Ph.D. Comprehensive Examination Written Report

HIGH THROUGHPUT PREPARATION AND TESTING OF SLUDGE DEWATERING POLYMERS

by

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PART I: Literature Review

1.1. Introduction

Sludge dewatering is an essential unit operation in wastewater treatment processes. Basically, it consists in the reduction of the moisture content of sludge or biosolids, which primarily serves in the reduction of processing, environmental and handling costs during the treatment process [1], specially before the sludge drying, an energy intensive process [2].

Several physical methods for moisture removal have been implemented, but none of them provide a high efficiency method of capturing solids without the use of dewatering chemicals (coagulants and flocculants) [1] that allow retaining the solids in the previous flocculation step. Therefore, the development of dewatering aids is an important topic in the wastewater treatment industry.

On the other hand, high throughput techniques, including combinatorial synthesis and fast characterization methods have been developed and implemented in the fields of polymer science, pharmaceuticals, and material science with great success, giving researchers an opportunity to develop new materials faster and more effectively [3–5].

This research proposal report will focus and examine different high throughput techniques in the development of new dewatering polymers for wastewater treatment.

1.2. Sludge dewatering process

Sludge is described as the slushy or muddy mass mixed with water at different levels. The wastewater sludge is a complex system conformed by different substances like: organic and inorganic matters, dead cells, microorganisms, dissociated molecules on the surface of particles, etc. These form structures known as "flocs" [6]. Most of these suspended solids are colloidal, containing large amounts of water in their structure as chemically bound water, surface water and interstitial water (Figure 1), which give them a low settleability, low density and difficult mechanical dewaterability [7].

Figure 1: Dewatering methods depending on the water distribution in the sludge (Reprinted from ref. [7]).

The conventional mechanical dewatering methods include: filtration, in which a difference of pressure is applied causing the fluid to flow through small holes of a cloth, impeding large particles of flowing through the material, forming a porous cake on top; sedimentation, in which the particles are separated by gravitational forces, acting over the various density and size of the particles; centrifugal separation (filtration or sedimentation), similar to the previous method mentioned, but this time the driving
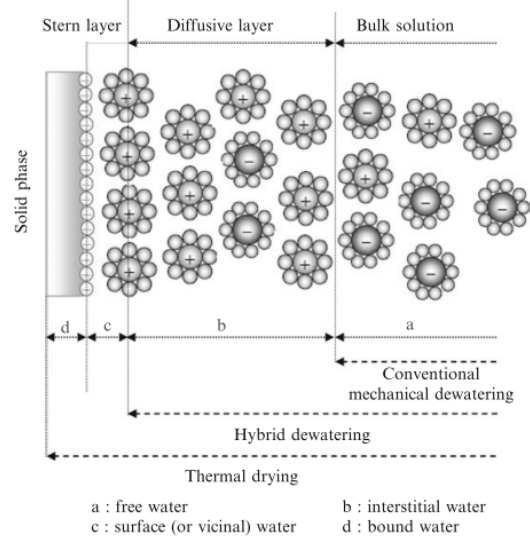
force that allows separation are centrifugal forces; and finally mechanical size reduction, where the particles and broken mechanically and separated according to size [8].

In this report cake filtration will be one of the methods used characterize dewatering properties under different additives; therefore, modeling on cake filtration can be used as Holdich [9] explains in his book, by defining that Darcy's law is applied to the filter medium and the forming filter cake, obtaining:

$$\Delta P = \frac{\mu*L}{A*k}\frac{dV}{dt} + \frac{\mu*L_m}{A*k_m}\frac{dV}{dt}$$

Eq. 1

Where ΔP is the membrane pressure drop (N/m^2), μ is the viscosity of the liquid (Ns/m^2), A is filtration area (m^2), k and k_m the permeability of the cake and medium, L and L_m the cake height at time t and medium height (m), V is the filtered volume (m^3) and t is filtration time (s). From there, The resistance of the medium and the cake are equal to $R_m = L_m/k_m$ which is constant during filtration and $R_c = L/k$ in which L will increase as the filtration goes. R_c can be also expressed in terms of α specific resistance of the cake (m/kg^1), for incrompressible cakes would be: $R_c = \alpha cV/A$, where c the dry cake mass of cake per filtered volume (kg/m^3).

In the case of working with compresible cakes, the concentration of the cake (C) is a function of the pressure drop applied during filtration. Hence, the specific resistance (α) is related to C and to the porosity (ε) through the permeability (k) of the solid cake, following these equations:

$$\alpha = \frac{1}{kC\rho_s} \, , k = \frac{\varepsilon^3}{K(1-\varepsilon)^2 S_v^2}$$

Eq. 2, Eq. 3

Where ρ_s is the density of the solid (kg/m^3), K is the Koseny constant (often considered 5), and S_v the specific surface area per volume (1/m).

Therefore, a fundamental step in the dewatering process is the pretreatment with chemical additives in which the flocs are formed. This step is called flocculation, and the dewatering polymers are prefereably flocculants that help the sludge particles to form big and stable flocs to aid the dewatering mechanical process.

1.3. Sludge dewatering polymers

Sludge dewatering aids are generally divided in coagulants and flocculants. Most of the literature mentions the terms "coagulation" and "flocculation" as different stages of particle aggregation, even though some of them are not very clear on their differentiation [10–12].

In this report, coagulants are additives that help the colloidal particles overcome the barrier of inter-particle repulsive energy, causing their aggregation [13]. This process is explained by different approaches to the DLVO theory (Deryagin & Landau, Verwey & Overbeek) that combines electrostatic, hydrophobic and hydration forces to describe mechanisms of destabilization or compression of the electrical double layer [14], [15]. Typical coagulants agents used in the industry include cationic inorganic salts, as aluminum (III) and iron (III), however, their cost, poor ability to form strong flocs and their dangerous disposal due to corrosion [12] give them considerable disadvantages.

On the other hand, flocculants are agents that assist to continue the coagulation process and by the transport process of flocculation, cause the collision of the destabilized particles in larger particles, the aforementioned flocs [12], [16]. The most used flocculants in the industrial market are: cationic in nature as quaternary amines derivatives (e.g. polyDADMAC) and cationic polyacrylamides, anionic flocculants, generally composed of acid carboxylic derivatives of polyacrylamide, and non-ionic in nature like polyacrylamide, as natural polymers as some polysaccharides [11], [17].

Furthermore, systems with one or more flocculants acting at the same time have shown their enhanced action in the coagulation/flocculation process. Lee and colleagues [13] have reviewed and classified "hybrid" materials developed as flocculants, by their production (structurally, chemically-bound or functionally hybridized materials), and by combinations of their nature (inorganic, organic, natural polymer and bio-polymer).

In this report focus will be geared towards the system "organic-natural", or better referred as "synthetic-natural" hybrid polymers, since their enhanced biodegradability could be an important asset for the treatment plants nowadays. Several studies for natural polymers with synthetic polymers have been done, where the main streams include polysaccharides grafting onto either polyacrylamide (PAM) [18–21] or cationic quaternary ammonium compounds [22–25].

1.4.   Flocculation mechanisms

The mechanisms of action of these polymers can be divided in three types: bridging, electrostatic patch and depletion flocculation; the first two the most important in the flocculation in wastewater treatment process [11], [26]. These mechanisms rely in the polymer adsorption onto the sludge particles (as noted in Figure 2); in the case of bridging flocculation, the polymer chains adsorb onto one or more particles and link them together to generate bigger particles. The polymer can have different conformations when adsorbing the surface of the particles, like loops (segments spreading into the solution) and trains (segments absorbed onto the particle surface) [26]. Under this mechanism, the polymer adsorption should be enough to adsorb onto a few particles at the same time, because if the attraction is too strong, the polymer chains will totally adsorb onto the surfaces, provoking re-stabilization of particles [27]. According to Bolto [11], the practical systems of flocculation have optimum dosages of polymers about 1 mg of polymer per gram of suspended solid or less, and also stated that high molecular weight and linear polymers should work preferably with this type of mechanism. On the other hand, electrostatic patch mechanism happens when high charge density polymers adsorb onto the opposite charged particles, generating "patches" with different charge onto the particles [16]. This produces an attraction between positive patches and negatively charged areas from other particles, causing flocculation (Figure 2). The electrostatic patch flocculation is not expected to form stronger flocs that bridging flocculation, however in the
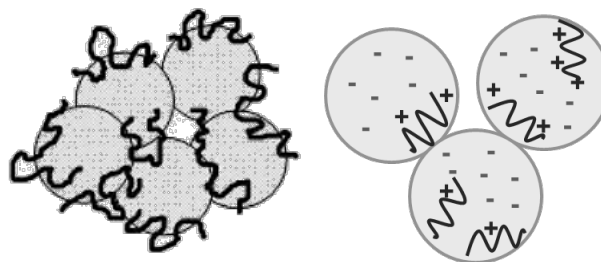

Figure 2: Left: Bridging flocculation. Right: Electrostatic patch flocculation. (Adaptation from ref. [11]).

case of flocs breakage, re-flocculation occurs more easily in patch flocculation than in bridging flocculation [28].

