

Article

Simultaneous Treatment of Agro-Industrial and Industrial Wastewaters: Case Studies of Cr(VI)/Second Cheese Whey and Cr(VI)/Winery Effluents

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Abstract: Hexavalent chromium (Cr(VI)) was co-treated either with second cheese whey (SCW) or winery effluents (WE) using pilot-scale biological trickling filters in series under different operating conditions. Two pilot-scale filters in series using plastic support media were used in each case. The first filter (i.e., Cr-SCW-filter or Cr-WE-filter) aimed at Cr(VI) reduction and the partial removal of dissolved chemical oxygen demand (d-COD) from SCW or WE and was inoculated with indigenous microorganisms originating from industrial sludge. The second filter in series (i.e., SCW-filter or WE-filter) aimed at further d-COD removal and was inoculated with indigenous microorganisms that were isolated from SCW or WE. Various Cr(VI) (5–100 mg L⁻¹) and SCW or WE (d-COD, 1000–25,000 mg L⁻¹) feed concentrations were tested. Based on the experimental results, the sequencing batch reactor operating mode with recirculation of 0.5 L min⁻¹ proved very efficient since it led to complete Cr(VI) reduction in the first filter in series and achieved high Cr(VI) reduction rates (up to 36 and 43 mg L⁻¹ d⁻¹, for SCW and WW, respectively). Percentage d-COD removal for SCW and WE in the first filter was rather low, ranging from 14 to 42.5% and from 4 to 29% in the Cr-SCW-filter and Cr-WE-filter, respectively. However, the addition of the second filter in series enhanced total d-COD removal to above 97% and 90.5% for SCW and WE, respectively. The above results indicate that agro-industrial wastewater could be used as a carbon source for Cr(VI) reduction, while the use of two trickling filters in series could effectively treat both industrial and agro-industrial wastewaters with very low installation and operational costs.

Keywords: hexavalent chromium; second cheese whey; winery effluents; co-treatment; trickling biofilter

1. Introduction

Nowadays, the removal of inorganic (heavy metals, such as Cr, Pb, and Cd) and organic pollutants (proteins, carbohydrates, fats, and nucleic acids) from industrial/agro-industrial wastewaters remains a huge challenge, leading to an important problem in the field of wastewater purification [1].

Chromium has found a wide range of applications and under normal conditions exists in two stable oxidation states: hexavalent (Cr(VI)) and trivalent (Cr(III)). Cr(VI) is much more mobile in

the environment than Cr(III). In contrast, Cr(III) has low solubility in water ($<1 \mu\text{g L}^{-1}$) and readily precipitates (formation of insoluble hydroxide, K_{sp} of $\text{Cr}(\text{OH})_3 = 6.3 \times 10^{-31}$, in the pH range between 7 and 10) [2]. Of the two, Cr(VI) compounds have the most significant effects on health. On the contrary, Cr(III) is less toxic than Cr(VI) and is listed as an essential element and a micronutrient. It is poisonous only at high concentrations [3]. Consequently, the removal or reduction of Cr(VI) to Cr(III) from contaminated waters and wastewaters is a very important process [4–6]. Most countries, including those in the European Union, have regulated the permitted limit for the total chromium in drinking and surface waters to 0.05 mg L^{-1} [7,8].

Several methods have been reported for chromium removal. The most widely used techniques for Cr(VI) removal are: adsorption, biosorption, reduction, filtration, ion-exchange, electrochemical, phyto-remediation, flotation, and solvent extraction [9]. Various chromium-contaminated waters such as groundwater, drinking water, tannery wastewater, electroplating wastewater, and synthetic industrial wastewater were used. The success of the applied technologies, in terms of Cr(VI) removal rate and efficiency, depends on the physicochemical conditions that are occurring in the aquatic environment. Treatment systems based on Cr(VI) reduction can be biological (microorganisms can catalyze redox reactions by a combination of several mechanisms, including enzymatic extra-cellular reduction, nonmetabolic reduction by bacterial surfaces, and intra-cellular reduction and precipitation) or abiotic (reduction by iron salts, sulfur compounds, and metals), or a combination of these [10]. Reductive precipitation, commonly by adding Fe(II), is a well-known treatment process for the removal of Cr(VI) from groundwaters that usually contain relatively low Cr(VI) concentrations. Firstly, Cr(VI) is reduced to Cr(III), while Fe(II) is oxidized to ferric iron (Fe(III)). This process has proven to reduce Cr(VI) concentration to sub-ppb levels in relatively short reaction time ($<20 \text{ min}$), significantly reducing the capital and operating costs [11]. Similar to the other treatment technologies, the greatest challenge with the operation of this process is disposal of the waste backwash water, which will contain all the Cr removed from the water as well as all the iron added. According to the literature, for a RCF (Reduction-Coagulation-Filtration) system using ferrous sulfate (FeSO_4) to reduce Cr(VI) to Cr(III), the total water (treatment) cost is $4.5\$ \text{ kgal}^{-1}$ (0.216 € m^{-3}) for 250 gpm ($1362.75 \text{ m}^3 \text{ d}^{-1}$) and initial Cr(VI) concentration of $25 \mu\text{g L}^{-1}$ [12]. Membrane filtration (ultrafiltration–UF, nanofiltration–NF, and reverse osmosis–RO) has received significant attention, however it cannot be easily applied for the removal of Cr(VI) from water sources at sub-ppb levels. These processes appear to offer significant efficiency, relatively simple operation, and save space [10]. However, their high capital and operating costs has become the greatest obstacle to the development of this technology in full-scale systems.

