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Microalgae Immobilization Using Hydrogels for Environmental Applications: Study of Transient Photopolymerization

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Immobilization of microalgae has emerged as a useful technique for effective environmental applications as removal of undesirable compounds from water, culture collection handling for CO_2 capture, development of biosensors, and production of clean energy among others. In this work, polymerization of hydrogels is evaluated in order to generate adequate nanoporous morphology for microalgae immobilization via use of transient light intensity. Hydrogels were polymerized using a UV light intensity range between 140 and 700 mW/cm^2 during 0.8 h and characterized using rheology evaluation using an angular frequency of 1 rad/s for defined monomer, initiator and solvent amounts.
Results shows that transient light polymerization has a significant effect on average pore size and pore size distribution, obtaining different gel points between 1,300 and 1,700 s and modules between 4,000 and 13,000 Pa, allowing to adjust nanoporous morphology of hydrogels improving the attach viability of species of microalgae with variable sizes and shapes, and allowing to develop better hydrogels for novel microalgae immobilization-based applications.

1. Introduction

Currently, the nanoporous hydrogels are under research owing to their physicochemical characteristics of both solid and liquid, mechanic elasticity and the ability to assemble dimensional networks, so as trapping solvents in large quantities until equilibrium swelling. Polymer matrix can be used for trapping desired chemical and/or biological species or controlled release of components of interest, harnessing these hydrogels to applications such as microalgae immobilization for environmental purposes due to the capacity of algae to accumulate potential hazardous elements or the creation of biosensors for the measurement of environmentally relevant elements (Carrilho et al., 2003). The technique of polymerization by ultraviolet light in presence of a photoinitiator has emerged as an easy and efficient method for obtaining hydrogels. It has been shown that ultraviolet light intensity to which a hydrogel photopolimerize, affects its mechanical properties and its nanostructure (Wang et al., 2008). Furthermore, one of the most important advantages of photopolymerization is the control of reaction kinetics with only enable or disable the UV light source at any point in the reaction.
Microalgae biomass is under study for several environmental applications as hydrogen production (Altimari et al, 2014), flue gas CO_2 capture (Iancu et al., 2012), biofuel production (Gonzalez-Delgado et al., 2013) and immobilization for environmental applications and water conservation. Immobilization relies on the encapsulation of living cells on a polymeric matrix which allows the movement a suspension with nutrients and other elements trough the polymer creating a bio-filtering process (Cohen, 2001), this method has the advantage of high growth rate on a reduced space and the elimination of physico-chemical processes of concentration as flocculation or coagulation (Aslan & Kapdan, 2006; Moreno-Garrido, 2008). Perullini et al. (2014), encapsulated a microalgae gender inside a nanoporous silica matrix with a high initial viability (96% of the population remained active 48 h after encapsulation). They found that the procedure allows the organisms to remain in liquid culture during the synthesis of silica matrix that would immobilize and isolate the small liquid

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culture from the surroundings. The silica matrix is mechanically stable and non-degradable by microorganisms. Additionally, its porosity can be tuned from the synthesis parameters to allow free diffusion of high molecular weight molecules. Its controlled porosity and the possibility of silica surface derivatization could allow for selective transport of particular pollutants, conferring different selectivity to each module in the arrangement.

Another way to inmobilizate microalgae is by the use of natural polysaccharides such as agars, carrageenans or alginates. Regarding to this, Praveen & Loh (2015) encapsulated *Chlorella vulgaris* in alginate beads and were added into a bioreactor treating synthetic wastewater using *Pseudomonas putida*. Authors found that during continuous operation, the removal efficiency at 500 mg/L glucose increased from 73 % without aeration to 100 % in the presence of immobilized microalgae. The initial microalgae concentration was critical to achieve adequate aeration, and the removal rate increased with increasing microalgae concentration.