1.4.1. Flocculation kinetics

Generally, the flocculation kinetics can be classified in two processes: perikinetic and orthokinetic flocculation. In the first one, perikinetic flocculation or micro-flocculation, (driven by Brownian motion of fluid particles) is the aggregation of destabilized colloidal particles to start forming bigger conglomerates or flocs. The second one, orthokinetic flocculation or macro-flocculation, is the aggregation of colloidal particles by inducing velocity gradients, in which fast particles can collide against slow particles to produce aggregation and growth [1], [27].

Thomas and collaborators [29] presented a complete review in flocculation modeling, in which they presented the Smoluchowski theory of coagulation kinetics of colloids and described their assumptions and limitations, as for example: the collision efficiency is equal to 1 for all collisions (of highest efficiency), laminar shear as fluid motion, all particles have the same size, there is not breakage of agglomerates, all particles are spheres, even before and after collision happens, and only two particles are present in the particle collision.

The applicability of this theory to real systems has been extensively studied in order to decrease the use of these assumptions [30–32]. For instance, Camp and Stein [31] in 1943 studied the effect of orthokinetic flocculation in tanks, and re-established Smoluchowski's equation in terms of operation and design in flocculation mixers by using the average velocity gradient (G) as the key parameter, associated to the τ detention time (s), Q is the flow rate (m^3/s), P is power requisite (W), V equipment volume (m^3), and μ the dynamic viscosity (N*s/m^2). Unfortunately, this equation cannot be used in perikinetic flocculation, since the highest values of G cannot produce an small enough eddy to provoke the removal of particles less than 10 μm [1], [33].

Also, according to latter research, the floc size and the mass distributions are determined by the balance between floc formation and breakage [34]; Yeung and Pelton [35] also described that the model of agglomerates breakage depends on the floc compactness. Therefore, more compact flocs tend to reflocculate more and follow the erosion breakage model, while less compact flocs tend to break by fragmentation. The morphology of the flocs is being described by fractal geometry theory develop by Mandelbrot, and explained for flocs in dewatering by Li [36].

For those reasons, Yang and coworkers [37], have recently published a model that combines the modified Smoluchowski model [38] and fractal theory, in which they employed them in a real flocculation system by using two equations:

$$\frac{d\left(\frac{N_t}{N_{initial}}\right)}{dt} = -N_{initial} * k_1 \left(\frac{N_t}{N_{initial}}\right)^2 + k_2 \left(\frac{N_t}{N_{initial}}\right) \qquad \text{Eq. 4}$$

$$I \propto Q^{-D_f} \qquad \text{Eq. 5}$$

Where in $N_{initial}$ and N_t are the number concentrations of particles at initial time and time t, k_1 and k_2 kinetic constants for the aggregation and breakage of the particles respectively, and, I the

scattered light intensity, Q the modulus of the scatter vector and D_f the fractal dimension. Therefore, using light scattering technique in a Malvern mastersizer, the flocculation kinetics can be analyzed for experimental data.

In the case that the measurements of large flocs would not be possible in a high throughput fashion by light scattering; there is an additional way to indirectly compare flocculation kinetics, by using gravity settling experiments, since according to Stoke's equation settling velocity is related to the particle size [39]. In hindered flow (when a large number of particles is present) the Stoke's equation is valid for laminar flow (Reynolds number < 1) with the introduction of a correction factor ($\varepsilon^2\psi_p$) that depends on the volume fraction of the slurry (ε) and an empirical correction factor ($\psi_{p)}$), giving a relationship for the settling velocity (υ_t) (m/s):

$$\upsilon_t = \frac{gD_p{}^2(\rho_p - \rho)}{18\mu} * (\varepsilon^2\psi_p)$$

Eq. 6

Where the particle diameter is (D_p) (m), the densities of the solid particle and liquid medium (ρ_p and ρ) (kg/m^3), the viscosity of the medium (kg/ms), and the gravitational acceleration (g) (m/s^2). As shown by Geankoplis [8], the settling velocity can be experimentally obtained by measuring the interfaces heights in a batch settling test. This approach might be useful in the following sections of this proposal.

1.5.   High throughput techniques

The development of new materials during the last two decades has been greatly influenced by high throughput screening (HTS) methodologies [4]. Basically, HTS is a process that brings the parallel production of several materials, in conjunction with different measurements (also high throughput) to characterize either, intrinsic or performance properties in the target materials [40]. These techniques respond to the need of covering the wide possibilities of combinations that components can have, referring as components as every single variable, as materials compositions, operation conditions and process factors that are possible to change in a determined development.

In order to successfully achieve a high throughput development, the automation of experiments, miniaturization, data collection and data management are key aspects [41]. Also, it is important to mention that polymer discovery has many challenges as mentioned by Meredith [42] like: most of the variables are continuous (just a few yes/no tests available), no universal screen for properties, or sometimes the properties are "size-dependant".

In the current proposal, the use of statistical tools as Principal Component Analysis (PCA) and Partial Least Squares regression (PLS) to analyze the data obtained will allow us to produce Quantitative structure–property relationship (QSPR), a modeling technique that will help us to refine our development. QSPR is based in the fact that after producing a sizeable amount of data for a high throughput workflow, the resulted property of that set of compounds can be related to a descriptor, an independent property of the materials, in that way the design of experiments and models become more robust [43], [44].

Principal Component Analysis (PCA) consists on the analysis of a matrix of uncorrelated and orthogonal variables (principal components), generated from the product between two matrices

of correlated variables (original observations) [45]. In that way, the component with the highest source of variance in the data is obtained. This model does not differentiate between the dependencies of variables; in consequence, it may have ambiguous results sometimes.

On the other hand, Partial Least Squares regression (PLS), comprises the identification of correlations between blocks of data for X (independent) and Y (dependent) variables; being a powerful tool to find hidden correlations among the data, and create QSPR models from there. Dr. McGregor and colleagues from McMaster University have dedicated several research projects on the fundamentals and applications of these models [46], creating a multivariate program, ProSensus ® Multivariate, that easily assists in the analysis of large sets of data, especially if many variables are involved.

PART II: Research Proposal

2.1. Literature pertinent to the proposal

As mentioned above, this proposal will be focused the preparation and testing of "synthetic-natural" dewatering polymers. The grafting of polysaccharides onto polyacrylamide is one of the sections of this report, and Singh and colleagues [19] have done extensive research on grafting some natural polymers onto polyacrylamide, especially interesting: Glycogen (a highly branched polymer of glucose, similar to amylopectin) [47], and Dextran [48] (polymer of glucose with a 95% of α-(1→6) glycoside linkages) [49].