Microbiological Cr(VI) reduction is regarded a safe and sustainable process which has been focused on extensively even in wastewaters with high Cr(VI) concentrations [1,13,14]. Taking into account the nutritional requirements of the microorganisms involved, the addition of an external organic carbon source is necessary to enhance Cr(VI) reduction [15]. Various carbon sources have been tested (mainly in pure rather than mixed cultures) including lactose, maltose, glucose, fructose, humic acid, sodium acetate, sugar, molasses, etc., with significant Cr(VI) reduction rates [16–20]. However, costs for some of these feedstocks (which serve as an electron donor) might become prohibitive in actual field applications. The use of industrial-grade molasses may provide a more cost-effective alternative to any of the above-mentioned carbon and energy sources [21–25]. According to the literature, the treatment cost when using molasses ($0.13 \text{ € (Kg molasses)}^{-1}$ in Greece) amounts to about $0.39 \text{ € (m}^{-3} \text{ of wastewater)}$ [25].

To further reduce treatment cost, researchers have used agricultural wastewater as feed for bacterial growth [26]. Cheese whey, an organic by-product (resulting from cheese production), has been examined as a low-cost carbon source for Cr(VI) reduction. According to Panousi et al. [16], the cost of treatment using cheese whey (in a suspended growth system operating as a Sequencing Batch Reactor, SBR, using initial Cr(VI) concentration of $1.0\text{--}2.0 \text{ mg L}^{-1}$ and low feed chemical oxygen demand-COD of just $100\text{--}200 \text{ mg L}^{-1}$) varies between $0.16\text{--}0.34 \text{ €}$ and $0.68\text{--}0.86 \text{ € m}^{-3}$ for treatment capacities of $100 \text{ m}^3 \text{ d}^{-1}$ and $10 \text{ m}^3 \text{ d}^{-1}$, respectively. Cheese whey is considered to be the most

important pollutant of dairy wastewaters. This is not only because of its high organic load, but also because of the large volume generated. Only a few studies have been published using cheese whey for Cr(VI) reduction [16,22,27–33] and most of these were performed for in-situ groundwater and soil remediation [22,30–33], while the remainder took place in pilot-scale suspended growth systems (activated sludge technology) [16,27–29]. Another low-cost carbon source that was examined for Cr(VI) reduction was liquid pineapple waste by Zakaria et al. [20]. Zakaria et al. [26] studied the ability of a pure culture (*Acinetobacter haemolyticus*) to reduce Cr(VI) in a packed bed reactor using various initial Cr(VI) concentrations (15–240 mg L⁻¹). Successful application of cheese whey and liquid pineapple waste as organic substrates to promote the biotic reduction of Cr(VI) was reported in the above-mentioned studies, however limited information exists on the use of attached growth systems and the organic load (d-COD) removal of wastewater.

Second cheese whey (SCW), which is a by-product of cottage cheese production, is characterized as a high strength organic pollutant [34]. The use of SCW for Cr(VI) bioreduction has been examined only by our research group in a previous work, using pilot-scale horizontal subsurface flow constructed wetlands [35]. Constructed wetlands proved to be effective as they achieved 100% and 50–70% removal of Cr(VI) and dissolved chemical oxygen demand (d-COD), respectively. However, the initial Cr(VI) and d-COD concentrations that were used in the constructed wetlands were relatively low (0.4–5 mg L⁻¹ and 1300–4100 mg L⁻¹, respectively).

Winery effluents (WE) also contain high concentrations of readily biodegradable soluble organic matter (BOD is about 0.4–0.9 of the COD value) [36–38], however, to our knowledge, it has not yet been used as a carbon source for hexavalent chromium reduction.

The present paper focuses on the co-treatment of Cr(VI) (in high concentrations in the range of mg L⁻¹, present mainly in industrial wastewaters) with SCW or winery effluents (WE) in attached growth (trickling filter) aerobic biological systems. To achieve both Cr(VI) and d-COD removal, two pilot-scale trickling filters were operated in series in each case. The first filter was used for complete Cr(VI) reduction to Cr(III) and partial COD removal from SCW or WE, while the second filter in series was used for further COD removal. Indigenous microorganisms originating from industrial sludge were used for Cr(VI) reduction and indigenous microorganisms that were isolated from SCW or WE were also used for COD removal. To the best of our knowledge, the present study is the first to report on Cr(VI) reduction using SCW and WE in trickling filters, and the simultaneous removal of d-COD from the wastewater.

2. Materials and Methods

2.1. Wastewater Characterization

SCW and WE were obtained from the “Papathanasiou” cheese factory that is located in Agrinio city (western Greece) and the local “Grivas winery” in Agrinio, respectively. SCW had acidic characteristics (pH 4.5–6) with high salinity (about 5.25 ppt), conductivity of $8455 \pm 1000 \mu\text{S cm}^{-1}$, and high d-COD ($43,000 \pm 2000 \text{ mg L}^{-1}$). WE derived from water used for washing tanks after grape processing. The WE presented pH values between 6 and 7, conductivity of about $300\text{--}700 \mu\text{S cm}^{-1}$, and high d-COD ($60,000 \pm 3000 \text{ mg L}^{-1}$).

