a good image quality.

A.2 Result and Discussion

In order to investigate the differences in microstructures of PNIPAAm and PNIPAAm-PVA semi-IPN hydrogels, which may be able to illustrate the differences in
swelling kinetics, the morphology of the hydrogels were observed using an electron microscope.

![ESEM photos of PNIPAAm and PNIPAAm-PVA semi-IPN hydrogels at swollen and deswelled states](image)

**Figure A-1** ESEM photos of PNIPAAm and PNIPAAm-PVA semi-IPN hydrogels at swollen and deswelled states

For swollen gels, we observed that the morphology of PNIPAAm exhibits a homogeneous, dense architecture with smooth and nonporous strut walls, while the semi-IPN10 gel have uneven, porous structures and walls. According to the literature, the number of pores in semi-IPN gels may increase with PVA content. In addition, it is possible that the larger pore size may have contribute to an increased swelling capacity of hydrogels. In the deswelled status, the PNIPAAm gels retained their honeycomb structure, while semi-IPN gels shrunk and formed a porous but flat and loose surface.
Appendix B

Gel Dewatering Device Design

B.1 Introduction

An efficient device is required for gel dewatering process. In this research, beakers were chosen as the reactors because of the relatively low culture and gel load. For industrial scale dewatering processes, large quantity of algal cultures may be required to be processed. Hence, the dewatering device should have a large capacity to hold and process large volume of liquid. The dewatering device is also required to be constructed with an inlet and an outlet to allow the dilute culture flowing in and the concentrated culture flowing out. Mechanical strength of hydrogels should also be considered in the dewatering device designing since gel pieces are brittle and heavy at their swollen state. The big pressure drop and the frictions among the swollen hydrogel pieces may make the hydrogels have large potential to break, and then reduce the gel lifespan and increase the cost for gel replacements. In this chapter, three generations of dewatering device, including beakers, sieve and tray, and gel column, had been developed and applied in gel dewatering process.
B.2 Gel dewatering process in beakers

Beakers are simple devices for gel dewatering process. We have successfully used beakers as the dewatering reactors, whose results are shown in Chapter 2. The photos taken for the 5 dewatering stages are shown below.

*Figure B-1* Gel dewatering process carried in beakers using semi-IPN gels
Figure B-2 Swollen gels were recovered in an oven after swelling

B.3 Sieve and tray model

We performed algae dewatering experiments using a sieve and tray device. Dry gels were placed on the sieve and contacted with the dilute algal culture. This allowed the swollen gels to be easily recovered after absorbing medium from the culture. Dry gel to culture volume media ratios of 2:1 and 20:1 were used. From these experiments, we found that at low gel loadings, four swelling-deswelling cycles were required to increase the concentration of the algal culture from \(~1.1\) g/L to \(7.4\) g/L. Including swelling and deswelling time, at low gel loadings, we would need a process residence time of \(~9\) h. At
higher gel loadings, the same final concentrations were achieved in 1 cycle (~1.75h). Thus, high gel loadings are more desirable since process residence time is short.

**Figure B-3** The conceptual sieve and tray device design; step 1: the dilute culture is pumped into the tray and swelling starts; step 2: after swelling, the concentrated culture is pumped out; step 3: warm media/water is injected into the tray for recovering the swollen gels; step 4: recovered medium is collected and recycled.

Compared to the dewatering process run in beakers, the overall medium absorbing rate was observed having and increase when using sieve and tray device, because hydrogel pieces were spread as a thin layer so the gel pieces was able to well contact with culture. The amount of absorbed biomass is close to the one when using the beakers (around 10% w/w). The hydrogels were still need to be squeezed to ensure all the concentrated culture being collected. The lifespan of the hydrogels may be extended.
because of the low pressure drop in the thin layer of gel pieces. Thus, the risk of gel damage could been reduced.

*Figure B-4* A dewatering process shows variations in gels and algal culture. (a) the dehydrated/deswelled hydrogels are placed on a mesh in the shallow tray; (b) pump in the dilute algal culture and the swelling process starts; (c) the mixture of the swollen gel and the concentrated culture; (d) the swollen gels after removing most of the concentrated culture.
Table B.1 Dewatering performance for hydrogels on sieve and tray device. Obviously, the semi-IPN gel has much higher separation efficiency.