Among environmental applications where immobilized microalgae can be used, is the wastewater treatment. In this case, an important variable to consider is organic material consumption and removal of contaminants, which are nutrient for algae. Caporgno et al*.,* (2015) cultivated the freshwater microalgae species *Chlorella kessleri*, *Chlorella vulgaris* and the marine microalgae species *Nannochloropsis oculata* in urban wastewater. The freshwater species demonstrated the possibility of growing in urban wastewater reaching high biomass production and nutrient removal when cultured in batch mode using a flat-panel airlift photobioreactor. Both microalgae species reached a nitrogen concentration reduction around 96% and 95%, and a phosphorous concentration reduction around 99% and 98% respectively.

Therefore, the high biocompatibility obtained between microalgae and syntethic polymer, as well as the results drawn about contaminants removal and the advantages of photopolymerization, specifically the possibility to control hydrogels average pore size and pore size distribution, suggests that the proposed technique could be applied to many other microalgae species, becoming a promising way to wastewater treatments.

2. Methodology

2.1 Hydrogel preparation

Polyacrylamide hydrogels were photopolymerized from a solution of acrylamide, N,N'-Methylenebisacrylamide (MBAm) and water. All substances were mixed in appropriate proportions to obtain a concentration of 9 % T and 5 % C (% T refers to monomer and crosslinking total mass per unit solution volume, and % C refers to crosslinking mass per unit crosslinking and monomer combined mass). 0.1% of Ammonium persulfate (APS) were added to the solution immediately before each experiment. Each sample (180 uL) was polymerized in Anton Parr MCR parallel-plate rheometer with UV curing system.

2.2 Hydrogel photopolimerization

Hydrogels were polymerized using a UV light intensity range between 140 and 700 MW/cm^2 during 0.8 h. all experiments were performed by triplicate: average pore size, pore size distribution and storage modulus (G') were calculates. two hydrogels were polymerized with the minimum and maximum values of ultraviolet light intensity as control system. For transient polymerization, light intensity was increased and decreased at a specific times of photopolymerization. In increase experiments, polymerization started at 140 mW/cm^2, and after an stablished time UV light intensity was suddenly increased to 700 mW/cm^2, and maintained at that value until the polymerization was completed. The disruption was performed for each sample at a different time of photopolymerization process, starting at 1300 until 1700 with a DetlaT of 100 s. In decrease experiments, UV light intensity was decreased from 700 to 140 MW/cm^2, starting from a few seconds after the onset of gelation (400 seconds) and increaseng the moment of the disturbance in 100 seconds. A prgressive variation was also evaluated by changing UV intensity in 12.5 mW/cm^2 every minute during the photopolymerization, 62 mW/cm^2 every 5 minutes during the process and variation in 556 MW/cm^2 at a specific time of polymerization. These variations are increases in the case of starting the hydrogels photopolymerization at 140 mW/cm^2, or decreases, in the case of photopolymerization begin at 700 mW/cm^2.

2.3 Hydrogels properties: thermoporometry and rheology experiments

All experiments were done at 20 °C and a solvent trap was used to prevent evaporation. The measurements were performed at a strain amplitude of 1%, and an angular frequency of $\omega = 1$ rad/s. During photopolymerization, Storage modulus G' was calculated using Eq (1); where n is the number density of active crosslink junction points (mol m^{-3}), G is the plateau value of the storage modulus, R is the ideal gas constant, and T is the temperature. Since rheological analysis allows us to estimate the molecular organization of the polymeric materials, and also to predict their dynamic properties (Dias et al., 2013), average pore was calculated using Eq (2), where N_A is Avogadro's number.

$$G^{'}(Pa) \cong G = nRT \quad (1)$$

$$L(nm) = \left(\frac{1}{nN_A}\right)^{1/3} = \left(\frac{RT}{G^{'}N_A}\right)^{1/3} \quad (2)$$

2.4 Microalgae immobilization and stability on hydrogel

In order to test the stability of the produced hydrogel as a immobilization matrix for algae, an experiment was designed as follows, 3 hydrogels were inoculated with 100 mL of 15 days old culture of *Chlorella vulgaris* UTEX 1803, the algae and the hydrogel were maintained on Bold Basal Culture Media (BBM) on 300 mL flask and mixed using filtered air (0.2 μm membrane filter) with 1% (v/v) of CO_2, after 15 days of culture the hydrogels were transferred to 100 mL of BBM and cultured over 20 days, once the time was completed the total biomass produced on the hydrogels and the biomass released (free biomass) form the hydrogels to the media was measured by the method described by Borowitzka & Moheimani (2013).