2.2. Indigenous Microorganisms/Enrichment and Culture Conditions

Mixed cultures of microorganisms were used for the reduction of Cr(VI) and degradation of d-COD. Initially, industrial sludge obtained from the Hellenic Aerospace Industry S.A. was used to grow indigenous microorganisms able to reduce Cr(VI). In two different Erlenmeyer flasks (SCW-flask and WE-flask of 2 L) 10 g of sludge were diluted with SCW or WE (concentration of $1000 \text{ mg d-COD L}^{-1}$) and 1.5 mg L^{-1} Cr(VI) (added as $\text{K}_2\text{Cr}_2\text{O}_7$). When Cr(VI) concentration dropped below 0.2 mg L^{-1} , 0.9 L of the SCW-flask's and WE-flask's liquid was replaced with fresh SCW or WE and chromium (45% volume exchange), in order to achieve the desired final d-COD and Cr(VI)

concentrations of 1000 mg L^{-1} and 1.5 mg L^{-1} , respectively, within the flasks. This process completed one operating cycle. A series of operating cycles was performed while a start-up time of approximately three weeks was necessary to reach maximum Cr(VI) reduction rates. A minimum period of about 2.0 and 1.90 h^{-1} was needed, using SCW and WE, respectively, for Cr(VI) concentration to fall below the maximum permitted limit of 0.2 mg L^{-1} . The liquid in the flasks was kept under oxic conditions through aeration (Dissolved Oxygen D.O. $> 5 \text{ mg L}^{-1}$), while agitation was at 400 rpm with a magnetic stirrer. It is known that the pH of a solution has a very significant effect on bioreduction experiments. According to the literature, many microbes are capable of successfully reducing Cr(VI) from aqueous solutions at a wide range of pH [1,10]. The pH of the media affects the solubility and ionization state of Cr(VI), and bacterial growth is also affected by change in the pH of the media [9]. The most important redox species at pH values > 6.5 is chromate (CrO_4^{2-}), while $\text{Cr}_2\text{O}_7^{2-}$ and HCrO_4^- are mainly relevant to pH values < 6.5 and its speciation depends on Cr(VI) concentration and pH value [2]. The pH range in this study was about 4.5–6.0, without adjustment (making the process easier to apply in full-scale units), while the temperature was maintained at $22 \pm 2 \text{ }^\circ\text{C}$. In this way, a natural selection process led to the predominance of species that are able to reduce Cr(VI) by using SCW or WE as a carbon source. These two enrichment cultures (Cr-SCW-EC and CR-WE-EC) were used to set up the pilot-scale packed bed reactors for Cr(VI) removal (Cr-SCW-filter or Cr-WE-filter).

Indigenous microorganisms from SCW or WE were isolated for the aerobic biological degradation of residual d-COD in the post-treatment step (SCW-filter or WE-filter). The procedure described by Tatoulis et al. [34] was followed, in which a natural selection process led to the predominance of indigenous species able to biodegrade SCW or WE in the SCW-filter or WE-filter, respectively. Specifically, 0.5 L of SCW or WE (concentration of $23,000 \pm 1500 \text{ mg L}^{-1}$) was placed into two different 1 L Erlenmeyer flasks. The solution in the flasks was kept under aerobic conditions (D.O. $> 5 \text{ mg L}^{-1}$) and was mixed constantly. As soon as d-COD degradation increased, 90% of the liquid volume was discarded and the flask content was then brought back to 0.5 L volume by adding tap water and SCW or WE in appropriate amounts that were sufficient to achieve the desired final concentration of $23,000 \pm 1500 \text{ mg L}^{-1}$. A series of operating cycles were performed while a start-up time of approximately one month was necessary to reach maximum d-COD degradation rates. The minimum duration of the operating cycle was about 72 and 70 h^{-1} for SCW and WE, respectively. These two enrichment cultures (SCW-EC and WE-EC) were used to set up the pilot-scale packed bed reactors for d-COD removal (SCW-filter or WE-filter).

2.3. Packed Bed Reactors (Pilot-Scale Filters)

The packed-bed reactors that were used in this work have been described in detail by Michailides et al. [25] and Tatoulis et al. [34]. All of the packed-bed reactors consisted of a Plexiglas tube, 1.6 m high and 9 cm internal diameter (Figure 1). Plastic tubes (1.6 cm internal diameter, 3 cm length, specific surface area (A_s) of $500 \text{ m}^2 \text{ m}^{-3}$, and filter porosity (ϵ) of 0.8) were used as support material inside the reactors. The depth of the support media in the filters was 1.43 m. For each wastewater used (SCW or WE) two pilot-scale trickling filters were operated in series. The first filter was used for complete Cr(VI) reduction and partial d-COD removal of SCW or WE, while the second filter in series was used for further d-COD degradation. Initially, 0.5 L volume of each enrichment culture (Cr-SCW-EC or CR-WE-EC and SCW-EC or WE-EC, Section 2.2) was used to set up the corresponding filter (Cr-SCW-filter or Cr-WE-filter and SCW-filter or WE-filter, respectively). The pilot-scale filters were first operated as batch reactors to ensure microorganism attachment (development of a biofilm layer) onto the support material. The sequencing batch reactor (SBR) operating mode with recirculation (SBR with recirculation) was then applied. Three different recirculation rates were tested in the Cr-SCW-filter or the Cr-WE-filter (0.5, 1.0, and 2 L min^{-1}), while the SCW-filter or WE-filter was operated as batch reactor at a constant recirculation rate of 0.5 L min^{-1} . The Cr-SCW-filter or Cr-WE-filter were loaded with various concentrations of Cr(VI) (5, 10, 20, 30, 60, and 100 mg L^{-1}) and SCW or WE (1000, 5000, 13,000, 21,500, and $25,000 \text{ mg d-COD L}^{-1}$). According to Owlad et al., Cr(VI)

concentrations in industrial wastewaters are estimated to be between 0.1 and 200 mg L⁻¹ [9]. It should be mentioned that the concentrations of Cr(VI) in the wastewaters of many industries can reach up to 100 mg L⁻¹, such as from chrome tanning plants (3.7 mg L⁻¹), electropolishing plants (20.7 mg L⁻¹, 42.8 mg L⁻¹), hardware factories (60 mg L⁻¹), and tanneries (100 mg L⁻¹) [9]. The feed SCW or WE concentrations of 1000 to 25,000 mg d-COD L⁻¹ examined in this work simulate cheese factory and winery wastewater effluents (including factory washing waters) [34]. Finally, experiments under continuous operating mode and continuous operating mode with recirculation were also performed. The total duration of these experiments was about twenty months.