These polymers are non-ionic flocculants and they are produced by using Ceric IV ammonium nitrate $(NH_4)_2Ce(NO_3)_6$ as a free radical initiator, an inert atmosphere that generates free radicals from the polysaccharide backbone through the mechanism proposed in Figure 3. After grafting, the polymer is generally precipitated in acetone, filtered and dried. These grafting reactions have the advantage to be accomplished in water, under fairly mild conditions (50 °C and nitrogen atmosphere) that can be extrapolated to high throughput syntheses.
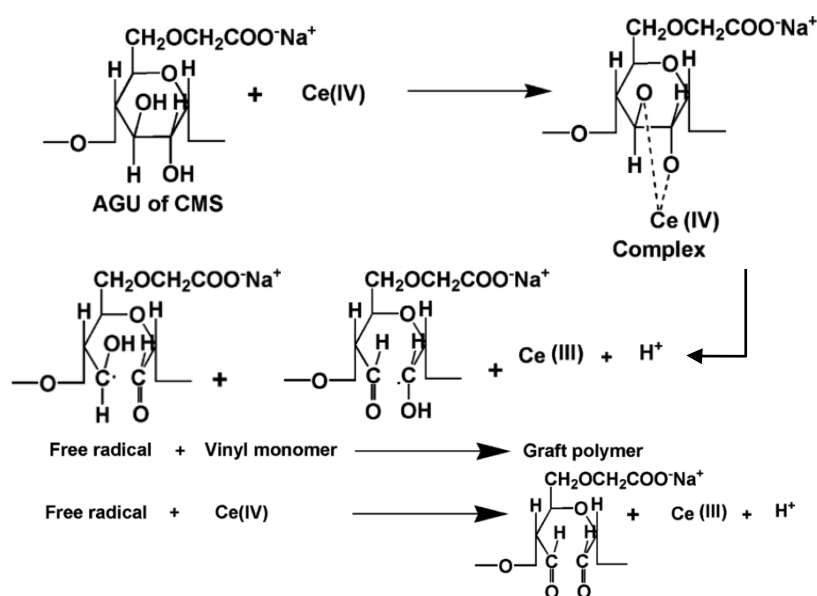

Figure 3: Mechanism of synthesis of carboxymethyl starch – PAM grafted polymers by using Ceric (IV) ammonium nitrate as initiator (Adaptation from ref. [82]).

Further typical characterization techniques used for these flocculants include FT-IR spectroscopy, X-ray photoelectron spectroscopy (XPS), viscometry, elemental analysis and differential scanning calorimetry (DSC). Additionally, typical flocculation techniques such as turbidity, sedimentation rate, and the jar test were tested as well [20], [24], [48], [50], [51].

Additionally, cationic hybrid polymers are also of interest in our scope, and the use of quaternary ammonium compounds with glycidyl moieties and long hydrocarbon chains looks promising for high throughput techniques.

In our interest, Sajomsang and colleagues [52] quaternized Chitosan (a polysaccharide composed by glucosamine and N-acetyl glucosamine moieties), and Nichifor and collaborators [53] have done extensive research quaternizing Dextran and some other polysaccharides with several quaternary ammonium chloride compounds.

They summarized about 10 years of research development with these compounds, proposing a procedure to generate quaternized compounds by varying the substituents in quaternary and tertiary amines. The synthesis comprised the use of epichlorohydrin, tertiary amines and a polysaccharide as raw materials, in water as solvent, without any catalyst, keeping the temperature at 45° C for 24 hrs., as shown in Figure 4. The product was purified by precipitation in mixtures of methanol/acetone, and then re-dissolved in water and dialyzed against water in an ultrafiltration cell until the conductivity of the dialysate was close to the conductivity of deionized water.

For characterization they used several methods, being elemental analysis, FT-IR spectroscopy, Nuclear Magnetic Resonance (NMR), potentiometric titrations and antibacterial activity (the most important) [25], [54]. Also, they reported some flocculation studies for a "surfactant-like" polymer created with an octyl hydrocarbon substituent in the tertiary amine. Those results were investigated by turbidity, zeta potential and particle size measurements, in which the larger the polymer initial concentration, the larger the flocculation window [55].
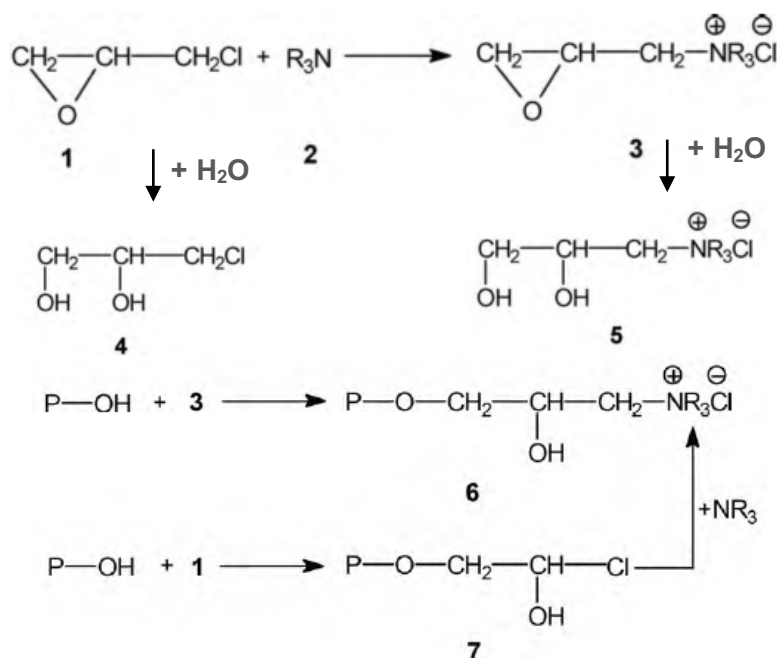

Figure 4: Proposed mechanism of polysaccharide quaternization with equimolar mixtures of epichlorohydrin/tertiary amine, where P-OH is a polysaccharide (Adaptation from ref. [53]).

2.2. Proposal objectives

The hypothesis of this proposal is to establish that: dewatering water-soluble polymers, specifically eco-friendly hybrid polymers, can be successfully prepared and characterized using high throughput (HT) techniques; also, their efficiency as sludge dewatering aids can also be tested by the use of these kinds of methods.

In order to verify this statement, the short-term objectives are:
- To develop a high throughput (HT) production sets of eco-friendly dewatering polymers by using: polysaccharides (dextran, glycogen and chitosan), and acrylamide.
- To repeat the previous point but this time by using quaternization of polysaccharides with tertiary and quaternary amine derivatives.
- To develop HT techniques for characterization of these polymers like FT-IR spectroscopy and MALDI-TOFF mass spectroscopy.
- To validate the combinatorial methods of fabrication of polymers by characterizing the prepared polymers via HT techniques.
- To develop and validate HT techniques for analyzing dewatering characteristics as: flocculation efficiency, sedimentation rate, floc structure and specific resistance of filter cake.
- To validate the proposed HT tests for sludge dewatering characteristics by generating similar assays in a classical way, and compare.
- To work in collaboration with other research groups or industrial partners to develop robotic pieces of equipment that allow us to automatize essential techniques of characterization of polymers like DLS and zeta potential analysis.
- To create statistical models for multivariate analysis of all the results at different stages of this proposal.
- To identify the best combinations of new hybrids polymers for sludge dewatering, without the need to generate large batches and time consuming methods for testing the efficiency of dewatering.

Additionally, the long-term objectives include:
- The creation of a database complemented with statistical tools to have easy access to the properties and results of hybrids dewatering polymers that were synthetized, characterized and tested by using HT methods.
- The establishment of a combinatorial production and HT characterization of new dewatering polymers which are composed from a natural and a synthetic compounds, giving the opportunity to create hybrid polymers that are more environmentally friendly in the water treatment field.
- The routine implementation of fast and HT assays for measuring the dewatering characteristics of the new flocculant polymers, to analyze large amount of different polymers with only small amounts of sample.
- The transfer of these HT methods to the industry of water treatment, by offering collaboration University-Industry, creating application patents, implementing some HT analysis in water treatment plants, and fabricating these polymers in partner companies that supply these types of additives to the water treatment industry.

