ATTI

Ricerca e applicazione di metodologie ecotossicologiche in ambienti acquatici e matrici contaminate

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INDICE

ASSESSMENT OF THE ECOTOXICOLOGICAL EFFECTS RELATED TO THE USE OF NATURAL ORGANIC COAGULANTS IN THE PRIMARY TREATMENT OF WASTEWATERS AT URBAN AND INDUSTRIAL LEVEL.

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Abstract - Natural coagulants have been receiving much attention in wastewater treatments and sludge conditioning for their eco-friendly and safety characteristics. In this field, the LIFE Adnatur project is aimed at demonstrating the efficacy of new natural based coagulants based on tannin extracted from the Black Acacia bark. Among the products tested in the project, Ecotan Bio 90D was chosen for the evaluation of the potential toxicity effects. Results were compared with a common inorganic metal based coagulant (i.e. $FeCl_3$). The natural coagulant toxicity in aquatic systems was determined by measuring the bioluminescence inhibition of *Vibrio fischeri* while the potential effects on the biological compartments of a conventional waste water treatment plant were evaluated by estimating the activated sludge activity inhibition by means batch respirometric tests. Moreover, in order to define the coagulant biodegradability, in both aerobic and anaerobic conditions, respirometric techniques and batch test for the assessment of the potential methane production (BMP test) were coupled. BMP tests were carried out on the sludge settled during Jar tests which permitted, by simulating the coagulation/flocculation process, to define the optimal natural coagulant dosage for sludge recovery maximization. The natural coagulant showed to not imply any toxic effects for aquatic life and activated sludge biomass. Moreover the product showed to have a good degree of biodegradability both in aerobic and anaerobic conditions.

Keywords: natural coagulant, ecotoxicity, biodegradability, biomethane potential production, respirometry.

Introduction

The main applications of polyelectrolytes in wastewater treatment plants (WWTP) are in coagulation-flocculation processes, in dewatering and thickening the settled sludge (Bolto B., Gregory J. 2007). In recent years there has been an increasing interest in the development of natural coagulants produced or extracted from microorganisms, animal or plant tissues. In comparison with synthetic or inorganic coagulants, natural based products has shown to be safer for human health and environmental ecosystems. Among the results highlighted by several studies (Vijayaraghavan G. *et al.*,2011; Bolto B., Gregory J. 2007), the use of natural organic coagulants implies advantages in energy and resources saving, reduction of coagulant dosage, elimination of other chemicals such as neutralizing agents and substitution of hazard chemicals. Moreover, advantages are identifiable for the improvement of biological processes and sludge conditioning.
In this context, LIFE Adnatur project (LIFE12/ENV/ES/00265) is focused on the validation, assessment and demonstration of the efficacy of a new developed product line based on natural coagulants for the urban and industrial wastewater primary

treatment. The developed technology is based on tannin extracted from the Black Acacia bark (*Acacia mearnsii*) and further chemically modified to improve the coagulation efficiency. Preliminary analysis on the effectiveness of coagulation and flocculation process using the natural coagulants in urban, ceramic and textile wastewater treatments have been highlighted by executing several lab tests (*www.adnatur.com*).
The actual study was directed to assess the biological implications connected to the use of a cationic, low molecular weight coagulant named Ecotan Bio 90D, in primary WWT and, more in general, the potential environmental impacts and ecotoxicological risks associated with its use. The coagulant aquatic toxicity, the potential effects on the activated sludge biomass as well as its biodegradability, in aerobic and anaerobic conditions, were evaluated combining biological essays and batch test techniques.

Materials e methods

The experimental plan defined in this paper was based on the comparison of the Ecotan Bio 90D with ferric chloride as one common metal based coagulant in WWTP. *Vibrio fischeri* bioluminescence inhibition assay was employed to evaluate the two coagulant aquatic toxicity. The test was carried out by using the Lumistox™ 300 system according to the ISO 11348-3 (1998) standard for the chronic toxicity evaluation. The procedure was slightly modified by correcting the pH up to 5.5 (for both the sample and the control) avoiding the precipitation of coagulant solutions. The bacterial bioluminescence was read at 30 minutes.
The activated sludge inhibition was evaluated by means respirometric techniques based on the rate of O_2 consumption measured as OUR (Oxygen Uptake Rate) (OECD 1993a; Polo *et al.*,2011). The sludge biomass for the batch tests was taken from the aerated basin at a municipal WWTP located in Bologna and the equipment employed was an automatic respirometer (MARTINA™, SPES). The respiration rate of activated sludge samples (500 ml) was evaluated firstly by spiking an easily biodegradable substrate (i.e. Sodium acetate trihydrate, NaOAc) followed by the addiction of the same amount of NaOAc together with increasing coagulant concentrations. Data achieved from *Vibrio fisheri* essay and from the batch respirometric experiments were computed for the inhibition percentage calculation (H%) and, further, the log concentration–log response data were fitted with a gamma-function for EC50 estimation. The aerobic Ecotan Bio 90D biodegradability was evaluated by means batch respirometric experiments, following the procedures reported by MARTINA™, which permitted to quantify the coagulant biodegradable share (BCOD) expressed as a percentage of the chemical oxygen demand (COD) content.
The batch tests in anaerobic conditions were based on the estimation of the biomethane production rate achievable from primary sludge settled performing Jar tests where the coagulants were applied for urban raw sewage samples treatments. The tested natural coagulant dosages, from 0 to 300 ppm, indicated 300 ppm as the most effective for primary sludge recovery. Consequently, the same dosage was applied conducting Jar tests with ferric chloride. The assessment of the settled sludge anaerobic biodegradability was carried out in batch test (BMP test, Angelidaki *et al.*, 2009) by using a commercial laboratory instrument (AMPTS, Bioprocess Control, Sweden). The gas-tight glass bottles were inoculated with the liquid fraction of digested sludge (TS and VS content resulted in 52.8 and 35.9 g/kg_{wet} respectively) drawn from a mesophilic farm-scale anaerobic digestion plants. Each closed vessels were maintained in mesophilic conditions for at least 30 days applying substrate/inoculum ratio around 3 gVS/gVS. The daily production of biogas