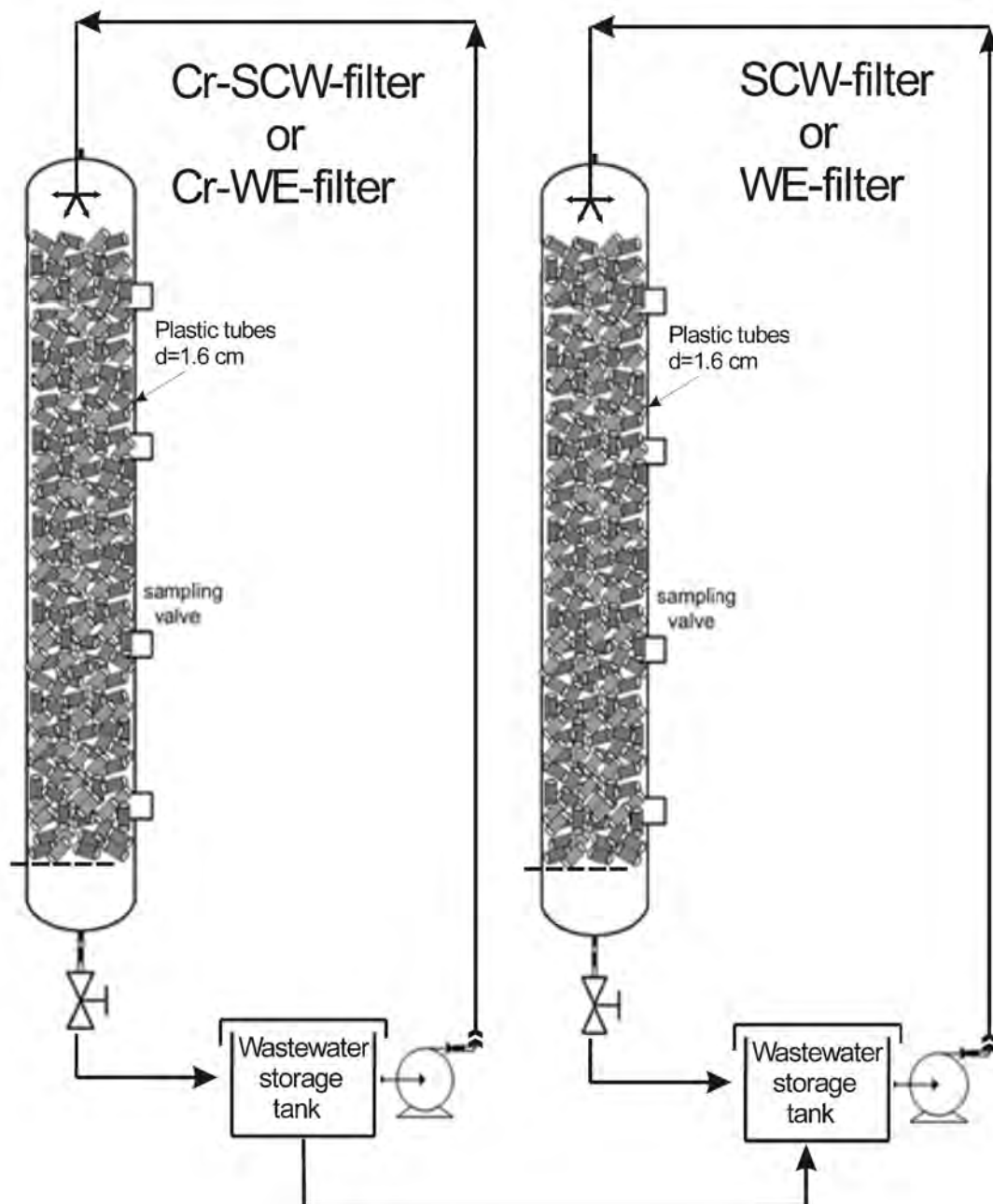


Figure 1. Scheme of the pilot-scale bio-filters.

The working volume (pore volume after biofilm formation) of all filters used was 6 L. Filter backwashing due to pore clogging was necessary using water (8 L min⁻¹ for all filters used,

Cr-SCW-filter, Cr-WE-filter, SCW-filter, and WE-filter) and air (upflow velocities of 10 L min^{-1}). Backwash frequency for the Cr-SCW-filter or Cr-WE-filter depended on the initial hexavalent chromium concentration tested (i.e., once every 3–4 days for Cr(VI) concentrations above 30 mg L^{-1} and once every eight days for lower concentrations). For the SCW-filter or WE-filter, backwash frequency depended on the SCW or WE feed concentration and was determined by the loading time (less than 1 min) of the working volume of polluted water into the filter, thus indicating pore clogging.

2.4. Sample Collection and Analyses

Samples were collected daily and were filtered through $0.45 \mu\text{m}$ -Millipore filters (GN-6 Metrical Grid 47 mm, Pall Corporation). Cr(VI) concentration was determined according to the “Standard Methods for the Examination of Water and Wastewater” (3500-Cr D Colorimetric method at 540 nm) [39], using a spectrophotometer (Boeco S-20). Total Cr concentrations were also measured using an atomic absorption spectrophotometer (Perkin-Elmer AAS-700), following the “Standard Methods for the Examination of Water and Wastewater” [39]. Cr(III) was estimated as the difference between the total chromium and Cr(VI). Measurements of total chromium concentrations were taken at the beginning and end of each experiment and their values were almost identical to those of the Cr(VI) (measurements of total chromium not presented here). d-COD concentration was measured by the closed reflux, colorimetric method using a Multiparameter Bench Photometer (HANNA C99). Total phenolic compounds (with respect to syringic acid) were determined spectrophotometrically using a Boeco (Germany, S-20) spectrophotometer, according to the Folin-Ciocalteu method [40]. A HANNA HI9828 multi-parameter meter was used to measure dissolved oxygen (D.O.) and pH.

