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Connecting physico-chemical characteristics with gelling properties of alginate like exopolymers recovered from wastewater granular sludge.

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Abstract: Alginate like exopolymers extracted from aerobic granules have shown interesting gelification properties. The composition of those extracts has been proven to be very complex, therefore the need to understand which molecules and mechanisms are responsible for the gelification properties is necessary. A fractionation method coupling anionic exchange chromatography with size exclusion chromatography has been used to separate molecules into fractions according to their global charge and size in order to determine which physico-chemical properties are the most important for their application as functional hydrogels. A miniaturized gelification screening method has been developed to compare the capacity of the collected fractions (corresponding to polymers with diversified charge and size) to gelify in standardized conditions.

Keywords: Alginate like exopolymers; fractionation; gelification capacity.

Introduction

Sludge granulation emerged in the last decade for biological wastewater treatment. This promising biotechnology enables microorganisms to form granular sludge and the secreted extracellular polymeric substances (EPS) are now considered as low-footprint gel-forming agents. EPS were proven to be rich in negatively charged molecules and in particular in polymers containing uronic acids (Lin et al., 2010). Acid precipitated EPS are called alginate like exopolymers (ALE) because of their ability to form gels with divalent cations. Until now there is a poor understanding of the characteristics of the gel forming molecules present in those extracts because of their complexity. Considering seaweed alginates, it was shown that alginate molecules with a higher molecular weight have a better gel formation kinetics and enhanced viscosities (Fernández Farrés & Norton, 2014). The goal of this work is to fractionate molecules present in the extract based on their charge and size to determine which characteristic is the most important in terms of gelifying capability.

Material and Methods

Dried granules produced in Eawag according to Layer et al., 2019 were submitted to the extraction protocol summarized in Figure 1. After acidic precipitation, dialysis, and freeze-drying, the lyophilizate named ALE was resuspended in 0.1M NaOH for further analysis. Their composition was determined using modified Lowry method for proteins and humic-acid like substances with respectively BSA and humic acid as standards; double anthrone method was performed for neutral and uronic sugars quantification with respectively glucose and glucuronic acid as standards (Schambeck et al., 2020).

ALE fractionation consisted of coupling anionic exchange and size exclusion chromatography with UV detection at 280 nm. Anionic exchange chromatography was performed with a Source 15Q column (Cytiva) with Tris-HCl 50mM, pH 9±0.01,

and 4 NaCl concentrations successively used (0M, 0.25M, 0.5M, and 0.75M) for elution of four fractions respectively named: Cationic and Neutral (CA/N), Low Anionic Charge (LAC), Medium Anionic Charge (MAC) and finally High Anionic Charge (HAC) molecules. The various Source Q eluted molecules were then injected in a SUPERDEX 200 column (Cytiva) to be separated according to their molecular weight into: LMW=Low Molecular Weight, MMW= Medium Molecular Weight and HMW= High Molecular Weight molecules.

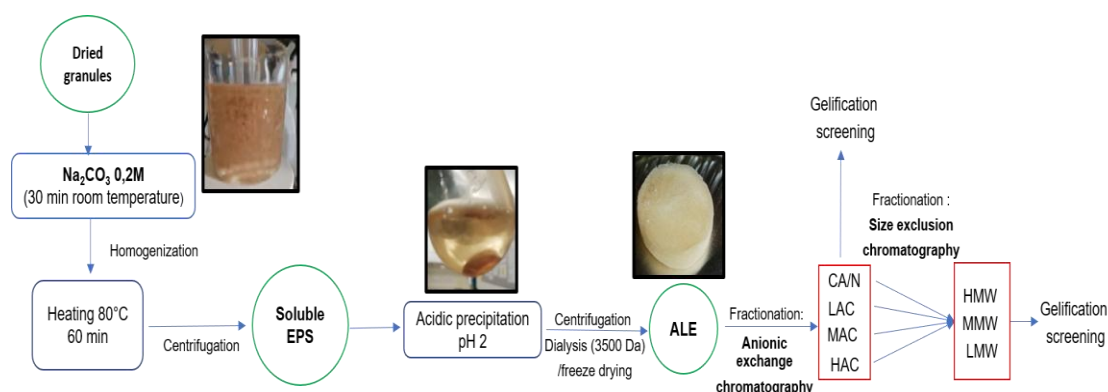


Figure 1 Protocol of extraction and fractionation of ALE from aerobic granular sludge.

A miniaturized gelifying capacity test was developed by the addition of CaCl_2 to measure ALE's viscosity using a low range viscosity rheometer (BROOKFIELD). A low viscosity alginate (sigma Aldrich) was used as a model molecule.