3. Results and discussion

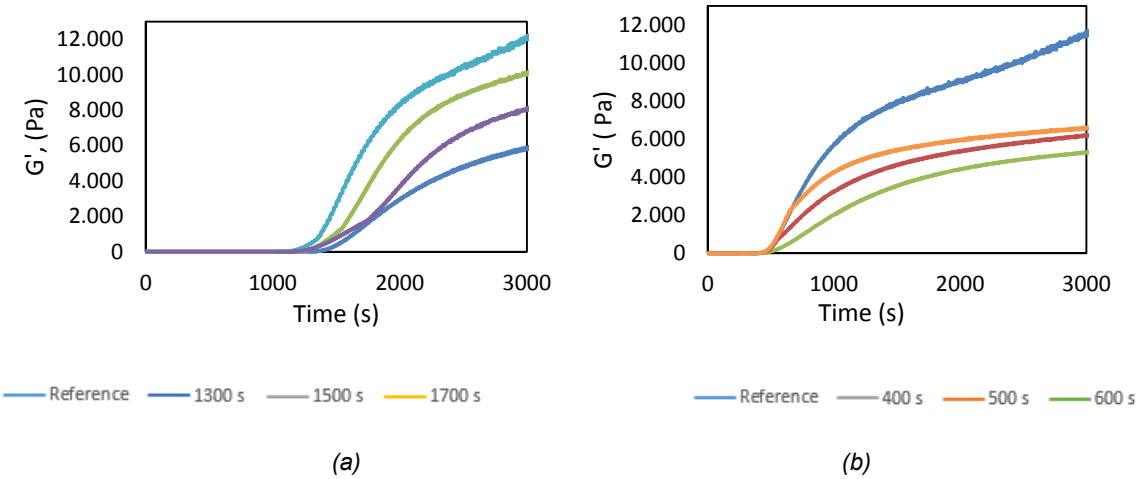
3.1 Effects of constant values of UV light intensity

The rheological characterization of the two hydrogels are presented in Table 1. The hydrogel polymerized at low UV light intensity has a greater average pore size. This is because low UV light intensity produces a slow dissociation of the photoinitiator, increasing the starting time of gelation and allowing long polymer chains be formed before crosslinking. Therefore, more structures with a greater pore size are obtained. The opposite case occurs with the sample photopolymerized at 700 mW/cm^2, where high light intensity, produces rapid reaction between acrylamide monomers and MBAm molecules, breaking long chains to obtain finally a dimensional network with smaller pores.

Table 1: Characteristics of photopolymerized hydrogels at constant light intensity

Concentration	UV intensity (mW/cm^2)	Gelation point (S)	Modulus G' (Pa)	Pore diameter (nm)
9 % T, 5 % C	140	385	5490	8.94
9 % T, 5 % C	700	1210	14100	6.58

3.2 Effects of instant shifts of UV light intensity



(a) *(b)*

Figure 1: Rheological behaviour of hydrogels that presented (a) instant increases and (b) instant decreases of UV light intensity during their photopolymerization process.

In increases experiments, perturbation causes a change in the hydrogels rheological behavior, as is shown in Figure 1 (a). It is observed that the three curves had a change in the slope of elastic modulus (G'), near to the point where it was made the disturbance, but the change is more abrupt when the instantaneous increase is

made closer to gelation point. In addition, elastic modulus value at the end of the polymerization was different for the three samples, but does not rise to be similar to any of the values shown in Table 1 for reference samples. From these results, it can be stablished instantaneous increases of light intensity affect the curing process, and the magnitude of the effect depends on the time when the increase takes place. In decrease experiments (Figure 1 (b)), all hydrogels reached the gelation point rapidly because no disturbance was made prior to this point, however, a strong decrease in their elastic modulus was observed after they reach the gelation point. This shows that a rough decrease in light intensity produces a decreasing of crosslinking rate. We can also see G' final values which are lower than those obtained when the light intensity increases.