2.5. Statistical Analysis

All of the analyses were carried out in triplicate with a relative standard deviation not exceeding 5%. Results were expressed as mean \pm standard deviation. Statistically significant differences between data were evaluated using the *t*-student confidence interval, for 95% probability. In this statistical analysis, the data was considered to follow the *t*-student distribution and the confidence interval for the difference of a pair of mean values was calculated.

3. Results and Discussion

3.1. Packed Bed Reactor Experiments in Cr-SCW-Filter and Cr-WE-Filter

3.1.1. Sequencing Batch Mode Operation

Initially, abiotic chromium reduction was investigated. Clean Cr-SCW-filter and Cr-WE-filter (without microorganisms) were loaded with SCW or WE (of concentration 5000 mg/L) and chromium at a final concentration of $10 \text{ mg Cr(VI) L}^{-1}$ and operated in batch mode. The system was aerated by an air pump and for an operation period of 24 h no significant change in the concentration of Cr(VI) was observed thus indicating the absence of abiotic Cr(VI) reduction. Therefore, the addition of microorganisms was necessary to achieve Cr(VI) reduction.

The pilot-scale Cr-SCW-filter and Cr-WE-filter were then inoculated with the enrichment cultures Cr-SCW-EC and Cr-WE-EC, respectively (as described in Section 2.2). The filter was first operated in batch operating mode with initial Cr(VI) and d-COD concentrations of 5 and 1000 mg L^{-1} , respectively. As soon as Cr(VI) reduction was completed the solution was discharged (and then used as an influent solution for the SCW-filter or WE-filter) and the new feed medium with the same Cr(VI) and d-COD concentrations was added into the filter. This process comprised one complete operating cycle. Cycles were repeated until the maximum removal rate of Cr(VI) was recorded for at least three cycles, while an air pump that was located at the base of the filter provided air ($\text{D.O.} > 5 \text{ mg L}^{-1}$).

According to the results (data not presented here), the Cr-SCW-filter and Cr-WE-filter were not able to achieve complete Cr(VI) reduction in all of the cycles performed under batch operation and

did not reach a steady-state condition. Additionally, the d-COD removal rate recorded in these filters ranged between 5 and 35%. This was due to rapid biomass growth along the Cr-filter, which led to spatial heterogeneity and insufficient exploitation of the filter. To avoid these problems, the filter was operated under SBR operating mode with recirculation.

3.1.2. Sequencing Batch Mode Operation with Recirculation

The Cr-SCW-filter or Cr-WE-filter was then loaded with SCW or WE and Cr(VI) at the final concentrations referred to in Section 2.3, while the recirculation rates of 0.5, 1.0, and 2.0 L min⁻¹ were tested. The Cr-SCW-filter operated successfully for the recirculation rate of 0.5 L min⁻¹ as complete Cr(VI) reduction was achieved for all of the initial concentrations of Cr(VI) (5–110 mg L⁻¹) and d-COD (1000–25,000 mg L⁻¹) tested. Table 1 presents Cr(VI) reduction rates (mg L⁻¹ h⁻¹ and g m⁻² d⁻¹) as well as percentage d-COD removal (%) achieved for all series of experiments that were performed in the Cr-SCW-filter for the recirculation rate of 0.5 L min⁻¹. Table 1 shows that, as initial d-COD concentration increased, higher Cr(VI) reduction rates were achieved. This was observed only for initial d-COD concentrations of up to 21,000 mg L⁻¹. For higher initial d-COD concentrations (25,000 mg d-COD L⁻¹), the Cr(VI) reduction rates were lower when compared to those that were recorded for 21,000 mg d-COD L⁻¹, but higher than those obtained for concentrations of 1000, 5000, and 13,000 mg d-COD L⁻¹. It is known in bioreactors that metals can be removed via weak adsorption onto organic substrates. In such a case, increase of organic load leads to higher Cr(VI) removal rates. However, in this study no loss of Cr(VI) was found through adsorption during the operation of the non-biomass reactors. Besides biological reduction, biosorption was reported to be significant for the efficient removal of Cr(VI) biologically in the Cr-SCW-filter. Biological reduction is the reduction by biological means of metallic ions that are normally multivalent. The reduction agent (reductant) can be organic compounds from living or dead biomass, natural organic matter, and industrial organic waste. Microorganisms may also act as a catalyst or medium for metal reduction. The reduction of Cr(VI) in this study can be achieved both by the presence of microorganisms and by just the presence of high concentrations of organic compounds in the examined wastewaters, acting also as reductant agents. However, the specific contribution between these two mechanisms is difficult to be assessed in such a complex system, as the studied one. Also, the combined effect of metals and organic carbon pollutants on microbial activity and community composition is not so clear, since few studies have addressed this point [41]. According to Nakatsu et al., [41] if species richness is reduced in sites contaminated with complex mixtures with high organic and metal concentrations, the communities may be less resilient. Also, according to Hawley et al., [10] various factors affect the rate of microbial reduction, including carbon concentrations. Therefore, the high organic load of 25,000 mg d-COD L⁻¹ of SCW may have affected the structure of the microbial community, and therefore the bioreduction rate.