produced was automatically recorded and the results were expressed in terms of Specific Methane Production (SMP: $Nm^3CH_4/tVSS$).

Results

The comparison between the use of Ecotan Bio 90D and $FeCl_3$ as primary coagulants achieved from the several tests carried out is reported in Tab.1.

Table 1. Results of ecotoxicological tests and coagulant biodegradability evaluations.

	Ecotan Bio 90D	$FeCl_3$
Bioluminescence essay	EC50 (mg/l) (95% Conf. Interval)	
	5326.1 (2870.8-9881.3)	339.9 (263.9-435.5)
Sludge inhibition activity	EC50 (mg/l) (95% Conf. Interval)	
	ND	313.4 (136.5-719.7)
Biodegradability	10% BCOD (COD 180 g/l)	
TS (VS% on TS)	6090 g/l (88.2%)	7690 g/l (55.9 %)
SMP	164.7 $Nm^3/tVSS$	113.6 $Nm^3/tVSS$
K_d	0.28 d^{-1}	0.16 d^{-1}

Vibrio fisheri essay showed for Ecotan Bio 90D a very high EC50 value which, however, came out of an extrapolation since the max biolumiscence inhibition achieved was around 50%. No toxic effects for Ecotan Bio 90D on activated sludge samples were experimented during the batch respirometric essays. Both the two ecotoxity tests carried out on $FeCl_3$ reported an EC50 value around 300 ppm. Fig. 1 shows two respirograms obtained for the same dosage of Ecotan Bio 90D and $FeCl_3$. Precisely, the former OUR peak is related to NaOAc addiction (20 mg/l) whereas the latter is due to NaOAc and coagulant dosage spike (20 mg/l of NaOAc and 120 mg/l of coagulants). Clearly, whereas the natural coagulant implied an increase in the respiration rate, the same $FeCl_3$ concentration caused a sludge inhibition effect. As confirmed from Ecotan Bio 90D biodegradability evaluation, the biodegradable organic fraction (BCOD) is about 10% of the total COD amount. This could easily explain the increase of activated sludge activity.

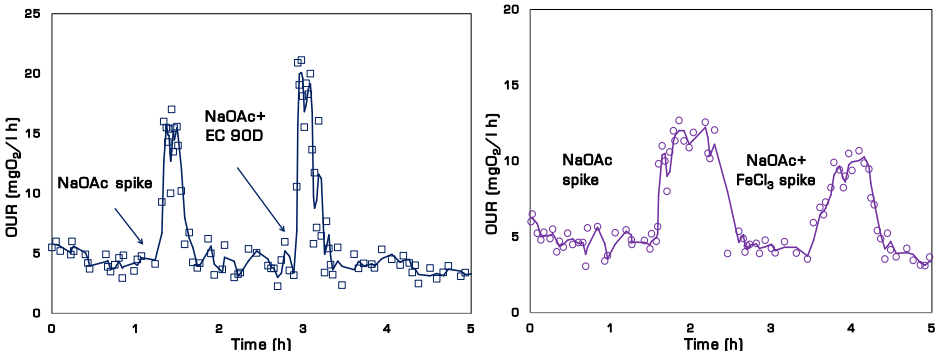

Figure 1. OUR profile of a) Ecotan Bio 90D and b) $FeCl_3$ for the activated sludge activity inhibition estimation.

The sludge settled during the Jar tests were characterized by determining the total suspended solids (TS) as well as the volatile content (VS). The primary sludge produced by the use of Ecotan Bio 90D showed a TS content equal to 6090 mg/L with a high volatile fraction (88.2% on TS), whereas the sludge settled with $FeCl_3$ reported a concentration of 7790 mg/l with a lower volatile fraction (55.9% on TS). This preliminary result suggested a great difference in terms of potential energy recovery between the two substrates in anaerobic conditions. In fact, at the end of BMP trials, the sludge linked to the use of Ecotan Bio 90D showed a specific methane production (164.7 $Nm^3/tVSS$) 45% greater than the sludge produced by $FeCl_3$ (113.6 $Nm^3/tVSS$).

