Int. J. Environ. Res., 10(4):593-600, Autumn 2016
ISSN: 1735-6865

Flocculation of Microalgae via pH Change in a Turbulent Medium and Subsequent Filtration

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Received 19 July 2016; Revised 23 Sep. 2016; Accepted 29 Sep. 2016

ABSTRACT: Traditional microalgae harvesting techniques consume a lot of energy. Flocculation, or the formation of aggregates, is an energetically favorable process to collect biomass. Flocculation is normally carried in tanks to allow the formation of the aggregates after stirring, however, this consumes time and physical resources. In this work, flocculation of *Chlorella vulgaris* and *Scenedesmus* sp. by acidification and alkalization was compared to chemical flocculation in a turbulent medium for short periods of time (30 seconds and 2 minutes). Flocculation with potassium hydroxide at pH=10 showed to be nearly as efficient as traditional flocculation by using ferric sulphatum after two minutes. Acid flocculation with nitric acid was not as effective, even at values of pH=4. Flocculation by pH does not generate toxic wastes and the remaining added flocculants turn into nutrients after harvesting. After flocculation and neutralization, the remaining cells in the medium were viable to recultivate. Since pH-driven flocculation does not allow harvesting the total microalgae culture, remaining cells can be used to keep growing. Based on these results a semi continuous harvesting method incorporated in the microalgae growing phase seems promising.

Key words: Chlorella vulgaris, Aggregation, Harvesting, Continuous culture, Alkaline medium

INTRODUCTION

Microalgae, photosynthetic microorganisms, are a potential source of vegetable oil and other products like carbohydrates and protein for energy and food production. Some species have reached more than 70% lipids on a dry weight basis when cultivated in controlled laboratory reactors (Ghasemi et al, 2012), and they grow exponentially under optimal conditions. Microalgae currently cost more to cultivate than traditional crops, and to make microalgae production economical feasible it is necessary to use most biomass compounds available for product development (Vanthoor-Koopmans et al, 2013). Moreover, lowering production cost due to technological development is also important and possible. One of the main obstacles in the production process of microalgae is the harvesting (Weschler et al., 2015). Most microalgae are naturally dispersed in the medium, and it is difficult to collect the biomass once it has reached optimal densities. Traditional harvesting by centrifugation can damage the cell under the gravitational forces, it needs expensive equipment and it presents high energy consumption (Chen et al., 2012). Harvesting via filtration is suitable for larger microalgae (Kim et al., 2015), but for small cells requires special membranes and flux reduction occurs after a while due to fouling (Ríos et al., 2012).

Flocculation, the formation of stable particle aggregates in an aquatic medium is a natural phenomena under certain conditions such as estuarine mixing. The physical properties of the medium, such as pH and temperature, and the presence of metal ions promote the flocculation and subsequent precipitation of solid aggregates (Shamkhali Chenar et al., 2013). In wastewater treatment, flocculation has been used to easily collect microalgae and other microorganisms via filtration or decantation (Chen et al., 2015). However, most microalgae do not flocculate in normal conditions due to the repulsion caused by the negatively-charged cell membranes. Nevertheless, flocculation can be induced by flocculants and has been proposed as a better alternative to harvesting microalgae than centrifugation or filtration or as a first

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step to improve the performance of both (Vandamme et al., 2013). In estuaring mixing, natural flocculation promotes the reutilization of certain nutrients (Karbassi and Heidari, 2015). Therefore, after flocculation of microalgae cultures, harvested medium could be reused depending on the flocculation conditions. Flocculation has shown to concentrate a dilute microalgae suspension of 0.5 g/l dry matter to a slurry of 10–50 g/l (Wileman et al., 2011). The requirements for a second and final dewatering step via centrifugation or filtration are easier to achieve or even unnecessary. Traditional flocculants like metal salts, chitosan, or polyelectrolytes have been studied and recommended for microalgae flocculation (Stephenson et al, 2010; Rashid et al., 2013; Gorin et al., 2015), but presents disadvantages like residual contaminants, long flocculation times and economical costs (Granados et al., 2012; Gerardo et al., 2015; Golzari et al., 2016).