2.3. Methods and proposed approach

The selected synthetic polymers are shown in Figure 5. All the experiments will be done at the Biointerfaces Institute at McMaster University, otherwise noted. The reason to choose these synthetic monomers was because their known ability as dewatering agents, interaction with natural polymers, and commercial availability. In the case of the natural polysaccharides, three were chosen for their commercial availability in different molecular weights and, in particular: Dextran for its comparative studies available [54], [56], [57]; Glycogen for its highly branched structure, and its identification with the best performance in flocculation tests by Singh et al [58]; and Chitosan for being the second largest natural polymer available in the world [59].
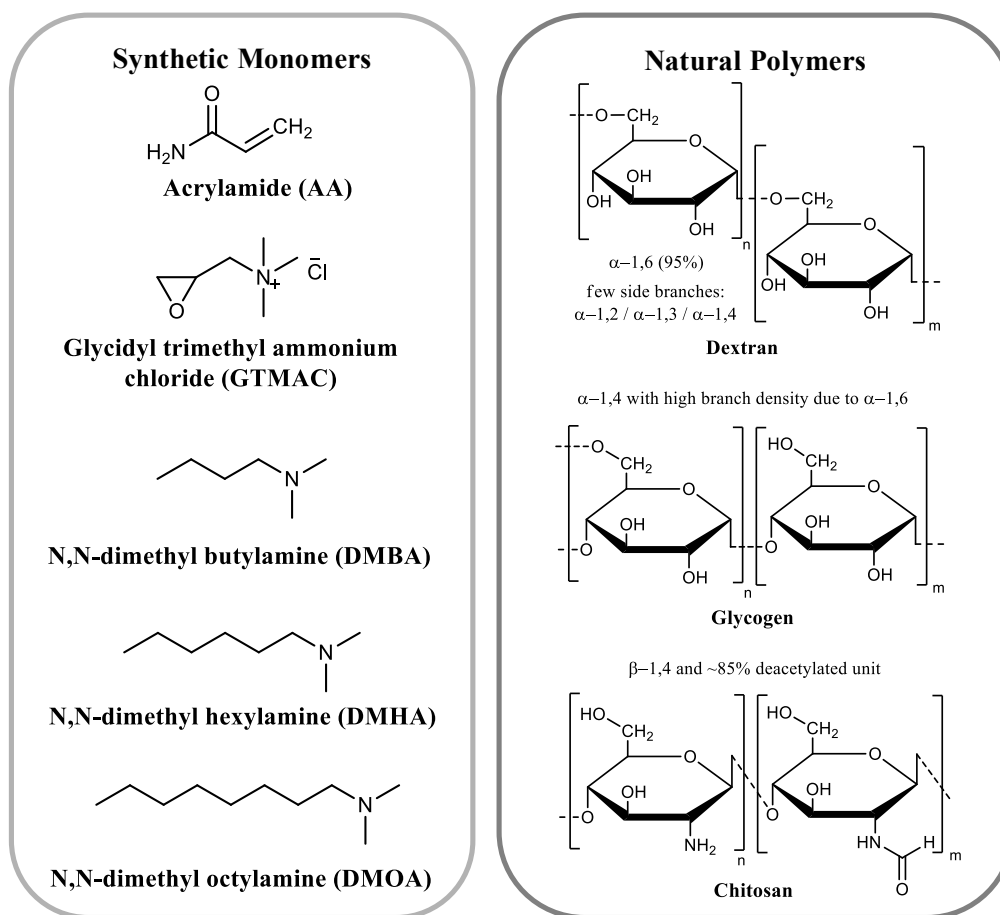
Figure 5: Selected compounds for high throughput synthesis approach.

2.3.1. High throughput synthesis of dewatering polymers

The grafting reactions will be run in a Tecan Evo 200 automated materials screen and synthesis system – specifically in a 24-mini reactors block shown in Figure 6, connected to a nitrogen atmosphere, with temperature and shaking controlled. Under this setup, up to 10 mL of each polymer can be synthetized in an aqueous media. The caps on each mini-reactor are provided with a septum, so the robot can dispense each of the reagents into each vial without human

interference. For that, all the solution reagents will be dispensed in glass test tubes or plastic rectangular reservoirs, in that way the pipetting arm can have easy access to them.

The procedures will be divided in 3 steps: reagents preparation, polymer synthesis and purification of products. Also, grafting reactions for each polysaccharide will be divided in 3 groups: reaction with acrylamide, reaction with glycidyl trimethyl ammonium chloride, and reaction with tertiary amine derivatives, ending up with 9 sub-groups of reaction conditions.
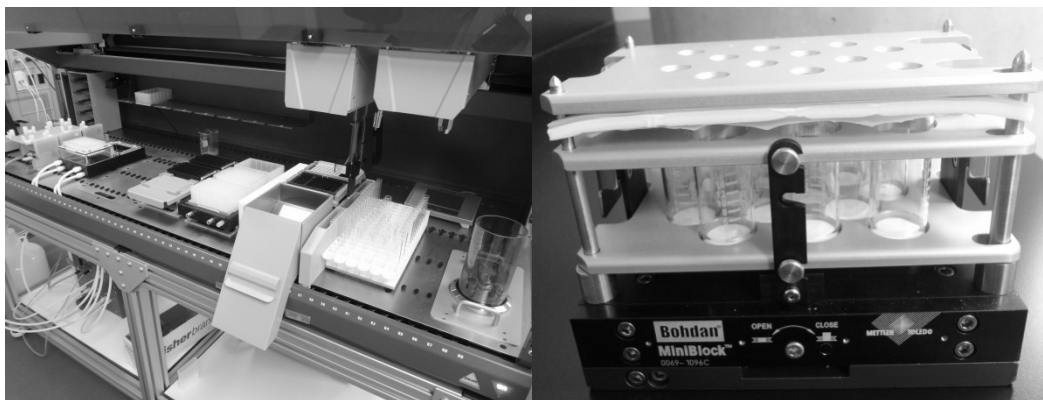

Figure 6: Left: Tecan Evo 200 automated materials screen and synthesis system.
Right: 24-mini reactors block.

All the polysaccharide reagents can be bought at Sigma-Aldrich. Dextran with 3 molecular weights (Mw: 6000, 40000 and 100000) will be used on each reaction; Glycogen from oysters (Type II) in 3 different initial concentrations will be used; and Chitosan of low molecular weight (viscosity of 20-300 cps), medium molecular weight (viscosity of 200-800 cps), and chitosan extracted from shrimp shells (viscosity of >200 cps) will be used. Dextran and glycogen will be dissolved in deionized water previous the reaction, to generate the reagent stock solution for each, while Chitosan can be diluted in 0.1 M acetic acid / 0.1 M sodium acetate buffer. Initial solutions will be 0.75% w/v in the corresponding solvent.

2.3.1.1. Preparation of polysaccharide with acrylamide (AA)

The preparation will proceed via Ceric (IV) initiation reaction, as explained in section 2.1 with some modifications to adjust the procedure to a high throughput operation. First, injection of the required amount of polysaccharide solution and 6 ml of deionized water into the mini-reactors will be done, followed by increasing the temperature to 30° C, and vortex agitation at 250 RPM to homogenize the system for 10 min under nitrogen atmosphere. The dissolved acrylamide amount will be injected into the reactors, and 30 min will past to allow homogenization. Consequently, 1 mL of a 0.03 mM Ceric ammonium nitrate aqueous solution will be injected, and the system under N_2, 30° C and vortex agitation will be kept for 24 hrs. for Dextran and Glycogen and 3 hrs. for Chitosan, according to reported classical procedures [47], [57], [60], [61].

Final soluble polymers in aqueous media are expected for each reaction, and the purification step will be: 1 mL of the product will be dispensed in a 2-mL deep well plate, then 1 mL of acetone will be dispensed and the deep well plate will be shaken for 5 minutes to allow polymer

precipitation. Following that, the solution excess is removed and a few more washing steps with acetone and agitation are executed to allow more precipitation. The product is re-dissolved in 2 mL of water and transferred to test tubes to freeze dry them in a final purification.

2.3.1.2. Preparation of polysaccharide with Glycidyl trimethyl ammonium chloride (GTMAC)

Dextran and Glycogen polymers will be dissolved in a solution of 5% NaOH, and GTMAC will be injected instead of polyacrylamide. The polysaccharide will be injected and the temperature will rise to 40° C with stirring for 10 min to homogenize. Then, the quaternary compound is injected and the conditions of temperature and stirring at 250 RPM are kept for 24 hrs. The same purification procedure is followed, with the only change that the precipitation solvent will be now a mixture of methanol/acetone and isopropanol for dextran and glycogen, respectively. Most of the procedure is done by the robotic system; therefore up to 24 samples can be prepared at the same time. Classical procedures are found in the literature [62], [63].

In the case of Chitosan derivative polymers, the procedure is modified due to the grafting can happen either in the hydroxyl and amine moieties in the polysaccharide, and chitosan is not very stable at high pH. Therefore, for GTMAC the preparation follows the same guidelines mentioned above, but it is added slowly in 3 portions separated by 2 hrs. each. After 24 hrs. of reaction at 40° C and constant stirring at 250 RPM, the solution is purified by mixture acetone/ethanol following the typical purification procedure mentioned above, in a modified process presented by Lim et al. [64].