The maximum Cr(VI) removal rate recorded was 35.99 mg L⁻¹ h⁻¹ for initial Cr(VI) and d-COD concentrations of 60 and 21,000 mg L⁻¹, respectively (Figure 2a). For the filter of 9 cm diameter, or cross section area of 63.62 cm², the mass of Cr(VI) removed per square meter of the filter's surface per day was 815.1 g m⁻² d⁻¹. Based on the results of the statistical analysis, the initial d-COD concentration was found to have significant effects on Cr(VI) removal for many of the paired data of each initial Cr(VI) concentration that was tested. For each initial Cr(VI) concentration the pairs in which significant differences were observed are: 5 mg Cr(VI) L⁻¹: (1000–5000; 5000–13,000; 13,000–21,000), 10 mg Cr(VI) L⁻¹: (1000–5000; 21,000–25,000), 30 mg Cr(VI) L⁻¹: (5000–13,000; 13,000–21,000; 21,000–25,000), 60 mg Cr(VI) L⁻¹: (5000–13,000; 13,000–21,000; 21,000–25,000), and 100 mg Cr(VI) L⁻¹: (13,000–21,000).

Table 1. Hexavalent chromium (Cr(VI)) reduction and dissolved chemical oxygen demand (d-COD) removal rates for the different initial Cr(VI) and d-COD concentrations tested in the Cr-SCW-filter operating under sequencing batch reactor (SBR) with a recirculation rate of 0.5 L min⁻¹.

Initial Cr(VI) (mg L ⁻¹)	Initial d-COD (mg L ⁻¹)	Cr(VI) Reduction Rate (mg L ⁻¹ h ⁻¹)	Cr(VI) Reduction Rate (g m ⁻² d ⁻¹)	d-COD Removal (Cr-SCW-Filter) (%)	Total d-COD Removal (SCW-Filter) (%)
5.02 ± 0.08	1076 ± 22	2.23 ± 0.04	50.53 ± 0.9	25.28 ± 1.25	-
10.01 ± 0.14	1053 ± 33	1.48 ± 0.03	33.58 ± 0.68	22.74 ± 1.04	-
5.00 ± 0.04	5098 ± 87	5.00 ± 0.18	150.99 ± 5.43	17.21 ± 0.64	-
10.05 ± 0.13	5185 ± 36	10.05 ± 0.21	227.66 ± 4.75	26.65 ± 1.09	-
30.26 ± 0.02	5035 ± 121	10.12 ± 0.11	161.25 ± 1.75	29.69 ± 0.81	97.57 ± 1.12
60.10 ± 0.69	5018 ± 141	6.67 ± 0.19	142.13 ± 4.05	27.35 ± 1.19	-
100.94 ± 1.46	5010 ± 69	4.16 ± 0.08	94.27 ± 1.81	34.33 ± 1.21	-
5.01 ± 0.08	13155 ± 362	6.68 ± 0.24	151.19 ± 5.43	27.78 ± 1.03	-
10.03 ± 0.12	13200 ± 121	12.53 ± 0.29	283.59 ± 6.56	33.13 ± 0.66	-
30.02 ± 1.14	13295 ± 305	24.01 ± 0.34	543.81 ± 7.70	37.19 ± 0.99	99.06 ± 0.56
60.76 ± 0.18	13425 ± 251	12.79 ± 0.22	289.67 ± 4.98	33.89 ± 1.14	-
101.66 ± 0.29	13075 ± 315	4.59 ± 0.09	104.03 ± 2.03	42.49 ± 2.12	-
5.03 ± 0.04	21610 ± 805	8.68 ± 0.32	196.51 ± 7.24	12.26 ± 0.24	-
10.07 ± 0.03	21600 ± 925	14.28 ± 0.33	323.29 ± 7.47	15.25 ± 0.76	-
29.68 ± 0.11	22190 ± 358	29.68 ± 0.63	672.13 ± 14.27	19.69 ± 0.99	-
59.75 ± 0.29	21080 ± 287	35.99 ± 0.45	815.10 ± 10.19	18.22 ± 0.98	99.40 ± 2.04
102.00 ± 2.44	21710 ± 1002	5.36 ± 0.09	120.00 ± 2.02	24.74 ± 1.24	-
5.03 ± 0.01	25175 ± 1205	8.98 ± 0.35	202.57 ± 7.89	16.12 ± 0.86	-
10.06 ± 0.02	25200 ± 925	14.42 ± 0.40	326.46 ± 9.05	17.20 ± 1.09	-
30.05 ± 0.10	25413 ± 1102	14.45 ± 0.24	327.23 ± 5.43	22.09 ± 1.12	-
60.08 ± 0.25	25013 ± 852	23.29 ± 0.64	527.40 ± 14.49	21.64 ± 1.08	98.70 ± 1.22
101.87 ± 2.02	25425 ± 912	5.20 ± 0.07	151.29 ± 2.04	26.65 ± 1.34	-