Microalgae flocculation in response to pH variation has been previously studied in both directions, by alkalization and acidification (Pezzolesi et al., 2015). Subsequent neutralization to reuse the medium after harvesting has been proven feasible (Castrillo et al., 2013). Flocculation mechanism by alkalization is accepted to occur due to metallic hydroxide precipitates, which requires a pH value around 11 (Huo et al., 2014). However, flocculation is a complex process that not only relies on cell charges neutralization, but also on the probability of collision between cells. At alkaline pH below 11, flocculation has been observed when there is a high microalgae density (Schlesinger et al., 2012; Besson and Guiraud, 2013). The acidification process has been poorly studied but it seems to occur by neutralization of cell membrane charges as well (Liu et al., 2013).

Until now, flocculation as harvesting mechanism has been proposed as a multi-step process (Lucas-Salas et al, 2013; Collet et al, 2014), first to stop the medium flow and flocculate the culture; then harvest is conducted, and reconditioning the medium is the final step in the process. However, in larger scale microalgae production, every process step implies an increase in costs, both initial and operational (Barros et al., 2015). Considering this, it is important to know the behavior of pH induced flocculation in a non-steady medium. If flocculation, harvesting and regrowing of the remaining cells can be incorporated in a single step, the possibility of a continuous production and harvesting of microalgae can result in cheaper and smaller production units. Until now, there are few reports on the possibility to flocculate microalgae in a turbulent medium, i.e., inside a photobioreactor (Yahi et al., 1994), this previous work proposed a system for continuous flocculation of microalgae, achieving 95% efficiency with pH between 11 and 11.5 after a residence time of 5 minutes.

In this study, flocculation by pH variation is evaluated under non-steady conditions, comparing its separation efficiency after filtration with traditional flocculant salts. Factors like pH, microalgae concentration and time of flocculation are evaluated. The final medium is neutralized and viability assays are conducted in order to prove the feasibility of the culture.

MATERIALS & METHODS

Scenedesmus sp. and *Chlorella vulgaris* supplied by UAM-Iztapalapa Distrito Federal, Mexico were used in this study. The culture medium was prepared with water and 0.1% of a commercial foliar fertilizer (Bayfolan Forte©, Total N 11.4%, phosphorus as P_2O_5 8%, potassium as K_2O 6%). Both species were grown in eight different 1 L glass recipients, incubated at room temperature and illuminated using cool-white 9 W LED lamps 24 h/day and continuously aerated by bubbling air. Samples of 50 mL with different microalgae concentrations were used in the different experiments. Optical density at 750 nm (OD_{750}) of microalgae cultures was measured every 24 h, and the subsequent acidification and alkalization experiments explained in the next sections were conducted every 48 h. The cultures were maintained for 10 days.

A pH-meter Horiba model f-74BW was used to measure pH. The pH of each sample was acidified by adding 0.1 N HNO_3 and alkalized with 0.1 N KOH. A curve of pH variation in response to HNO_3 and KOH was obtained for different microalgae concentrations to determine the necessary amount of each solution to achieve the desired pH levels for both species.

Flocculation experiments were performed with 50 ml culture samples at six different initial microalgae concentrations. Flocculation efficiency was tested at pH 4, 5, 7.3 (control), 8.5 and 10. For every pH value two time lapses were tested, 30 seconds and two minutes. For every time lapse, for both species, three samples of every concentration were stirred at 350 rpm after achieving desired pH values. After the specified time the samples were passed through a 20 µm pore size filter. The OD_{750} the medium was measured before and after filtering to determine the efficiency of the biomass separation comparing initial and final OD_{750} measurements. A t-test was performed in order to determine if the measured OD_{750} in the different experiments were different from the control.

A test with ferric sulphate ($FeSO_4$) was performed in order to compare the efficiency of pH driven

separation with a standard flocculant used in water treatment. Four different experimental treatments, alkaline flocculation (pH=10 adding KOH 0.1 N), acid flocculation (pH=4 adding HNO_3 0.1 N), and two chemical flocculation substances ($CuSO_4$ 1 g/l, $FeSO_4$ 2 g/l) were performed for the same two time lapses (30 seconds and 2 minutes) for *Chlorella vulgaris*, and OD_{750} was read after filtering it.

After flocculation at the different pH levels (4, 5, 7.3, 8.5 and 10) and filtration, the remaining microalgae cultures were neutralized to pH=7.3 and were left to grow in the original conditions with the purpose to verify if both the microalgae cells and the neutered medium were reusable. OD_{750} was measured daily after flocculation and filtration during three days to measure growth rate.

