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Effect of Cr(III) on process performances of MBR systems

Luca d'Antonio, Massimiliano Fabbricino*, Francesco Pirozzi

Department of Civil, Architectural and Environmental Engineering, University of Naples Federico II, Via Claudio 21, Naples 80125, Italy, Tel. +39 081 7683438; email: fabbrici@unina.it

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ABSTRACT

The paper investigates the effect of Cr(III) on the heterotrophic biomass of membrane bioreactors (MBRs) to understand the consequences on process performances. Respirometric tests are conducted to evaluate the consequences of chromium injection on oxygen consumption and oxygen uptake rate for the sludge sampled from a MBR pilot plant and, comparatively, for the sludge sampled from a conventional activated sludge treatment plant. MBR sludge is found to be more resistant to chromium injection than conventional activated sludge. Measurements of residual metal concentration are carried out to understand the mechanism of chromium adsorption and assimilation. The release of soluble microbial products, which play an important role on membrane fouling, is also investigated. Obtained information is completed by microscopic observations, aimed at identifying the variation of biomass structure and composition due to the presence of Cr(III).

Keywords: Biomass; Chromium; Inhibition; MBR; SMP

1. Introduction

The extensive use of Cr(III) in numerous industrial processes, and especially its use for leather tanning, is responsible for the consistent amount of Cr(III) which is frequently found in the influent of wastewater treatment plants [1–3]. Although Cr(III) is included among the so-called essential trace elements [4] because of its biochemical role, at low concentration, in supporting the microbial growth, at high concentration it can be toxic for the biomass operating in the biological phases of treatment plants [5–7].

In the past years, several studies have been conducted to evaluate the inhibition effect of Cr(III) and other heavy metals either on single microbial species or on microbial consortia sampled from the aeration basin of conventional activated sludge (CAS) treatment plants [7–14]. Very little is known concerning the effect of Cr(III) on the biomass developed in membrane bioreactors (MBRs) [15,16]. MBRs differ from CAS treatment plants as the separation of the biomass from the treated wastewater is obtained via membrane filtration and not via gravity. The presence of a membrane allows increasing the quality of the effluent, and therefore MBRs are replacing CAS treatment plants in many situations [17–20]. Because of the different separation modality, the biomass operating in MBRs is different from that developed in the aeration phase of CAS treatment plants [21] and therefore it does not necessarily have the same response to the inhibition caused by metals.

The aim of the present study is to verify the effect of varying chromium concentrations on the heterotrophic biomass of MBRs, in order to understand the

*Corresponding author.

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consequences on the whole treatment process development. For this reason, the study includes not only the direct investigation on the biomass, but also the characterization of soluble microbial products (SMPs) that have an important role on membrane fouling.

2. Materials and methods

The experimental study was carried out on three different biomass. The first one, indicated as MBR Sludge 1 (MS1), was sampled from a MBR pilot plant fed with synthetic wastewater non-containing chromium, operating in steady state conditions. The second one, indicated as MBR Sludge 2 (MS2) was sampled from the same plant, fed with synthetic wastewater containing 10 mg L^{-1} of Cr(III). The third one, indicated as conventional sludge (CS) was sampled, instead, from a municipal treatment plant, adopting the CAS configuration.