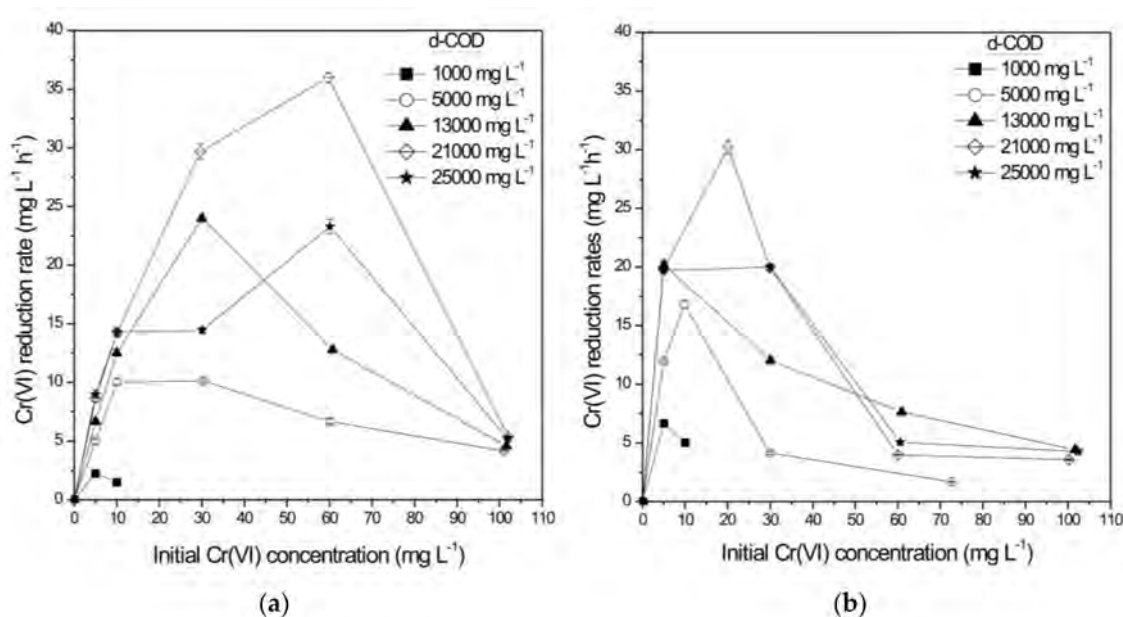


Figure 2. Cr(VI) reduction rates in the Cr-SCW-filter operating under SBR with recirculation mode for various initial Cr(VI) and d-COD concentrations and two different recirculation rates: (a) 0.5 L min⁻¹ and (b) 1.0 L min⁻¹.

The Cr-WE-filter also operated successfully for the recirculation rate of 0.5 L min⁻¹ as 100% Cr(VI) reduction was achieved for all the initial concentrations of Cr(VI) (5–110 mg L⁻¹) and d-COD (1000–25,000 mg L⁻¹) tested. Table 2 presents Cr(VI) reduction rates (mg L⁻¹ h⁻¹ and g m⁻² d⁻¹) as well as percentage d-COD removal (%) in the Cr-WE-filter for the recirculation rate of 0.5 L min⁻¹. Increase of initial d-COD concentration led to higher Cr(VI) reduction rates only for initial d-COD concentrations of up to 13,000 mg L⁻¹. It is probable that, as mentioned above, the high concentration of 21,000 mg d-COD L⁻¹ of WE affected the attached microbial community structure, making it less effective at Cr(VI) removal. However, for initial d-COD concentrations of 21,000 and 25,000 mg d-COD L⁻¹ the Cr(VI) reduction rates were higher than those obtained

for concentrations of 1000 and 5000 mg d-COD L⁻¹. The maximum Cr(VI) removal rate achieved using WE was 43 mg L⁻¹ h⁻¹ for initial Cr(VI) of 60 mg L⁻¹ (a higher removal rate compared to SCW, 35.9943 mg L⁻¹ h⁻¹), but was recorded for lower initial d-COD concentration, 13,000 mg L⁻¹ (21,000 mg d-COD L⁻¹ for SCW) (Figure 3a).

Table 2. Cr(VI) reduction and d-COD removal rates for the different initial Cr(VI) and d-COD concentrations tested in the Cr-WE-filter operating under SBR with a recirculation rate of 0.5 L min⁻¹.

Initial Cr(VI) (mg L ⁻¹)	Initial d-COD (mg L ⁻¹)	Cr(VI) Reduction Rate (mg L ⁻¹ h ⁻¹)	Cr(VI) Reduction Rate (g m ⁻² d ⁻¹)	d-COD Removal (Cr-WE-Filter) (%)	Total d-COD Removal (%)
5.12 ± 0.04	1151 ± 38	2.59 ± 0.04	58.62 ± 0.9	5.10 ± 0.06	-
10.22 ± 0.11	1109 ± 27	1.58 ± 0.02	35.76 ± 0.45	8.44 ± 0.12	-
4.56 ± 0.03	5108 ± 99	5.72 ± 0.09	151.05 ± 2.38	13.28 ± 0.24	-
10.01 ± 0.14	5005 ± 105	12.54 ± 0.27	284.28 ± 6.12	17.15 ± 0.39	-
30.82 ± 0.74	5010 ± 89	12.56 ± 0.31	279.08 ± 6.89	19.22 ± 0.52	91.10 ± 2.04
59.20 ± 0.33	5118 ± 152	7.89 ± 0.18	178.58 ± 4.07	25.15 ± 0.43	-
101.04 ± 1.03	5110 ± 122	4.36 ± 0.06	98.68 ± 1.36	27.33 ± 0.91	-
4.91 ± 0.05	13065 ± 208	7.55 ± 0.24	170.89 ± 5.43	17.08 ± 0.41	-
10.53 ± 0.18	13120 ± 305	17.55 ± 0.47	397.23 ± 10.63	18.23 ± 0.51	-
31.12 ± 0.22	13030 ± 287	30.12 ± 0.75	681.74 ± 16.97	21.85 ± 0.63	-
60.06 ± 0.49	13025 ± 299	43.01 ± 1.12	973.50 ± 25.35	29.25 ± 0.61	92.01 ± 1.89
98.7 ± 0.89	13295 ± 327	6.32 ± 0.25	143.05 ± 5.66	30.39 ± 0.78	-
5.03 ± 0.02	21800 ± 758	7.06 ± 0.31	227.70 ± 10.00	15.06 ± 0.44	-
10.27 ± 0.18	21250 ± 993	13.69 ± 0.47	309.86 ± 10.64	16.32 ± 0.57	-
29.55 ± 0.59	22000 ± 875	24.73 ± 0.61	636.20 ± 15.69	18.08 ± 0.61	-
60.77 ± 1.51	21380 ± 741	25.01 ± 0.41	566.08 ± 9.28	21.99 ± 0.49	91.80 ± 1.04
101.00 ± 2.01	21010 ± 974	5.06 ± 0.05	114.53 ± 1.13	25.89 ± 0.86	-
5.13 ± 0.03	25250 ± 1108	6.26 ± 0.19	232.22 ± 7.05	4.22 ± 0.04	-
10.10 ± 0.09	25450 ± 899	12.46 ± 0.22	304.66 ± 5.38	10.12 ± 0.21	-
29.05 ± 0.48	25150 ± 948	12.20 ± 0.34	298.77 ± 8.32	13.49 ± 0.38	-
59.88 ± 0.97	25300 ± 832	17.29 ± 0.52	536.61 ± 16.14	17.89 ± 0.47	90.50 ± 2.84
102.10 ± 1.98	25400 ± 1032	4.52 ± 0.05	102.23 ± 1.13	19.32 ± 0.64	-