RESULTS & DISCUSSION

In order to calculate the necessary volume of HNO_3 0.1 N and KOH 0.1 N required to change the pH to desired levels, a pH versus volume curve at different microalgae concentrations was obtained for acidification and alkalization (Fig. 1).

In the acidification curve -for both species- at pH values between 6.5 and 7, the acidification of the culture was very slow, and it behaves similar to a buffer solution. This behavior can be explained by the fact that at these pH values the charges in the microalgae cell membranes are being neutered by the protons (Liu et al., 2013). As the carboxyl groups in the cell membrane absorbs the H^+, the medium has a relatively stable pH, and when the addition of HNO_3 continues, the charges are totally neutered after which a quick drop in pH values is observed. This behavior can be confirmed by the fact that at higher microalgae concentrations more HNO_3 was required to acidify the culture.

In the alkalization case, the behavior was typical of a neutralization curve, though it seemed to behave similar to a buffer while approaching to a pH value of 11. These results suggest that, at pH values between 10 and 11, alkaline flocculation involves a membrane charge-related mechanism similar to the acid mechanism -for example, cation bridging- besides salt precipitations (Brady et al., 2015).

Results for OD_{750} changes after flocculation and filtration are shown in Fig. 2. The obtained values are shown in Table 1. For the acid experiments the separation after filtration was not very effective, and for the pH value of 5 there was no significant difference with the control ($t<2.22$ for $\alpha=0.95$). Flocculation, however, was observed as small aggregates after stirring. Nevertheless, after filtration, the OD_{750} for acid flocculation was only a fraction lower than the control. For the pH value of 4, a decrease of 30.43% ± 5.69% in OD_{750} for *Scenedesmus* sp. and 43.5% ± 10.52% for *Chlorella vulgaris* was observed. These results

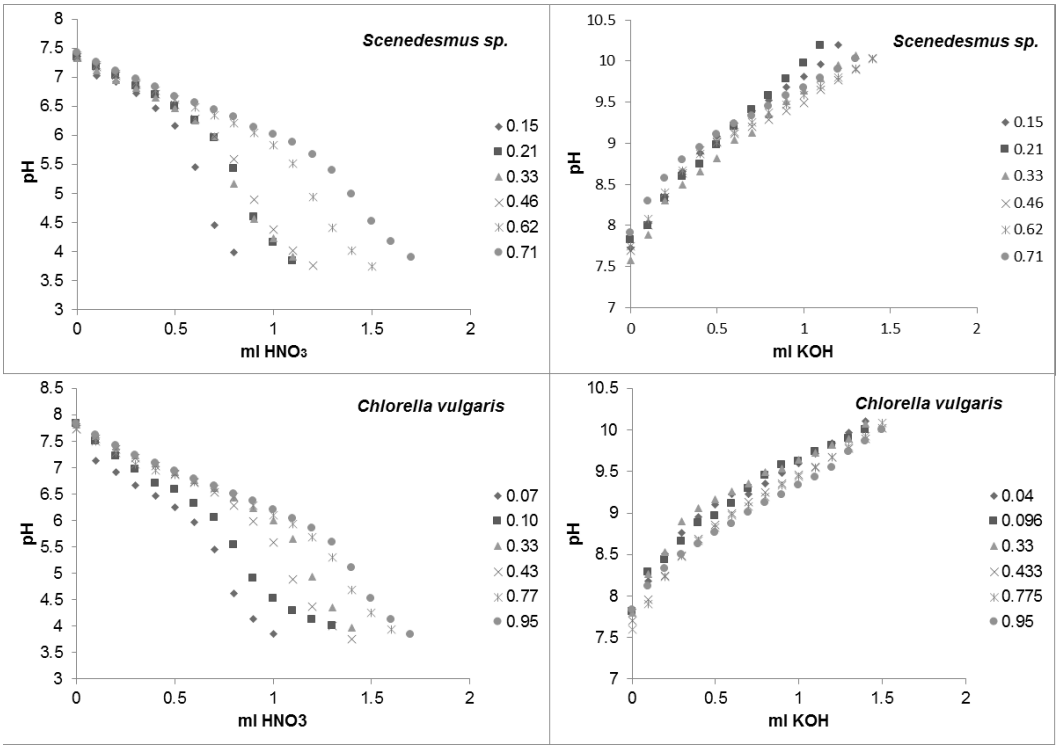

Fig. 1. Change of pH in response to addition of HNO_3 and KOH 0.1 N with different initial microalgae concentrations (OD_{750})

