Coagulation-flocculation of *Microcystis aeruginosa* by clay-polymer based composites

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INTRODUCTION

Cyanobacteria blooming episodes in water bodies are increasing due to global warming and nutrient enrichment arising from anthropogenic activities. Their presence in water poses a risk for the environment and human health due to release of toxins. Coagulation-flocculation has been proved to be a key treatment for removal of cyanobacterial cells along Drinking Water Treatment plants avoiding the release of endotoxins by cell lysis. The combined use of cationic polymer and clay minerals can be a good approach for their use in coagulation-flocculation of cyanobacterial suspensions, relative to more conventional chemicals used. In the current work, clay-polymer complexes were examined for removal of *Microcystis aeruginosa* in suspensions. These complexes were prepared from polymers derived of quaternized poly-4-vinylpyridine (PVP), which were sorbed and grafted on the surface of the clay mineral montmorillonite. Quaternization was performed by introducing methyl and hydroxyethyl moieties on the pyridinic N.

MATERIALS AND METHODS

Microcystis aeruginosa PCC7806 (hereafter referred to as *M. aeruginosa*.) was obtained from the Pasteur Culture Collection and grown in BG11 medium supplemented with 17.5 mM $NaNO_3$ and 10 mM $NaHCO_3$, under a bubbling mixture of CO_2/air (1% v/v) at 30ºC with a light intensity of 75 μE m^2/s under continuous light. Polyvinylpyridine (PVP) was quaternized by reaction with iodomethanol (QPVP) or 2-bromo-ethanol (OHQPVP). Sorption of OHPVP and QPVP was performed by adding a 3 g/L polymer solution to a 0.834 g/L bentonite clay yielding the composites CH_3- and OH-MMT, followed by centrifugation and further dry-freezing. The loading of the polymers was 4.3% w:w for OH-MMT and 6.9% w:w for CH_3-MMT. A grafted polymer based-composite (PGC) was synthesized by the "graft from" method as described in Gardi & Mishael (2018), that was further quaternized as described previously, leading to formation of CH_3-PGC and OH-PGC composites, their loading being of 33 and 29% w:w, respectively. The clay-polymer composites were characterized by infrared (FTIR) spectroscopy, thermal analysis (DSC-TG), X-ray diffraction (XRD) and zeta potential (ZP) analysis.

Polymer solutions and powder composites were added to a suspension of *M. aeruginosa* of $3.71x10^6$ cells/mL under continuous shaking for 1 h or 24 h. Addition of the polymer and composites were based on a final content in the suspension of 20 g/L of polymer. The content of cyanobacteria and its status were determined by flow cytometry.

RESULTS AND DISCUSSIONS

The characterization of the clay-based composites by combining XRD, FTIR and DSC-TG data suggested that OH-MMT and CH3-MMT posed a train configuration of the polymer on the clay surface instead of a loops and tails conformation. This is optimal because of the negligible release of external molecules adsorbed that may be detached from the surface, thus decreasing the risk of release of endotoxins due to the high toxicity of the QPVP and OHQVP polymers. The conformation of the polymers on the clay was completely different after grafting on

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the clay surface. The excluded volume interactions between the polymer chains yielded a stretched ("brush") conformation on the vicinity of the clay surface (Fig. 1).

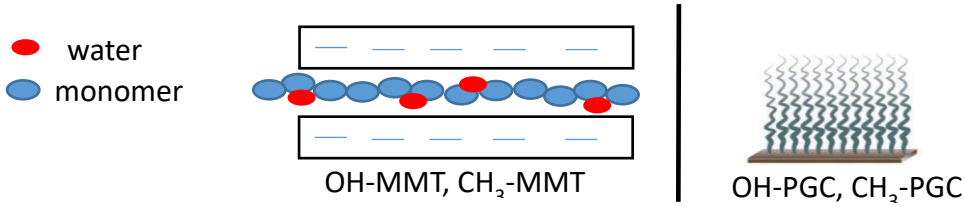

Fig 1. *Structure of the composites.*

Although the polymers per se are toxic, it can be observed that the fraction remaining in solution is mostly in a live status, the fraction of dead cells being very small and close to the fraction existing in the initial solution (not shown). Despite the fact that the cells are negatively charged due to dissociation of functional groups, mainly carboxylic, at the cell surface, and that the composites showed a positive surface potential, the retention of cells was minimal (Table 1), with the exception of the composite CH_3-PGC. This was explained by the combination of its high surface potential (+31 mV) reaching a critical value for cell adhesion together with the higher accessibility of the polymer when tethered to the cell surface in a brush conformation. The OH-PGC composite was not effective due to its lower zeta potential (+21 mV).

Incubation time: 1 h			
	Retained/Sorbed (%)	Solution, live (%)	Solution, dead (%)
PGC	2.71±2.02	86.61±2.09	8.98±0.07
CH3-PGC	66.08±5.84	30.97±5.36	2.95±0.48
OH-PGC	5.93±3.27	85.83±3.09	8.24±0.18
CH3-MMT	3.49±2.58	86.77±1.08	7.74±0.51
OH-MMT	4.09±3.05	85.28±2.00	10.63±1.05
Incubation time: 24 h			
	Retained/Sorbed (%)	Solution, live (%)	Solution, dead (%)
PGC	1.84±0.72	80.45±0.88	19.39±0.16
CH3-PGC	73.87±4.91	19.59±3.26	6.54±1.65
OH-PGC	3.23±0.14	78.47±1.50	19.84±0.69
CH3-MMT	1.08±0.23	79.69±0.11	21.39±0.12
OH-MMT	1.02±0.31	76.42±0.18	22.55±0.13

Table 1. *Cyanobacteria distribution after incubation as a function of time. Initial concentration of M. aeruginosa, 3.71x10⁶ cells/mL.*

CONCLUSIONS

The optimization of a clay-polymer based-composite for removal of cianobacteria as a coagulant/flocculant requires: (i) a high surface potential; and (ii) grafting of the polymer in a brush conformation. Polymer grafting allows retention of cells by direct contact with the polymer brushes, and prevents internalization of the polymer causing cell death and endotoxin release.

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