Respirometric tests were effectuated on MS1 and CS, using a flowing gas, static liquid (LFS) respirometer [22], following the Italian technical guidelines [23]. After sampling sludge concentrations were adjusted to 2.5 g L^{-1}. Tests were carried out using a food/mass ratio equal to 0.01, at pH 7.5 ± 0.1 and 20°C. The adopted organic substrate was a mixture of sodium acetate, ammonium chloride, and potassium dihydrogen phosphate. The food/mass ratio was kept to 1/20. Allythiourea (ATU) was mixed to the substrate to isolate the heterotrophic biomass, inhibiting the activity of nitrifying bacteria. Chromium was injected as anhydrous chromium sulfate, varying Cr(III) concentration between 10 and 500 mg L^{-1}. Before each test, two successive injections of substrate without chromium were effectuated to stabilize the biomass. The time between each injection was long enough to allow the biomass to reach endogenous respiration conditions. Three different test types were conducted (Table 1). Type I (blank test) was aimed at evaluating the reference values of OUR and ΔO_2 (OUR_{blank}, ΔO_{2blank}), therefore no chromium sulfate was added during the organic substrate injection. Type II (chemical test) was aimed at evaluating the chemical oxygen consumption due to the metal ($OUR_{chemical}$, $\Delta O_{2chemical}$) therefore only chromium sulfate was injected, without any organic substrate. Finally, Type III (inhibition test) was aimed at evaluating the biochemical inhibition due to different Cr(III) concentrations ($OUR_{inhibition}$, $\Delta O_{2inhibition}$). For each tested $Cr_2(SO_4)_3$ concentration (10, 50, 200, and 500 mg L^{-1}) one test Type I, one test Type II, and one test Type III were effectuated. Each test was carried out on several replicates to reduce the experimental errors.

During tests Type II and III on MBR sludge, at fixed times (1 and 19 h after substrate and/or chromium sulfate injection) 10 mL sample was withdrawn from the respirometer and filtered to measure the concentration of residual Cr(III) (Table 1). At the same times 10 mL more sample was withdrawn during tests Type I and III on MS1 to measure SMP concentration in terms of proteins and carbohydrates (Table 1). Cr (III) concentration was detected using an atomic absorption spectrometer (ERRECI Avanta, Italy). Proteins were measured according to the Lowry method, as modified by Raunkjaer et al. [24]. Finally, carbohydrates were measured using the phenol–sulfuric acid method [25].

Microscopic observations were effectuated on MS1 and MS2 within 1 h from the sampling, using a phase contrast microscope (ZEISS Standard 20, Germany), following the indications of Madoni [26]. Observations were aimed at identifying the structure and the abundance of the micro-fauna inhabiting the sludge.

All reagents used during the study were high purity degree. Only ultrapure water was used for the required dilutions.

3. Results and discussion

Results of respiromeric tests are summarized in Table 2. The table reports also the average inhibition indexes, evaluated as:

$$I_{OUR} = \left(1 - \frac{OUR_{inhibition} - OUR_{chemical}}{OUR_{blank}}\right) \times 100 \quad (1a)$$

$$I_{\Delta O2} = \left(1 - \frac{\Delta O_{2inhibition} - \Delta O_{2chemical}}{\Delta O_{2blank}}\right) \times 100 \quad (1b)$$

Table 1
Experimental procedure followed for the different respirometric tests

Test type	Injected solution	Residual Cr analysis	SMP analysis
I	$CH_3COONa + NH_4Cl + KH_2PO_4$ + ATU	No	Yes
II	$Cr_2(SO_4)_3$	Yes	No
III	$CH_3COONa + NH_4Cl + KH_2PO_4$ + ATU and $Cr_2(SO_4)_3$	Yes	Yes

Table 2
Oxygen uptake rate and oxygen consumption measured during respirometric tests