<table>
<thead>
<tr>
<th>Hydrogel</th>
<th>Swollen Gel to Culture Volume Ratio</th>
<th>Initial Concentration (g/L)</th>
<th>Final Concentration (g/L)</th>
<th>Time Required (hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNIPAAm</td>
<td>1:2</td>
<td>1.12</td>
<td>7.37</td>
<td>9</td>
</tr>
<tr>
<td>PNIPAAm/PVA Semi-IPN</td>
<td>2:1</td>
<td>1.08</td>
<td>7.38</td>
<td>1.75</td>
</tr>
</tbody>
</table>

Figure B-5 The variations in the culture volumes and colors when the concentrations were increased from 1.08 – 7.38 g/L.

To calculate the cost of hydrogels, several basis are assumed as following. The comparison of the costs for PNIPAAm and PNIPAAm-PVA semi-IPN gels are shown in Table B.2.
Design basis:

a) 1 ton/1000 tons of water (1g/L) to 1 ton/12.5 tons of water (80g/L)

b) Water to be removed (w) = 987.5 tons

c) Cost estimates are based on the bulk price of poly N-isopropylacrylamide (P)

= $0.5/kg

*Table B.2* Gel cost estimation for dewatering processes with PNIPAAm gel and PNIPAAm-PVA Semi-IPN gel.

<table>
<thead>
<tr>
<th>Parameters needed for calculations</th>
<th>Cost estimates for PNIPAAm Gel</th>
<th>Cost estimates for Semi-IPN Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹Swelling Ratio (R)</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>²Swelling/Deswelling Time (t)</td>
<td>4h</td>
<td>1h</td>
</tr>
<tr>
<td>³Gel Life-time (q)</td>
<td>90 days</td>
<td>90days</td>
</tr>
<tr>
<td>⁴Dry gel required achieve 80g/L</td>
<td>0.092tons</td>
<td>0.031tons</td>
</tr>
<tr>
<td>⁵Cost of the gel</td>
<td>$46.5</td>
<td>$15.5</td>
</tr>
</tbody>
</table>

¹Calculated as (wt. of swollen gel – wt. of deswelled gel)/ wt. of deswelled gel.

²Swelling and deswelling are assumed to be performed in parallel.

³Period over which the gel retains its swelling - deswelling characteristics without decline in performance. In this calculation, assume that the semi-IPN gel has the same lifetime with PNIPAAm gel.

⁴Calculated as = ((t*R) / (q)) * (w)

⁵Calculated as = P*W₉, can be lower than $15.5 depending on R, t, q and W₉.
B.4 Hydrogel columns

A column-shaped reactor was designed for gel dewatering process based on a packed-bed reactor. A syringe or pump were used for pumping the culture and air sparging. A sieve was installed on the bottom of the column to prevent gel pieces from being flushed away. The sieve was made of steel so it has enough mechanical strength to hold large amount of gels. The deswelled gel pieces were filled in the device manually before pumping in the dilute culture. During the swelling process, the gel-culture mixture was sparged with air for several times to enable the culture and gel well contact. Since a high gel loading 15g-20g was selected, the swelling time was restricted in 15min. This enable us to pump the concentrated culture out easily. We found that pump was much powerful than the syringe, which could result in less absorbed biomass (around 5% w/w in a single cycle). After pumping out the concentrated culture, the warm fresh medium was pumped into the reactor for recovering the gel pieces. Recovering process could be finished in 5min. The concentrations of culture before and after concentration and recovered medium were measured through T.S.S measurements.