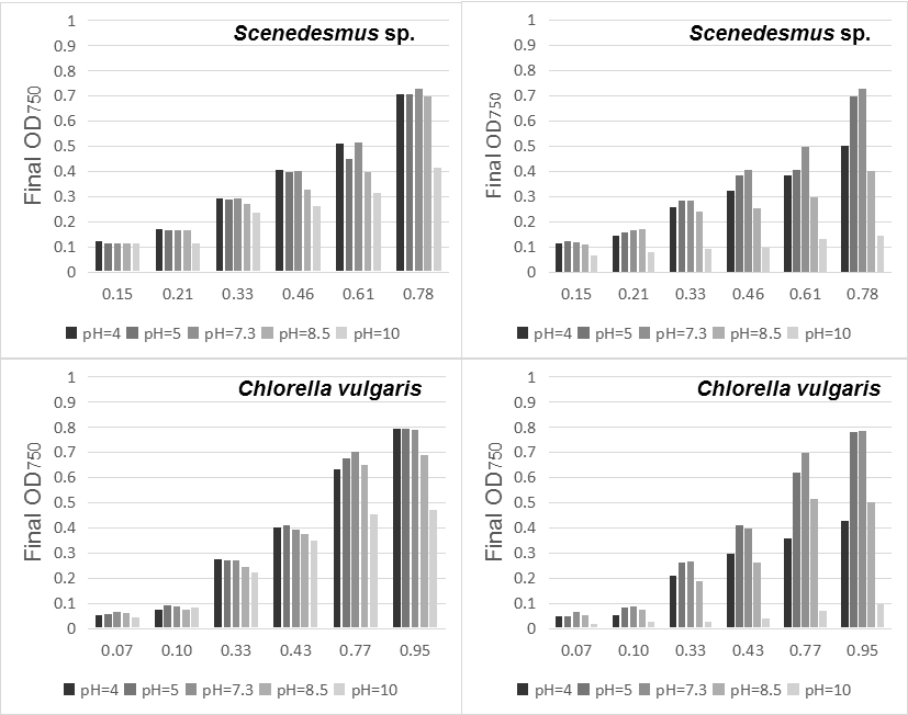

Fig. 2. Comparison of optical density (750nm) after different pH treatments allowing flocculation at different time intervals and passing through a 20 µm filter

Table 1. Average decrease compared to initial OD_{750} after pH change, stirring and filtration for both species

		Scenedesmus sp.		*Chlorella vulgaris*	
pH	**Time**	**Average decrease (%)**	**SD (%)**	**Average decrease (%)**	**SD (%)**
4	30 s	14.71	4.6	18.38	7.08
	2 min	30.43	5.69	43.5	10.52
5	30 s	18.17	7.3	13.98	5.16
	2 min	20.16	8.39	19.18	8.54
7.3	30 s	15.34	6.94	12.34	4.69
	2 min	15.67	6.19	12.63	6.03
8.5	30 s	23.11	8.67	20.23	6.65
	2 min	36.55	13.3	35.66	9.11
10	30 s	40.17	9.37	33.6	12.31
	2 min	72.07	10.1	86.35	7.32

indicate that acid treatment does not seem to form stable flocculates and the neutralization of the negative charge in the cell membrane is not enough to provide a highly effective flocculation in a non-steady culture. Acid flocculation of microalgae, however, cannot be discarded immediately, and more research is needed to explain the mechanism and the better conditions for this treatment.

In alkaline flocculation, a higher separation efficiency was obtained based on the optical density before and after filtration. Results on *Scenedesmus* sp. for OD_{750} measurement after filtration showed a minimum decrement of 23.11% ± 8.67% at pH=8.5 for 30 seconds stirring; and a maximum of 72.07% ± 10.10% at pH=10 for 2 minutes stirring. And for *Chlorella vulgaris*, a minimum decrement was obtained of 20.23% ± 6.65% at pH=8.5 for 30 seconds stirring; and a maximum of 86.35% ± 7.32% at pH=10 for 2 minutes stirring. Time is an important factor to consider, and results indicate that a 30 seconds lapse is not enough to achieve high levels of flocculation. The two minutes lapse showed better results.