		MBR				CAS			
Test	Cr(III) ($mg\,L^{-1}$)	$OUR_{inhibition}$ ($mg\,L^{-1}\,h^{-1}$)	$OUR_{chemical}$ ($mg\,L^{-1}\,h^{-1}$)	OUR_{blank} ($mg\,L^{-1}\,h^{-1}$)	I_{OUR} (%)	$OUR_{inhibition}$ ($mg\,L^{-1}\,h^{-1}$)	$OUR_{chemical}$ ($mg\,L^{-1}\,h^{-1}$)	OUR_{blank} ($mg\,L^{-1}\,h^{-1}$)	I_{OUR} (%)
1	10	27.8 ± 1.5	2.3 ± 0.2	23.0 ± 1.5	−10.8	35.0 ± 0.3	2.3 ± 0.5	26.6 ± 0.8	22.9
2	50	27.5 ± 0.3	2.8 ± 0.4	26.3 ± 0.3	6.0	19.7 ± 0.3	4.2 ± 0.2	19.3 ± 0.4	19.8
3	200	14.5 ± 0.1	3.9 ± 0.5	25.6 ± 0.6	58.3	7.6 ± 0.7	2.2 ± 0.2	23.4 ± 0.6	76.8
4	500	3.6 ± 1.1	2.5 ± 0.7	23.9 ± 1.0	95.5	2.2 ± 0.5	1.3 ± 0.4	28.1 ± 0.7	97.0
		$\Delta O_{2inhibition}$ ($mg\,L^{-1}$)	$\Delta O_{2chemical}$ ($mg\,L^{-1}$)	ΔO_{2blank} ($mg\,L^{-1}$)	$I_{\Delta O2}$ (%)	$\Delta O_{2inhibition}$ ($mg\,L^{-1}$)	$\Delta O_{2inhibition}$ ($mg\,L^{-1}$)	$\Delta O_{2chemical}$ ($mg\,L^{-1}$)	$I_{\Delta O2}$ (%)
1	10	14.3 ± 0.1	2.5 ± 0.1	12.4 ± 0.2	4.5	14.1 ± 0.1	2.8 ± 0.7	14.1 ± 0.4	27.5
2	50	10.2 ± 0.1	1.3 ± 0.1	10.9 ± 0.3	18.0	16.8 ± 0.7	5.3 ± 0.8	16.8 ± 0.9	42.4
3	200	12.1 ± 0.2	5.8 ± 0.0	12.1 ± 0.5	48.2	14.6 ± 0.1	1.2 ± 0.1	14.6 ± 1.1	51.3
4	500	2.8 ± 0.8	1.8 ± 0.6	10.3 ± 0.9	89.6	15.2 ± 0.3	0.6 ± 0.3	15.2 ± 0.2	94.8
1	10	14.3 ± 0.1	2.5 ± 0.1	12.4 ± 0.2	4.5	14.1 ± 0.1	2.8 ± 0.7	14.1 ± 0.4	27.5

where I_{OUR} is the inhibition index relative to the oxygen uptake rate (%). $I_{\Delta O2}$ is the inhibition index relative to the total oxygen consumption (%).

Data analysis shows that the effect of chromium injection, on both MS1 and CS, was of different nature for low dosages and for high dosages of $Cr_2(SO_4)_3$. The effect was also different in terms of immediate inhibition, measured by I_{OUR}, and long-term inhibition, measured by $I_{\Delta O2}$. For Cr(III) concentration lower than $50\,mg\,L^{-1}$, in fact, OUR values increased after each injection, indicating a positive effect of the metal on biomass metabolic activity, in agreement with previous findings [7]. The positive effect of chromium injection was not observed in terms of total oxygen consumption.

ΔO_2, in fact, decreased even in presence of the lowest tested Cr(III) concentration ($10\,mg\,L^{-1}$). Therefore, although low Cr(III) dosages did not have a short term inhibitory effect, they were responsible for non negligible long-term inhibition. As expected the inhibitory effect became more and more important increasing chromium concentration.

Inhibition indexes were generally higher for CS, which therefore resulted less resistant to Cr(III) toxic effect than MS1, although the differences were no more evident when Cr(III) concentration reached 500 $mg\,L^{-1}$. The half maximal effective concentration, evaluated considering OUR values, was equal to 105 mg L^{-1} for the conventional activated sludge and to 135 $mg\,L^{-1}$ for the MBR activated sludge.

Results of residual chromium concentration are summarized in Table 3. No residual chromium was detected in the samples up to $500\,mg\,L^{-1}$ of Cr(III) injection. In this latter case, residual Cr(III) was lower in tests Type II (i.e. tests effectuated without substrate injection) than in tests Type III, decreasing, as expected, with time.

Considering the following relationship:

$$Cr_{injected} = Cr_{adsorbed} + Cr_{uptake} + Cr_{residual} \quad (2)$$

where $Cr_{injected}$ is the amount of chromium added at the beginning of the test; $Cr_{adsorbed}$ is the amount of chromium adsorbed to the biomass; Cr_{uptake} is the amount of chromium absorbed by the biomass; and $Cr_{residual}$ is the amount of residual chromium; it was concluded that the amount of Cr(III) adsorbed by micro-organisms was higher in absence of organic substrate, which interfered with the adsorption process. As the adsorbed Cr(III) could not easily cross the cellular membrane if not transported by the substrate, Cr(III) uptake, in absence of substrate, was reduced.