2.3.1.3. Preparation of polysaccharide with tertiary amines and epichlorohydrin

The reaction will be carried in mini-reactors according to mechanism presented in Figure 4. The polysaccharide is injected in 6 mL of deionized water, and the system sets at 40° C and constant stirring at 250 RPM, followed by the injection of the required tertiary amine. Stirring will be kept for 5 minutes to homogenize and injection of epichlorohydrin is done, to let the grafting reaction to be carried out for 24 hrs. The purification will be done by precipitating the resulted polymer in acetone, following the procedure presented in section 2.3.1.1.

2.3.2. High throughput characterization of dewatering polymers

The most important parameters in characterization of these polymers are their functional groups analysis, molecular weight, particle size and surface potential. All these parameters can be obtained using different techniques and our focus will be to propose possible high throughput approaches to them.

2.3.2.1. Sample preparation for high throughput characterization

The samples can be required in different physical forms and formats depending of the experiment to be conducted. After purification, the samples will be in their solid form, ready to be dissolved in water.

Diluted samples: The dissolution can be done in the synthesis robot. The arm transports each empty tube to the Mettler Toledo SAG 204 balance connected to the equipment; weights are collected and storage in the software. Afterwards, the solids are manually dispensed on the weighted tubes, and the new weight is registered. Finally, a known volume of water (or any other solvent) is dispensed in each tube. If needed, the tubes can be stirred in a vortex.

Imprinted samples: Additionally, printed array with samples can be prepared at different scale size. If macro spots are needed Tecan Evo 200 automated materials screen and synthesis system can be used to dispense between 1-5 µL drops onto a surface (generally a glass slide or silicon wafer). In the case that micro spots are needed, the Scienion SciFlexArrayer S5 Non-Contact Microarray Printer with special coated glass slides can prepare microarrays with spot sizes between 80-250 µm, by dispensing between 50-800 pL per drop (multiple drops by spot can also be dispensed). It is worth to mention that the particle size for this application should not be above 200 nm otherwise the particles might clog the dispensing nozzles.
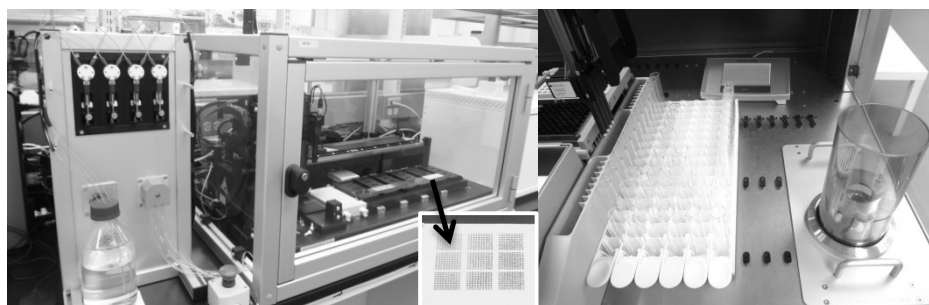

Figure 7: Left: Scienion SciFlexArrayer S5 Non-Contact Microarray Printer. Bottom-middle: 9 blocks of 10x10 microarrays (200 µm spot size). Right: Weighting and dissolution station.

2.3.2.2. Functional groups and degree of grafting

By using Fourier transform infrared spectroscopy (FT-IR) the functional groups on the polymer and the degree of grafting can be obtained. The method is based on the measurement of the adsorption due to the vibration that molecules present at some characteristic IR frequencies, when the sample is placed in the IR beam path.

Two high throughput options can be used in the new FT-IR equipment at the Biointerfaces Institute. First, solid samples can be dispensed in a Bruker HTS-XS 96-well Microplate Reader, and the equipment can obtain the IR spectrum for each sample in a transmission or reflectance mode (Figure 8). The second option is by using Bruker HYPERION FT-IR Microscope and a microarray produced by the method explained in the previous section. In this mode, 2D and 3D image (Figure 8) analysis of the surface in the different spots can be accomplished at different ranges of frequencies, to check differences in functional groups between samples.

Afterwards, the degree of grafting can be obtained from this analysis, by picking a characteristic peak for the substituent and analyzing the differences in signal in the spectra. Also, other typical and useful techniques for this calculation are chemical analysis or potentiometric titration.
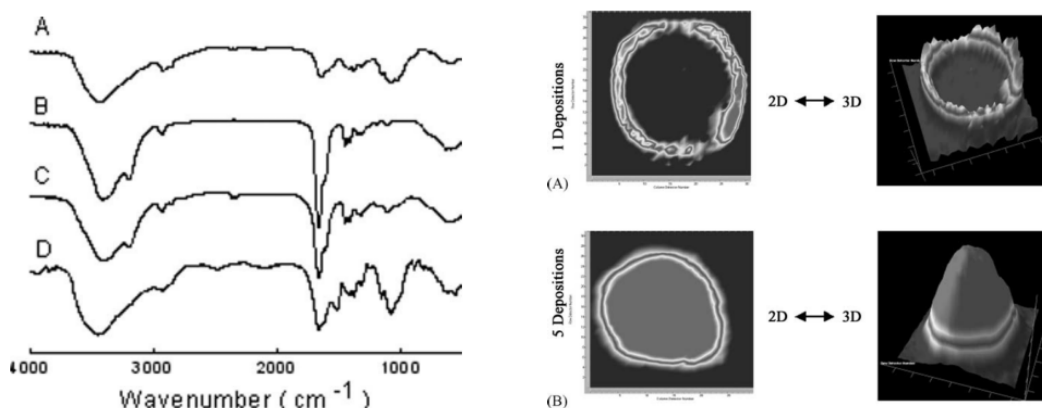
Figure 8: Right: Infrared spectra of Chitosan (A), PAM (B), and mixture of both (C). Reprinted from [61]. Left: 2D and 3D Chemical images from IR-microscope, integrated intensities between 1200 and 960 cm⁻¹ at different spotting deposition (1 deposition in A and 5 depositions in B). Reprinted from ref. [83].

2.3.2.3. Molecular weight characterization

The averages molecular weights can be obtained in a high throughput fashion by using matrix-assisted laser desorption/ionization - time of flight mass spectrometry (MALDI-TOF MS). In this technique, the analyte must be dissolved in a matrix, and the solutions are imprinted on microarray slides or MALDI plates (as explained in the previous section), to leave them to dry and co-crystalize at room temperature. After that, laser beams (patented IR radiation technology) impact a sample, in which the matrix compound adsorbs the laser irradiation, helping the analyte to desorb from the surface without suffers impact. Then the analyte molecules are ionized, and accelerated by an electric potential, therefore smaller ions will travel faster to the TOF detector than bigger, and the spectrum is acquired as in Figure 9. The ionization mechanism are still on debate [65].
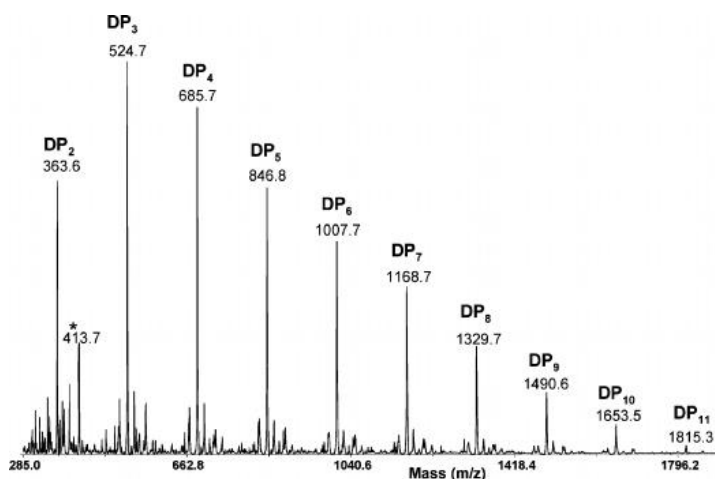
Figure 9: MALDI-TOF mass spectrum of the mixture of deacetylated chito-oligosaccharides (matrix: DHB) (Positive ion). The peaks show the different molecular weights found. Reprinted from ref. [84].

The following techniques are essential for the characterization of these polymers; however, the technologies in high throughput fashion are not available in the laboratory. Thus, some options to be developed are presented.

2.3.2.4. Particle size distribution and net surface charge

The particle size distribution can be accurately measured using Dynamic Light Scattering (DLS).