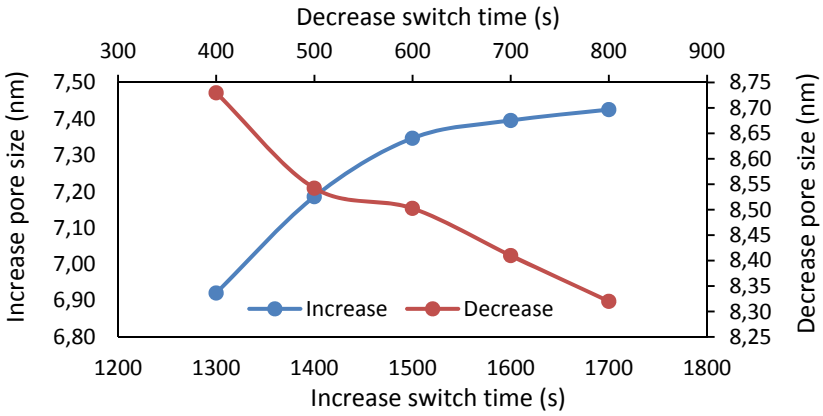


Figure 2: Average pore sizes for polyacrylamide hydrogels that suffered increase and decrease in light intensity during the photopolymerization

The effects of the instant increases in the average pore size of hydrogels at the end of photopolymerization can be seen in Figure 2, such increases reduces the average pore size of the hydrogel, being more remarkable the effect of the disturbance when this occurs earlier. In turn, while more advanced is photopolymerization process, decreasing the average pore size tends to be smaller; while time increases, the amount of unreacted MBAm is lower, being low the amount of crosslinker that responds to light intensity increases. Regarding to instant descreases, the effect observed was an increase in final pore size for all samples (red line) compared to the pore size should be if it had made no change in light intensity (6.58 nm). It was also noted that the final average pore size changes as changing the time in light intensity decrease, being the increase pore size larger if the disturbance occurred at the beginning of gelation and lower if this occurs when polymerization is already advanced. this behaviour can be explained due to the amount of crosslinker available at the disturbance time and the decrease in the free radicals production rate, although in this case, the polymerization started with a high light intensity, so the gelation point and the formation dimensional matrix was reached quickly, due to this, the time effect was less pronounced compared to instant increases.

3.3 Effects of gradual shifts of UV light intensity

The rate of light intensity changes in the time interval ($\Delta UV/\Delta t$) for each sample was calculated and plotted versus the average pore diameter obtained as is shown in Figure 3. It can be noted that the hydrogels which light intensity decreased show an increase in pore size to the extent that the disturbance becomes constant with time. Samples which light intensity increased presented a smaller pore size when a high magnitude change is instantaneous. In addition, pore size diameter increases while intensity change is distributed throughout the polymerization time. We may also observe that for any of the experiments, the pore size obtained is equal to the pore sizes that have these samples when they are not performed any disturbance.

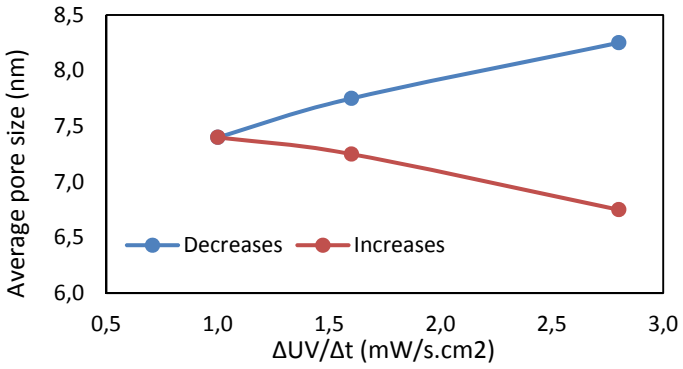


Figure 3: Pore sizes variation for polyacrylamide hydrogels as effect of gradual variations at different rates of change