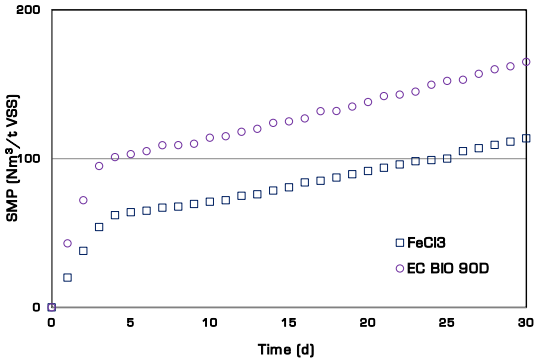

Figure 2. Potential Biomethane Production achieved from the settled sludge during the experimental trials.

Anaerobic degradation of complex organic materials has been described as a sequential process involving several steps such as hydrolysis, acidogenesis, acetogenesis and methanogenesis (Batstone D.J., *et al.,* 2002). Although, the hydrolysis of complex organic material has been considered the rate-limiting step of the anaerobic digestion process, Figure 2 shows that for both the samples, the most of the energy recovered from the substrates was reached within the first 5 days, when the methane production resulted more than 60% of the ultimate methane achieved. This aspect could be explained by the high inoculum to substrate ratio adopted in the experimental trials (Fernandez B. *et al.*, 2001). The greater anaerobic biodegradability of the settled sludge was confirmed by the value of the first order hydrolysis constant (K_d) as reported in Table 1. K_d values were calculated on the first part of the experimental curve according to Angelidaki *et al.* 2009. Indeed, the primary sludge produced by Ecotan Bio 90D showed a reaction rate of the hydrolysis step about 75% greater than $FeCl_3$.

Discussion

The *Vibrio fischeri* bioluminescence essay revealed for the natural coagulant a very elevated EC50 value which results to be too high respect to the typical coagulant dosages in primary WWTP (i.e. 50-200 ppm for $FeCl_3$). Moreover, the EC50 derived from a data extrapolation since the maximum inhibition percentage achieved was equal to 48%. At the same time, the tests carried out on activated sludge samples for the evaluation of respiration rate inhibition due to Ecotan Bio 90D applications reported no effective toxic effects. Moreover, by adding the natural coagulant (at

lower dosage) the sludge biomass activity appeared even stimulated. Batch respirometric tests highlighted as about 10% of Ecotan Bio 90D COD is readily biodegradable and this could explain the increase of the activated sludge activity. On the contrary the EC50 values calculated for $FeCl_3$ resulted to be close to the common ferric chloride dosage range applied in the primary treatment of sewage water. Comparable results were achieved from ecotoxigological tests. In fact, both the *Vibrio fischeri* bioluminescence essay and the activated sludge inhibition activity estimation reported EC50 values around 300 ppm. However it should be considered that downstream of the process of clariflocculation, the coagulant concentrations in the supernatant, hereafter directed to the oxidation basin, result to be lower than the applied dosages. Moreover, the efficiency and the reactivity of a specific coagulant depends on several parameters such as the pH and the presence of carbonates in the wastewater to be treated. On the basis of these considerations, ecotoxicological effects on the active biomass of a WWTP are excludable also for ferric chloride.
The results of BMP trials show clearly that Ecotan Bio 90D produced a primary sludge with a higher anaerobic biodegradability than $FeCl_3$. Likely, since the different origin and chemical characteristics of the two coagulants, the mechanism they promote during coagulation/flocculation processes are different. As showed, the sludge settled by the use of Ecotan Bio 90D reported a greater SV to TS percentage ratio in comparison with the sludge achieved by applying the inorganic coagulant.

Conclusions

Bioluminescence essay and sludge inhibition activity tests showed as the ECOTAN BIO 90D coagulant doesn't imply any toxic effects for bacterial community. The estimated EC50 values resulted to be too far from the typical coagulant dosages applied in primary wastewater treatments. The good level of biodegradability as well as the increase in methane production rate during the anaerobic tests are considered plausible results since the ECOTAN Bio 90D organic nature. As a matter of fact the natural coagulant has shown no toxic effects for both the aquatic life and the sludge biomass activity. Moreover. the product resulted to have a good level of biodegradability in both aerobic and anaerobic conditions. On the other hand, lab tests conducted in the Adnatur project partner labs have shown the efficiency of natural coagulants in coagulation/flocculation processes. The highlighted advantages connected to the use of natural products as primary coagulants in WWTP are to be demonstrated at industrial level by means two wastewater treatment prototype plants to be located in different real end-users facilities from textile, ceramic sector firstly and, then, at urban wastewater level.

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