In this technique the particles suspended in a solvent, in which a laser produces scattered light through the sample, and the rate of fluctuation of the light intensity due to Brownian motion, is measured by a detector. This value is related to the translational diffusion coefficient (D), using the Stokes-Einstein equation [66]: $D = kT/6\pi\eta R_p$, in which, k Boltzmann's constant, T is the temperature, η denotes the viscosity of the medium, and R is the hydrodynamic radius of a spherical particle. From there the hydrodynamic diameter can be obtained.

On the other hand, the surface electrochemical behavior of the polymers is measured by electrophoresis. For this method, an electric field is applied to the particle solution and makes them to move in the fluid with a determined velocity that is related to their electrophoretic mobility. From this measurement is possible to address the net surface charge by calculating the zeta potential [67],and its distribution by using Smoluchowski or Hückel theories, depending on the particle's characteristics [15].

The Biointerfaces Institute laboratory has a DLS Zetasizer Nano ZS from Malvern Instruments (Figure 10), which can accomplish both assays by inserting 1 mL sample in a 4-side clear cuvette and measuring into the chamber. For particle size distribution, the measurement for each sample is typically 1-2 minutes, and the system is manually assisted to insert the cuvette into the chamber. The particle size range of this equipment is 0.3 nm - 10 µm at 25° C. For measuring the surface charge, the equipment measures the particle velocity using a multi frequency phase analysis light scattering technique (M3-PALS), calculating the zeta potential and its distribution [68].

A high throughput method for these techniques can be developed by adding a robotic arm connected to the equipment in Figure 10 for inserting and removing the sample. Hence, the sample preparation can be done in the Tecan Evo 200 automated system. Another option in this development would be to implement an auto-sampler injection system, similar to the one used in high pressure liquid chromatography (HPLC) or size exclusion chromatography (SEC) [69].

Figure 10: DLS Zetasizer Nano ZS and MPT-2 Autotitrator (Malvern Instruments).

Additionally, an accessory of this equipment is a MPT-2 Autotitrator (Figure 10) could be acquired, permitting to obtain the zeta potential and the particle size measurements in the sample directly as function of pH, conductivity or additive concentration, depending on the titrant solution to be used.

Another possible option would be to acquire some new high throughput DLS apparatus that are available in the market (e.g. Zetasizer APS from Malvern Instruments or DynaProTM Plate reader TM from Wyatt Technology), which allow you to measure the hydrodynamic diameter and coagulation studies in a microplate setup [70]. However, the particle size boundaries are only 0.15 nm to 1 µm.

2.3.3. High throughput dewatering test of polymers

Among the literature in water treatment by dewatering polymers, the most used techniques of analysis are turbidity [71], [72], settling rate and volume [73], and the jar test [74], which in joint with particle size and zeta potential gives the most relevant information to decide whether a flocculant will help in dewatering process.

2.3.3.1. Flocculation efficiency test by turbidity

Turbidity measurements in function of time serves as good indicator of how efficient a flocculant can be producing instability. In typical spectrometers, the attenuation-detection angle is the method used [75], in which the light beam passes through the sample horizontally and the detector measures the attenuated light beam due to light scatter and adsorption. Alternatively, the same method is used in a microplate-based measurement, only that this time the light beam crosses the sample vertically, with the detector beneath the microplate [76]. Therefore, inherent disadvantages with absorbance measurements in a microplate are seen if the sample is too concentrated, for instance: if flocs sediment on the bottom it might block the beam completely. Some approaches as multi-reading per well in different positions and individual optimization of the sample concentration has overcome this inconvenience for coagulation on early stage of flocculation, but they do not help when the particles have already started to settle.

Therefore, in this approach an automated sampling at different heights in a deep well microplate, followed by an automated turbidity measurement in the aliquot is being proposed. The flocculation experimental design will follow guidelines from Sirin and colleagues [77] for algae harvesting flocculation tests with some modifications. The procedure will be done as follows:

1) A known volume of sludge sample in a determined concentration will be dispensed in a 96-deep-well plate, from column 3 to 11, and rows A to H.
2) A decreasing volume of each additive in duplicate will be dispensed from column 4 to 10, and rows A to H (therefore, 4 additives will be tested in duplicate and different dosages on each plate). An additional volume of additive will be dispensed in column 2.
3) Respective amounts of water will be dispensed to complete equal volumes on each well (1.5 mL), from column 2 to 10, and rows A to H.
4) The deep-well plate will be carried to the shaking station and will be shaken at 500 RPM for 2 minutes to allow mixing and diffusion of the additive into the sample.
5) 50 µL aliquots will be taken from the 1/3 height and 2/3 height in the deep well plate, and they will be poured in separated 96-microplates. Additionally, 150 µL of deionized water will be added to each well, and they will be stir at 200 RPM for 1 minute to homogenize.
6) Absorbance at 550 nm and 850 nm will be measured on each microplate and recorded.
7) Steps 4 and 5 five will be repeated using new microplates every 15 min until at least 51% percent of the aliquots shows a clear solution (absorbance similar to the deionized water).

Workflow of the experiment is shown in Figure 11, and the microplate layout for 4 candidates is presented in Figure 12. Optimal concentration of sludge and approximate time of sedimentation in water should be determined by previous analysis with additives, in order to optimize time and equipment in the laboratory.
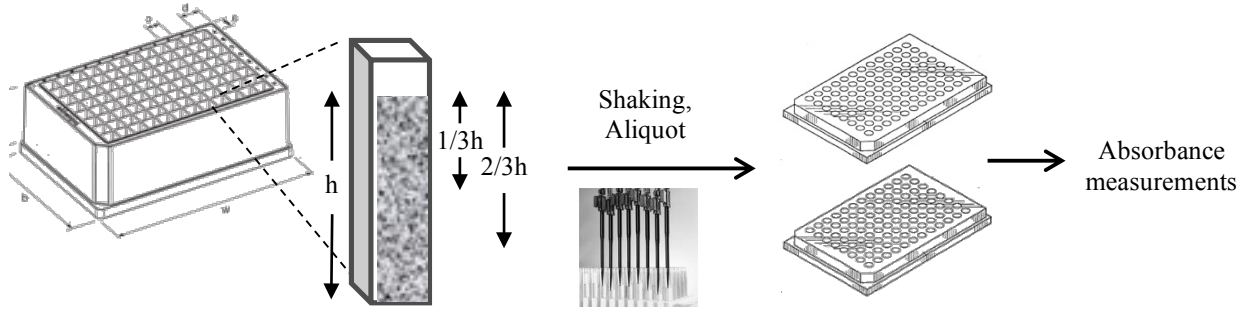
Figure 11: Workflow of flocculation experiments, and heights that will be determined in order to calculate the flocculation efficiency.

Measurements at different heights and periods will be acquired in order to obtain information about the flocculation efficiency, settling rate and optimal dosage. The flocculation efficiency (FE) can be calculated by using the following equation:

$$FE\ (\%) = \left(1 - \frac{A_{sample,t} - A_{ref,t}}{A_{control} - A_{ref,t}}\right) * 100 \qquad \text{Eq. 7}$$

Where $A_{sample,t}$ is the absorbance value , and $A_{control}$ is the initial absorbance of the sludge sample + water, and $A_{ref,t}$ is the absorbance of a mixture of polymer + deionized water, everything at time "t". If the polymer is clear in water $A_{ref,t}$ can be neglected. Candidates that produced higher flocculation efficiency will be selected and tested again in order to find their optimal dosage.