3.4 Microalgal Stability on hydrogel

Hydrogels made in decrease experiments showed a higher immobilization rate than those made from increase experiments, this can be explained by the closer range of average pore sizes during polymerization process, high immobilization rates lead to evaluate the environmental application of microalgae-polymer matrix for bionanosensors in which microalgae serves as indicator of undesired compounds in a effluent, and for decrease of microalgae cultivation area which decreases the amount of water required, with a positive environmental impact related to resources conservation. Taking into account last application mentioned, Figure 4 shows the microalgae biomass concentration using hydrogels, it is shown that *C.vulgaris* can effectively attached to the hydrogel and present a larger biomass production (2.3 g/L) than a normal culture (1,8 g/L) under the same cultivation conditions. One of the biggest milestones on the microalgae immobilization is the relation between size pore and cell size, mainly because of larger pores on the gel may not effectively retain the cells and allowing the algae go free on the media, on this experiment results shown that the release from the hydrogel to the media is less than 5% (0.1 g/L) of the total biomass produced; this results demonstrate that hydrogels produced in this way can retain the algae cells for as long as 20 days without losing significant amounts of biomass in the surrounding media.

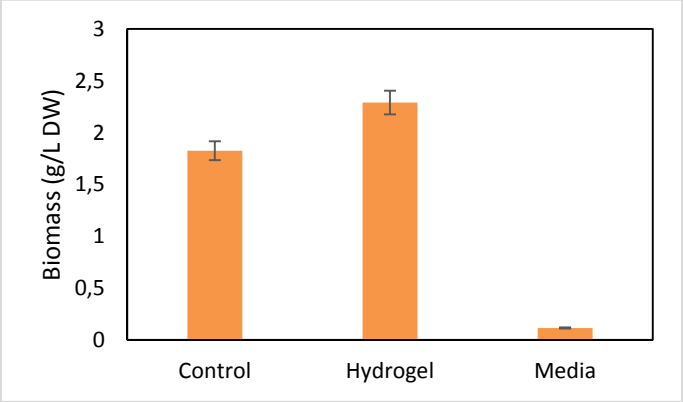


Figure 4: Comparison of Biomass production (g/L of DW) on a without immobilization (Control), the hydrogel and the cells released to the media from the hydrogel.

4. Conclusions

A variation of UV light intensity during the polymerization process has an inverse effect on the average pore size of hydrogels when the polymerization is completed. The final pore size depends on the time when perturbation is performed, the type of disturbance (increase or decrease of ultraviolet light intensity) and the way in which this is distributed during the curing process. Increasing the intensity of UV light during the photopolymerization of hydrogels of polyacrylamide crosslinked with MBAm, produces an asymmetric rheological behavior extended by a wide margin of average pore sizes for different times, while reducing the intensity of UV light produces a more symmetrical distribution with a tendency to stabilization at long times.

Proper manipulation of UV light intensity during the photopolymerization of polyacrylamide hydrogels, allows to obtain hydrogels with the same average pore size, but different matrix formation mechanisms, which is an interesting finding, since to date the only way to obtain gels with these characteristics was preparing two solutions of different crosslinking and monomer concentrations before polymerization, while manipulating the ultraviolet light allows to obtain the same concentration, on the other hand, incorporation of tailored hydrogels to microalgae cultivation units allow to retain the algae cells for as long as 20 days without losing significant amounts of biomass in the surrounding media, showing the promising ability of being used for environmental applications. Further studies can be focused on study of effect of transient photopolymerization on modification of gel point, with pose size distribution, and photopolymerization of hydrogels including cultivation media with microalgae during the process for evaluation of bionanosensors.

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