Overall, the results suggest that alkaline pH flocculation does not entirely depends on salt precipitations, since flocculation was observed at both alkaline pH values tested (8.5 and 10). A membrane-based alkaline flocculation has been suggested before. After experiments with different pH values and microalgae species, flocculation has been observed at pH=10 for several species, but mutant membrane deficient *Chlamydomonas reinhardtii* did not flocculate even at pH=12, which suggests that membrane charges are related to alkaline flocculation (Schlesinger et al., 2012).

The formed microalgae floccules with alkaline pH values were stable enough to be retained by a filter with a relative big pore size (20 µm), even with constant stirring, which can help reduce the difficulty of simple filtration for harvesting. Since flocculation also occurs in a turbulent medium, this process can be directly incorporated in the culture systems without the need of a second unit for harvesting.

Flocculation by pH change was compared to the traditional flocculant ferric sulphate and copper sulphate, metallic salts used for algal control in water. Results are shown in Fig. 3, OD_{750} after flocculation and simple filtration in turbulent medium using ferric sulphate is 0 after 30 seconds. This means ferric sulphate is highly efficient as complete harvest occurs. Efficiency of copper sulphate is higher than acid flocculation but lower than alkaline flocculation. Flocculation by pH change is not as effective as traditional chemical flocculation. However, if not all cells are separated from the medium, the remaining cells could be used to keep the culture growing in the same media after neutralization if they stay viable. If the highly effective ferric sulphate flocculation is used, residual salts could affect the reuse of the filtered medium (Rwehumbiza et al., 2012). Copper sulphate also is highly toxic for microalgae, and it is not a viable option if the intention is to reuse the medium to grow microalgae.

After the flocculation experiments, the remaining microalgae cells were able to keep growing after the pH was neutered to 7.3. This indicates that non filtrated cells remain viable and keep growing in the same medium. After returning to neutral pH conditions, the increase in OD_{750} as a function of population growth was measured for several days, and the behavior was similar in all cases for the acidification and alkalization treatments (Fig. 4).

A typical logistic population growth was obtained for all the neutered cultures. It can be seen that the control experiment was in lag phase for the first 24 hours, while the flocculated experiments were already growing. Also, it was observed that after the initial 24 hours, all the samples had similar behavior and only the cells flocculated at pH=5 presented a slightly lower OD_{750}. These results indicate that after pH-change induced flocculation, the remaining algal cells behave similar to a normal culture. The addition of potassium and nitrogen due to potassium hydroxide and nitric acid added to induce flocculation and neutralize the

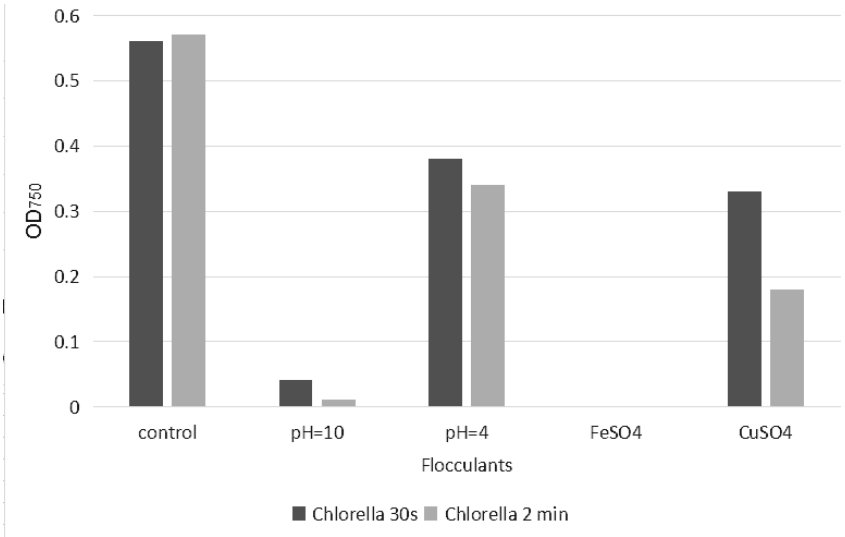

Fig. 3. Comparison of OD_{750} after different flocculation processes and times for *Chlorella vulgaris* (initial OD_{750}=0.889).