Settling rate could be interpreted from the relationship between FE and time, since depending the settling of particles would increase faster when the flocculant performance is better, as some simulated results show in Figure 12.
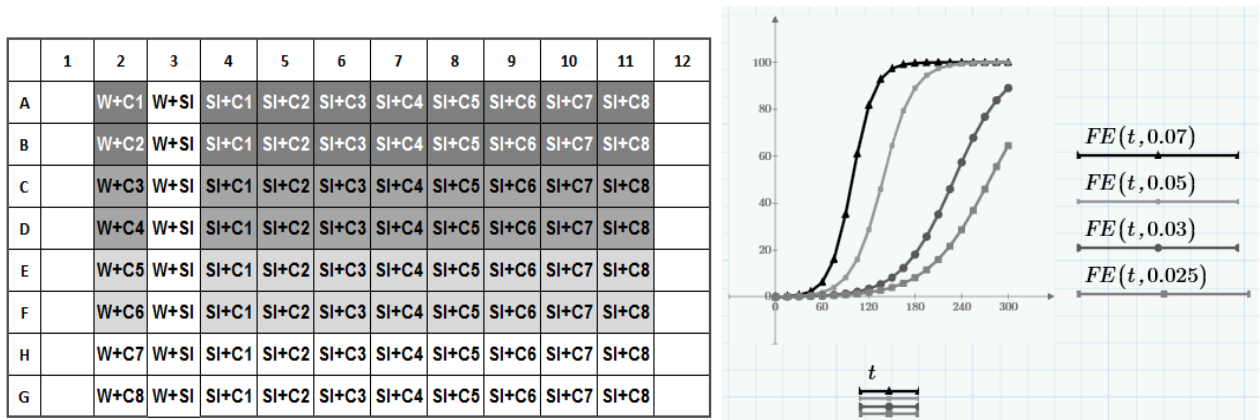
Figure 12: Left: Deep-well plate layout (Sl: Sludge, C1-C8: polymer concentrations, W: deionized water). Right: Simulated data for flocculation efficiency in 4 flocculants at the same concentration.

2.3.3.2. Flocculation and floc structure test by image analysis

This second experiment consists in recreating the experiment mentioned above, but instead of using a deep-well microplate, the element to be used will be 4-side clear polystyrene cuvettes.

This will allow us to take high resolution pictures to each row of 8 cuvettes for further image analysis.

The procedure will start by attaching each cuvette to a removable 4x8-cuvette holder (Figure 13) by using thin Velcro attachment. The holder is placed in the robot and the robot is programed to recognize and utilize this new labware. Then a new script is programed to follow the same procedure above mentioned, but instead each 8-cuvettes row will be momentarily removed from the robot and brought to the picture station in where high definition pictures will be taken to get additional information about the behavior of the sample during flocculation, pictures similar to Figure 13 would be expected.
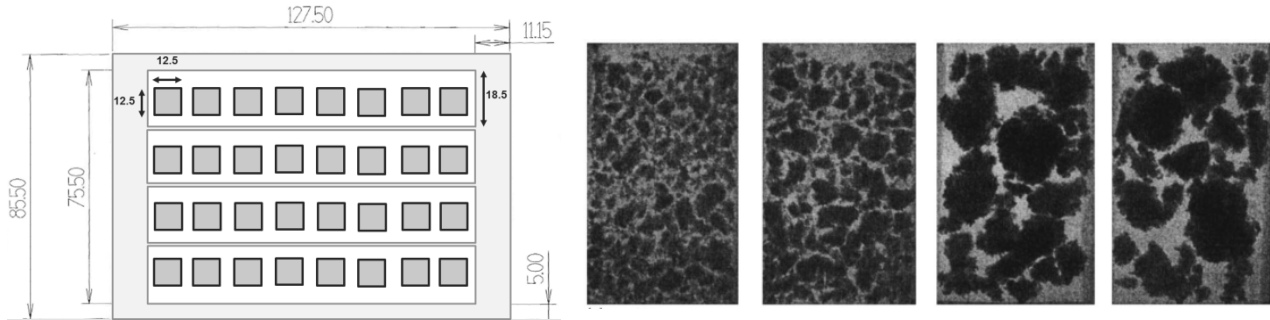

Figure 13: Left: 4x8-cuvette holder representation. Each grey square represent the space where a cuvette is going to be inserted. The dimensions are equal to a microplate. Right: Images obtained for different type of flocculants at different concentrations (picture width 2 cm) (Adaptation from ref. [85]).

This experiment could help to analyze the floc appearance, the elasticity of the supernatant, the height of the sediment cake and the height of the clear interface, to calculate the sedimentation velocity as described in section 1.4.1. The image analysis can the accomplished by inverting the colors in the image and applying calculations to get the amount of white in the picture. The greater the value, the more condense and small the flocs are. From here, statistical analysis of images can be done, by using fractal dimensions and particle size. That may be a good opportunity to apply Eq. 4 and Eq. 5, in collaboration with other research groups or other industrial partners.

2.3.3.3. Specific cake resistance test by filtration

The third experiment to be presented can give us useful information about the cake resistance after flocculation. A known volume of sludge sample will be mixed with a known volume of polymer. In similarity to the previous procedure, the samples will be shaken and will be transferred to the 96-filter plate, with a controlled dispensing rate. A vacuum system will be connected to the filter plate, therefore the pressure differential during the filtration is known. PVDF membranes will be used in this proposal due to their good chemical resistance and available size range in filter plates [78]; however, ester-cellulose membranes are also greatly used in water treatment [1]. The pore size can vary between 0.22 – 5 μm (Even though 1.2 μm or even higher should work since the flocs are expected to be bigger in size).

The filtered volume will be dispense in a 1.2 mL 96-well Cluster Tubes, and the total time of filtration, weight and volume of the filtered will be recorded in the computer connected to the

robotic system. Then, the experiments will be repeated but this time the filtered volume will be acquired at different intervals of time, by stopping the pump momentarily. For example: if the total time of filtration a plate was 10 min, then 5 samples in intervals of 2 min will be taken in the tubes, and the weight and volume on each tube will be measured by using the robotic arm and the balance, and the liquid detection option by conductive tips in the robot, respectively.
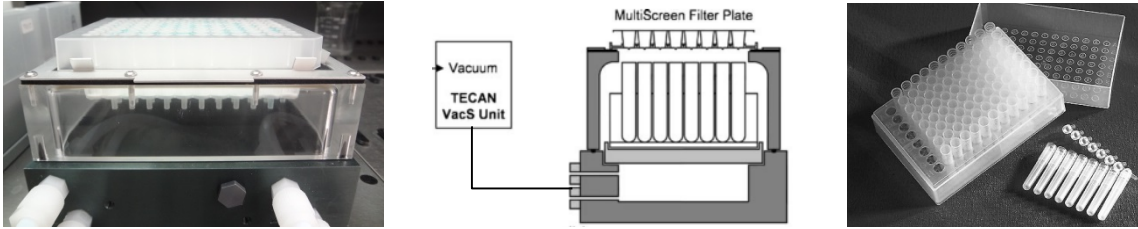

Figure 14: Left: 96-well filter plate placed in the vacuum system. Middle: cross-section of a single vacuum filtration system showing the filter plate on top and the collection cluster plate below (adaptation from ref. [78]). Right: 96-well Cluster Tubes picture, Corning Life Sciences.

These values can be used to determine the cake filtration specific resistance by using the equations proposed in the introduction and considering pressure filtration constant [9]:

$$\frac{t}{V} = \frac{\mu \alpha c}{2A^2 \Delta P} V + \frac{\mu R_m}{A \Delta P} \qquad\qquad \text{Eq. 8}$$

Where R_m is the membrane resistance (constant). If all the data is determined and c is obtained from the moisture ratio (m_r), the specific resistance of the cake (mkg^{-1}), α, can be determined from the slope of a plot like Figure 15.

Therefore, the lower the specific cake resistance, the better the flocculant. Jackson and coworkers used this relationship in a parallel analysis of microfiltration operations [78], in which they tested the specific cake resistance on Echerichia Coli under different solutions and determined that the yeast extract (typical flocculant of E. Coli) lowered the cake resistance in the bacteria sediment, while a buffer solution increased it.

2.3.3.4.Bacterial flocculation tests

Another important factor in sludge dewatering operations is the high amount and diversity of biological residues like bacteria that can be found in the sample to be treated [79], [80]. Therefore, a fourth high throughput experiment is proposed, in order to find possible candidates that improve the flocculation of bacteria, specifically, Escherichia Coli in different conditions of pH and ionic strength.
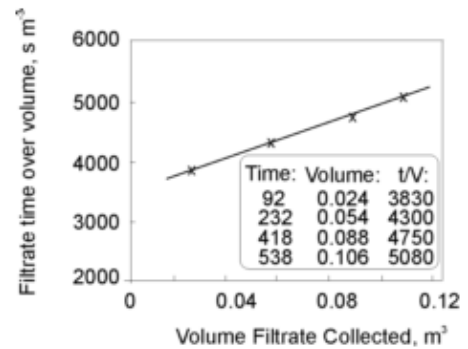
Figure 15: Example of data analysis by Equation 8 (Reprinted from ref. [9]).