Results and Discussion

The total amount of ALE extracted from the granules was of 342.29 ± 12.85 mgVS/gVS and were mainly composed of uronic sugars equivalent to 643.71 ± 31.58 mg/gALE, then of 49.64 ± 6.59 mg/gALE of neutral sugars, proteins with a quantity of 152.47 ± 37.43 mg/gALE and humic acids equivalent to 308.24 ± 10.96 mg/gALE. These results show the complexity of the extracted ALE (Schambeck et al., 2020) and show the preponderance of uronic acids. Therefore, a fractionation is essential to separate molecules to understand the gelification of ALE.

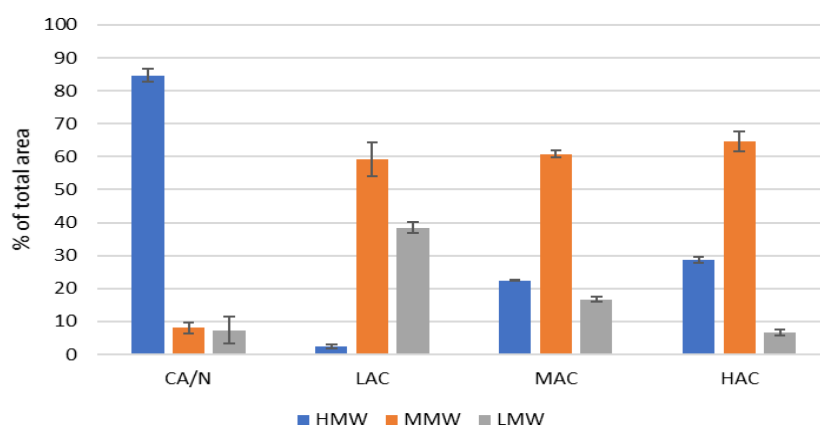


Figure 2 Distribution of molecular weight in the fractions collected after anionic exchange chromatography performed at pH 9. Values are presented as a percentage of total area after integration of peak areas at 280 nm.

After the anionic exchange chromatography and integration of peak areas at 280 nm, the results showed that the sample was made of $50.42 \pm 4.30\%$ CA/N molecules,

25.85±2.28% of LAC, 14.35±1.25% of MAC and finally 9.38±1.53% of HAC. All fractions were injected in size exclusion chromatography and it was noted that CA/N molecules eluted at a pH 9 were composed mainly of HMW (85%). Negatively charged molecules (LAC, MAC and HAC) on the other hand are composed of a majority of MMW (around 60%), results are shown in Figure 2.

Finally, a screening method for gelification was developed using alginate as a pure well characterized gelling polymer (Figure 3). This test allows to evaluate hydrogel formation by measuring low viscosity at concentrations of alginates ranging from 0,05 to 0,8%. When applied on complex ALE, the test was able to detect hydrogel formation for very low concentrations ranging from 0.2% to 0.5% that correspond to 1 to 2.5 mg of freeze-dried ALE. This test shows thus good sensitivity for screening all the molecules recovered after ALE fractionation. Therefore, this test will help to determine what are the ALE molecules showing key physico-chemical properties (size/charge) for hydrogel formation.

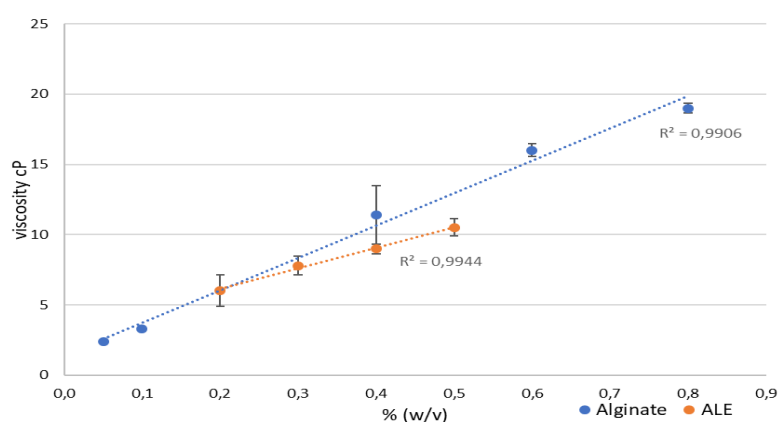


Figure 3 Evolution of alginate's viscosity with 10 mM calcium at different concentrations compared to the evolution of ALE's viscosity with 30 mM calcium.

Conclusions

In addition to their well described chemical complexity, this work shows the size and charge diversity of ALE. It also provides a fractionation protocol and a miniaturized gelification screening method helpful to determine which physico-chemical property (size/charge) is correlated with the best gelification capacity in the case of natural biopolymers recovered from wastewater treatment.

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