From Table 3, it can be also observed that the amount of Cr(III) adsorbed in 1 h was extremely high, explaining why it was not possible to detect residual

Table 3
Residual chromium concentration for $500\,mg\,L^{-1}$ of Cr(III) injection

Test Type	Time (h)	MBR Cr(III) ($mg{\cdot}L^{-1}$)
II	1	96.0 ± 0.1
	19	8.8 ± 0.1
III	1	175.0 ± 0.2
	19	18.6 ± 0.1

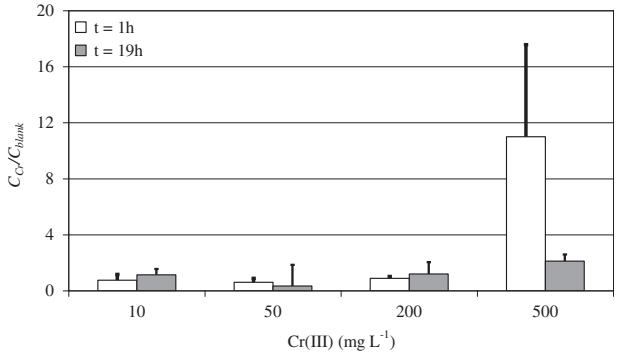

Fig. 1. Ratio between carbohydrates concentration with and without Cr(III) injection.

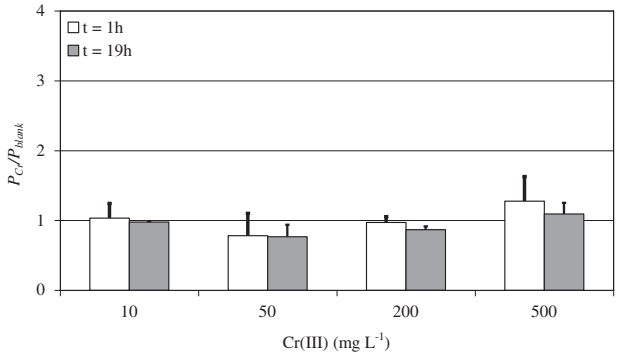

Fig. 2. Ratio between proteins concentration with and without Cr(III) injection.

chromium concentration whenever the injected amount was lower than the maximum tested value.

Figs. 1 and 2 show the concentration of carbohydrates (*C*) and proteins (*P*) measured on samples withdrawn at different times. Data are reported in terms of ratio between the values measured during inhibition tests (C_{Cr}, P_{Cr}) and those measured during blank tests (C_{blank}, P_{blank}). Generally the differences between carbohydrates and proteins released in presence or in absence of chromium were not relevant, at least up to 400 mg L^{-1} of Cr(III). Nonetheless 1 h after the injection of 500 mg L^{-1} of Cr(III), it was detected a substantial increase of carbohydrates, and a less important increase of proteins. The observed increases were almost abated 19 h after the injection. Most probably this effect was due to the cellular lysis of biomass caused by Cr(III), which resulted in an immediate release of SMPs. In agreement with the death-regeneration hypothesis [27] produced SMPs were successively consumed by micro-organisms, so that their concentration decreased with time.

Microscopic observations conducted on MS1 and MS2, summarized in Table 4 and Fig. 3(a) and (b), clearly indicate that Cr(III) injection modified the composition and the structure of the micro-fauna developed in the plant. *Vorticellae convallariae* and *Testatae amoebae*, which denote stable conditions of the plant and excellent quality of the treated wastewater [28] were absent after Cr(III) injection, indicating the scarce resistance of these micro-organisms to the toxic effect of the metal. The number of crawling ciliated protozoan, index of good plant performances [28] decreased in MS2 respect to MS1, while the number of

Table 4
Micro-fauna in the MBR sludge in absence (MS1) and in presence (MS2) of Cr(III)