The procedure will follow guidelines from Strand and colleagues [81], with modifications according the high throughput setup. Bacteria and culture conditions will be prepared accordingly, while the concentration of polymers to be tested will be dispensed in deep-well microplates by 4 successive dilutions (columns 3-6 and 7-10), in similarity to the flocculation

efficiency test previously presented. After that, Acetate/NaCl buffer solution at pH = 4 was dispensed (columns 7-10), and finally, a known volume of bacteria suspension will be dispensed in columns 2-10. The deep-well plate was treated according procedure in section 2.3.3.1.

Same procedure is repeated, but now with Acetate/NaCl buffer solution pH = 5, PSB buffer solution pH = 6.5 and 8, and at different ionic strength by adding NaCl solutions (0.01 M, 0.1 M and 1 M). In that way, the efficiency of bacterial flocculation will be obtained at different pH and ionic strength for each additive in duplicate.

2.3.4. Statistical analysis of high throughput experiments

All the valuable information from the different dewatering tests proposed in conjunction with the analysis of the polymer candidates give a robust data set to be treated in a multivariable analysis way, like PCA or PLS models, most useful approaches to analyze data from high throughput experiments. By using 3-variable representation like 3D plots or contour plots, variables can be associated and be analyzed in a straight forward way. However, the use of PCA and PLS models allow us to analyze many more variables at the same time, finding even unthinkable correlations among the data. Figure 16 illustrate typical plots for coefficients (shows the contribution of variables to the principal component), and scores (shows correlations between samples) in PCA analysis. Also, this information can be relevant to generate new prediction sets of dewatering polymers, tuning the possibilities from preparation of polymers to the ones with the best correlation, as explained in the introduction by using QSPR models.
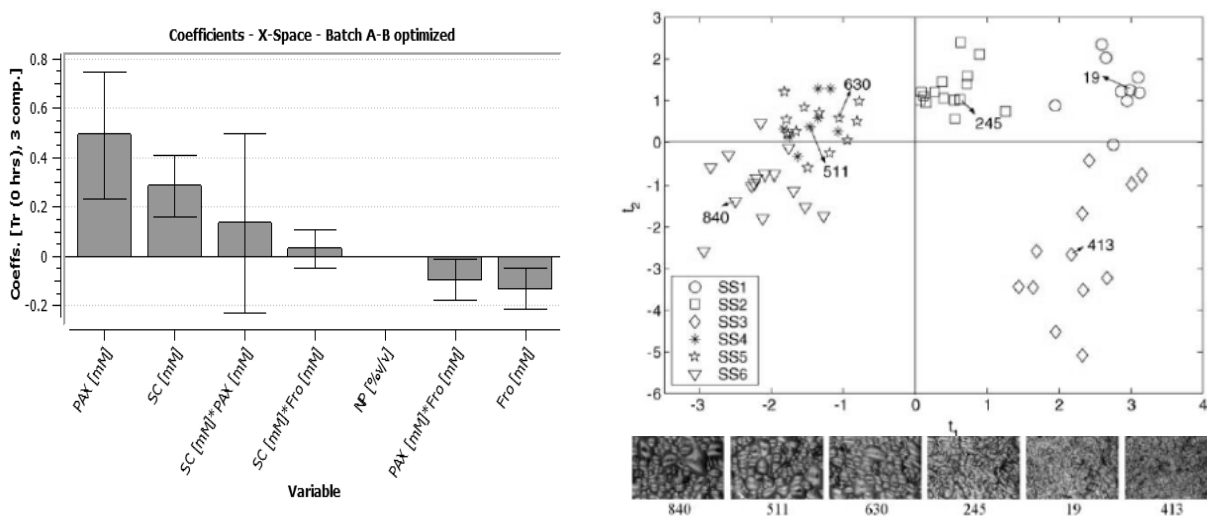
Figure 16: Left: Coefficient plot for a PCA model (Reprinted from ref. [86]). Right: Score plot from PCA analysis in images of flotation operation (underneath: selected steady-state) (Adaptation from ref [87]).

2.4. Challenges

The first and greatest challenge is the introduction of high throughput techniques that are normally applied to combinatorial chemistry or biological assays into an industrial field like water treatment, since many times the typically used additives, old practices and regulations are

playing against the new alternatives that may bring financial, environmental and health benefit in a long term but need significant investment in the short term.

Also, during the design of experiments, several challenges can be detected, like:

- Automation of experiments always require a high amount of time in planning, programming and checking all the raw materials, labware and workstations involved; which increases the time spent in robots and the cost of the experiments. Only effective training and trial/error experiments will overcome this challenge.
- Time analyzing data is a real challenge; therefore, expertise in several options of software and collaboration outside the laboratory is essential to succeed in this research proposal.
- Possible problems in one of the vessel during the parallel reactions might affect the other reactions as well. The same can happen in microplate experimentation, where a problem in one part of the process could affect the whole process.
- Some results like grafting efficiency in polymers should be validated by methods no high throughput as chemical analysis or potentiometric titration, which may bring time consuming tests for these many samples. This can be diminish by running the dewatering performance experiments before final characterization, in that way, only the good candidates can be analyzed by special techniques.
- The lack of publications in the exactly same research proposed experiments is a challenge to fix problems during experimentation by finding out what others have done.
- Unexpected chemical or physical interactions between compounds or results with high variability might bring the need to optimize the experiments in a non-high throughput fashion, generating possible delay in the timeline of the proposal.

2.5. Milestones of proposal

The proposed milestones for a Ph.D. candidate full time and next 2 year of a Post-Doctoral fellow are outlined as follows:

- **First year:** the candidate should get training on high throughput techniques presented and learn about details on experimentation. Trial/error experiments of the characterization techniques and the sludge dewatering tests with commercially available dewatering polymers (e.g. polyacrylamide and polyDADMAC), and sludge models (as kaolin or silica beads) should be done, in order to validate the methods, and to find any other special situation that could alter the experimental design in any way. Likewise, conversations with a water treatment plant should start in order to get real sludge samples on time for real experiments. Also, to create collaboration within research groups or industrial partners to acquire additional robotic equipment as needed might be expected.

- **Second year:** the candidate should analyze all the gathered information during the first year by using required statistical and image analysis, and optimize the parameters in any experiment as needed; re-validation of assays should be done. Also, trial/error experiments with the first set of new hybrid polymers (polysaccharides + acrylamide) should be prepared, picking some of the combinations and prepare them by classical and high throughput

techniques and compare their results, in order to validate the use of the high throughput synthesis.

- **Third year**: optimization of high throughput synthesis in the first batch will be done. Previously optimized experiments of sludge dewatering characteristics using sludge models and real sludge samples should be applied to the first set of hybrid polymers, in order to start feeding the database of these new polymers. The best candidates will be brought to more detailed characterization techniques (in some cases non-high throughput techniques as well). Also, trial/error experiments with second and third sets of new hybrid polymers (polysaccharides + quaternary and tertiary amines) should start.

- **Fourth year:** optimization of high throughput synthesis and testing of dewatering characteristics should be accomplished for the second and third sets of polymer candidates. The best combinations should be analyzed further, and the completion of statistical and image analysis, and the generation of QSPR models for new development is expected in order to finalize experimental part and write Ph.D. thesis.

- **Fifth and sixth year** (Post-Doctoral fellow): Implementation of QSPR models and these high throughput analyses as routine basis to help other students in the development of new sets of polymers should be priority. Similarly, the focus on publication of work done and/or the development of application patents is expected. Finally, implementation of these techniques in industrial partners sites and expand collaboration within Universities is desired.

2.6.   Anticipated significance of the work

The development of high throughput techniques applied directly to the waste water treatment industry would have a great positive impact in different entities in our society. First, at an academic level, the possibility of creating new techniques for preparation of more eco-friendly dewatering polymers and fast testing of their properties will improve the standing of the research group and University, showing our concern for the environment and our most precious resource: water.

This will open new interest for investment from most probably the private sector (specially supplier companies), the treatment plants (for implementing these techniques to accelerate their non-online quality control process), the robotic and engineering companies (to generate sponsorship in the work related to this industry), and even the government, which might have interest to apply these techniques in their own regulations, treatment plants, and environmental organisms (like Environment Canada).

In that way, the novelty and significance of this work is demonstrated in terms of new high throughput techniques in non-typical applications and industries, generating the space needed to take advantage of new technologies available in other fields of science.

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