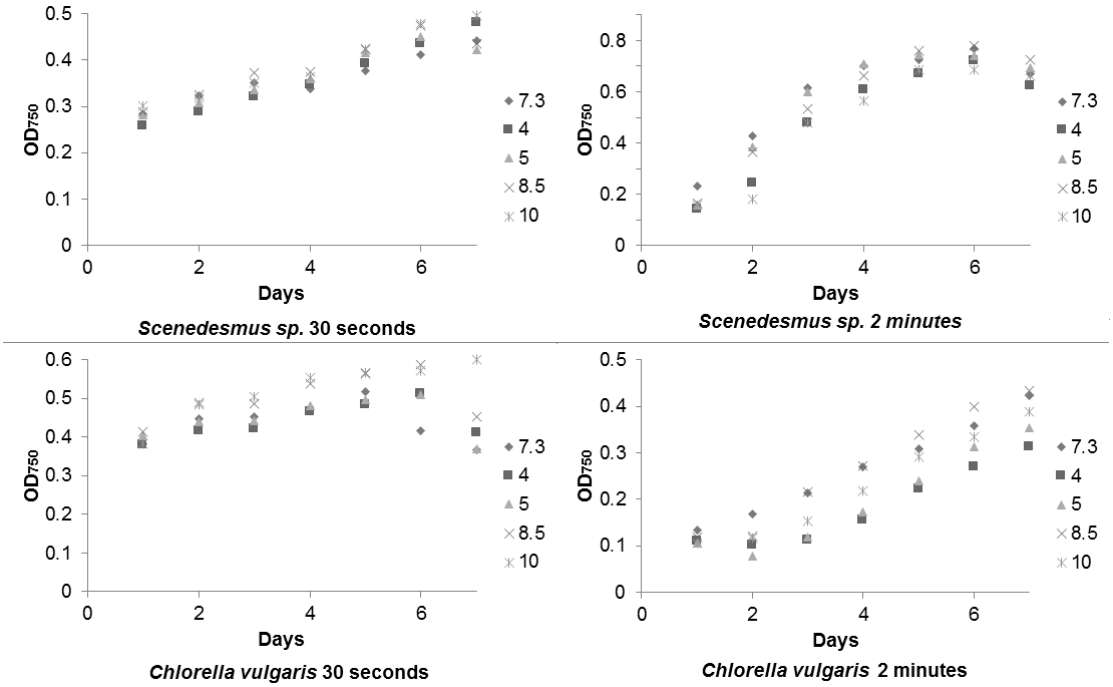

Fig. 4. Microalgae growth curve measured in optical density (750nm) after flocculation and filtration at different pH levels

medium could explain the difference in behavior between the treated samples and the control, as the salts can be assimilated as nutrients by the microalgae. Also, the decline of growing after several days for the 2 minutes experiment for *Scenedesmus* sp. and for the 30 seconds experiment for *Chlorella vulgaris* was observed for the treatments and the controls, and this might not be related to the variables used in this experiment but it could be linked to other stress-inducing factors.

CONCLUSIONS

Alkaline flocculation was highly effective for *Chlorella vulgaris* and *Scenedesmus* sp., even when the medium is in constant agitation; this could allow to adapt a semi-continuous inline harvesting system in a typical bioreactor with no need of a separation tank. Alkaline flocculation efficiency is not as effective as typical $FeSO_4$ chemical flocculation. However, it presents very acceptable efficiency rates, and once the flocculated microalgae are harvested and the medium neutered, it is possible to reuse the medium immediately after neutralization. Acid flocculation does not form stable floccules in turbulent conditions for both species; hence, it limits the application of this mechanism to a two-step process. Even when the behavior of the two species used on this experiment was similar, more research is needed in order to observe how pH induced flocculation is related to particular species, specifically to its membrane properties.

ACKNOWLEDGMENTS

This research was finantially supported by Universidad Autónoma de Querétaro, and Consejo Nacional de Ciencia y Tecnología (CONACYT) through FOMIX QRO-2014-C03-250295 project. The authors want to thank Centro de Estudios Académicos sobre Contaminación Ambiental (CEACA-UAQ) for the equipment collaboration and Andrea Macías for the help provided in the laboratory.

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