The cylindrical-shaped dewatering column was designed with large diameter and less dead volume below the sieve. The small diameter of the column will not work well since the swollen gel pieces may block all the sieve pores, thus the air bubbles and culture may be difficult to travel through the swollen gels. At the same time, the swelling time was restricted short since the large size of swollen gel pieces would not allow all the concentrated culture to be easily separated, which would result in more absorbed biomass.
Figure B-6 The conceptual design for gel dewatering in a column
Appendix C

Gel Lifespan and Recovery

Hydrogel lifespan is one of the major factors which may contribute to the cost of gel dewatering process. A short lifespan or difficulties in gel recovery will result in frequent replacements of hydrogels, and then increase the gel cost. Since the swollen hydrogel pieces are brittle, attention should be paid when handling the gel pieces.

To extend the gel lifespan, the gel pieces should own enough mechanical strength to prevent themselves from being broke because of the high shear and compression existing in the dewatering process. Conventional PNIPAAm hydrogels are relatively weak and brittle. After multiple dewatering stages, plenty of tiny pieces appeared which were difficult to be separated from the culture. After introducing the PVA chains into the PNIPAAm networks, the developed PNIPAAm/PVA semi-IPN hydrogel network showed much better mechanical properties. The PNIPAAm/PVA semi-IPN hydrogels were characterized and show stronger mechanical strength compared to the conventional PNIPAAm gels. Seldom tiny gel pieces could be observed after the multiple swelling cycles. According to the experiments, the lifespan of semi-IPN gels could last several months. Considering the mechanical characteristics of hydrogels, the dewatering should
be carried out under a relatively mild environment (with limited stirring or avoid rapid culture flow motion).

**Figure C-1** Completely recovered gels without any biomass sticking on the surface

The semi-IPN hydrogels were tested over 50 swelling/deswelling cycles without any decline on their swelling kinetics. Swelling rate could become even faster after multiple swelling and deswelling since the gel network was stretched and became looser to enable gels absorbing more water. In conclusion, the swelling kinetics may not affect the lifespan of hydrogels.

In addition, to ensure a long lifespan, the hydrogel pieces used for dewatering the algal slurry were proved being able to be recovered without biomass sticking on the surface, which is shown in Figure C-1.

In a gel dewatering process, the major energy consumption happens during the gel recovery. Heat is provided to increase the gel temperature above their LCST. If using warm media/water bath to recover gel pieces, the temperature of the bath can be
maintained by providing the flue gas or other waste heat sources. Swollen gels can also be recovered in an oven, however, the time required for gels being completely deswelled can be longer due to the lower heat transfer rate. For both methods, the temperature should be maintained around 40°C to ensure the adsorbed biomass having good viability.
Appendix D

Swelling Kinetics of Semi-IPN Gels in Distilled Water and Microalgal Culture

![Swelling kinetics of semi-IPN10 gels in cell-free media, water, and microalgal culture, respectively. Semi-IPN gels have the best swelling kinetics in distilled water, however, the media containing salts or microalgal culture containing algal cells may inhibit the gel swelling.](image)

*Figure D-1* Swelling kinetics of semi-IPN10 gels in cell-free media, water, and microalgal culture, respectively. Semi-IPN gels have the best swelling kinetics in distilled water, however, the media containing salts or microalgal culture containing algal cells may inhibit the gel swelling.
Appendix E

Hydrogel Chemical Component Structures

Ammonium persulfate (APS)

\[
\left[ \begin{array}{c}
\text{H} \\
\text{N} \\
\text{H}
\end{array} \right]^+ \left[ \begin{array}{c}
\text{O} \\
\text{S} \\
\text{O} \\
\text{O} \\
\text{S}
\end{array} \right]^{2-}
\]

N, N'-methylenebisacrylamide (BIS)

\[
\text{H}_2\text{C} = \text{N} - \text{N} - \text{C} = \text{CH}_2
\]

Sodium metabisulfite (Meta-BIS)

\[
2\text{Na}^+ \left[ \begin{array}{c}
\text{S} \\
\text{O} \\
\text{O} \\
\text{O}
\end{array} \right]^{2-}
\]

N-isopropylacrylamide (NIPA)

\[
\text{H}_2\text{C} = \text{N} - \text{C} = \text{CH}_3
\]
Poly (N-isopropylacrylamide) (PNIPAAm)

Polyvinyl alcohol (PVA)