Micro-fauna	MS1 (No. 25 μL)	MS2 (No. 25 μL)
Sessile ciliated protozoan	72	47
Vorticella convallaria	66	44
Vorticella microstoma	2	1
Opercularia	2	2
Epistylis	2	
Crawling ciliated protozoan	8	1
Swimming cliliated protozoan		2
Sarcodina protozoan	28	
Testate amoebae	26	
Naked amoebae	2	
Flagellates protozoan		1
Metazoan	6	39
Rotifera	6	37
Nematoda		1
Larvae		1
Total	114	90

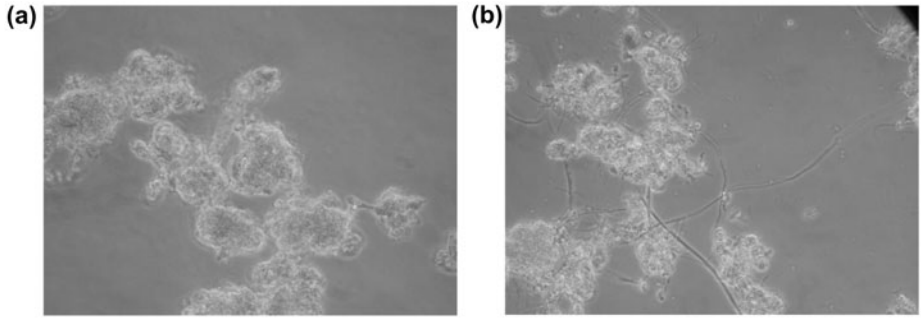

Fig. 3. Microscopic characteristics of the sludge (100×): (a) MS1, treatment plant working without Cr(III). (b) MS2, treatment plant working with 10 mg L^{-1} of Cr(III).

metazoans was much higher in MS2 than in MS1, most probably because metazoans are characterized by a larger number of lysosomes which are able to accumulate a large amount of toxic compounds, acting as detoxifiers for the cells. Flocks structure of the two sludges was completely different, as it can be easily deduced by comparing Fig. 3(a) and (b). Flocks were scarcely formed and very disperse in MS2, with abundance of frustules without colonies. Finally in MS2 many filamentous micro-organisms were individuated, including the following species: Type 0041, Type 0675, and Haliscomenobacter hydrossis. Nonetheless, although microscopic characteristics of the micro-fauna developed in presence of Cr(III) indicated a sludge which would have caused a reduction of performances in CAS treatment plants, because of the different separation system adopted in MBRs no practical effect was produced in the pilot plant in terms of effluent quality.

4. Summary and conclusions

According to the obtained results the presence of Cr(III) affects the metabolism of the heterotrophic biomass of CAS and MBR systems and, for these latter, seems to be responsible for an increase of SMPs release. Moreover the results suggest a possible alteration of the structure and the composition of the micro-fauna inhabiting MBR sludge, although only more advanced analysis in molecular microbiology can point out the effect of microbial changes. Nonetheless, as the half maximal effective concentration is around 140 mg L^{-1}, for MBR and 100 mg L^{-1} for CAS systems, the inhibitory effect becomes relevant only at very high Cr(III) concentrations, which are seldom found in real wastewater. Similarly, as the increase of SMPs is caused by cellular lysis produced by Cr(III) doses corresponding to 500 mg L^{-1}, no worsening of membrane fouling is generally expected. Finally it has to be highlighted that the observed presence of filamentous micro-organisms is not expected to affect the quality of the final effluent of MBR treatment plants because of the presence of a membrane filtration unit, characteristic of these systems.

List of symbols

I_{OUR} — inhibition index relative to the oxygen uptake rate (%)

$I_{\Delta O2}$ — inhibition index relative to the total oxygen consumption (%)

$Cr_{injected}$ — amount of chromium added at the beginning of the test

$Cr_{adsorbed}$ — amount of chromium adsorbed to the biomass

Cr_{uptake} — amount of chromium absorbed by the sludge

$Cr_{residual}$ — amount of residual chromium

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