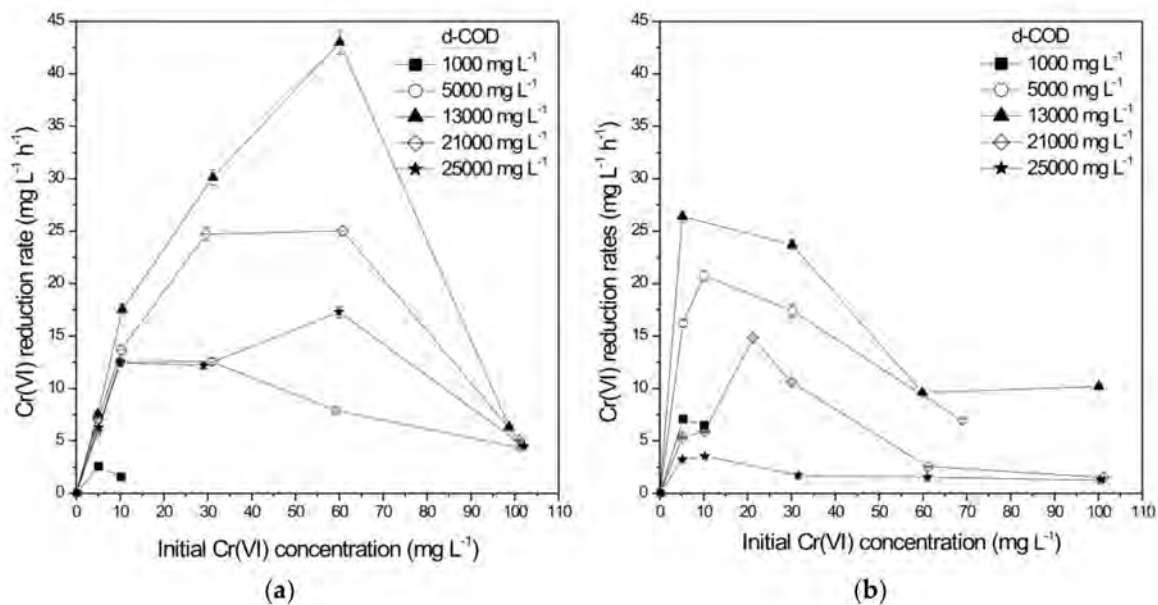


Figure 3. Cr(VI) reduction rates in the Cr-WE-filter operating under SBR with recirculation mode for various initial Cr(VI) and d-COD concentrations and two different recirculation rates: (a) 0.5 L min⁻¹ and (b) 1.0 L min⁻¹.

Similar to SCW, initial d-COD concentration of WE was found to have significant effects on Cr(VI) removal for many of the paired data of each initial Cr(VI) concentration tested. For each initial Cr(VI) concentration, the pairs in which significant differences were observed are: 5 mg Cr(VI) L⁻¹: (1000–5000; 5000–13,000), 10 mg Cr(VI) L⁻¹: (1000–5000; 5000–13,000; 13,000–21,000),

30 mg Cr(VI) L⁻¹: (5000–13,000; 13,000–21,000; 21,000–25,000), 60 mg Cr(VI) L⁻¹: (5000–13,000; 13,000–21,000; 21,000–25,000), and 100 mg Cr(VI) L⁻¹: (5000–13,000; 13,000–21,000; 21,000–25,000).

When considering the results of Tables 1 and 2, it is clear that WE led to significantly higher Cr(VI) removal rates than SCW for initial d-COD concentrations of up to 13,000 mg d-COD L⁻¹. The same was not observed for initial d-COD concentrations of 21,000 and 25,000 mg d-COD L⁻¹, since, when the d-COD concentration was 21,000 mg d-COD L⁻¹, the use of WE led to a significantly lower Cr(VI) reduction rate when compared to SCW, while when d-COD concentration was 25,000 mg d-COD L⁻¹, Cr(VI) reduction rates had no significant differences between SCW and WE. It is probable that, in these experiments with the less diluted WE, the high concentration of d-COD and/or the (poly)phenolic component of WE (that emanates from the seeds, skins, and piths of grapes), which is the least degradable fraction of WE and associated with phytotoxicity and microbial toxicity, had a negative impact on Cr(VI) reduction [42,43]. According to Ramond et al., [43] microbial communities are significantly impacted by winery wastewaters depending on their spatial locations, probably due to the toxicity of the influent stream (high COD, phenolic content, and low pH). Specifically, they mention that winery wastewaters (with concentrations of about 100 mg L⁻¹) have been shown to induce structural changes in environmental microbial communities [43]. It is worth mentioning that in this study the percentage contribution of total phenolics to the COD was small, ranging from 0.5 to 1.5%. Also, minor phenol removal ranging between 0 and 20% was observed during all of the experiments.

Direct comparison with previous studies using cheese whey [16,27–29] is not currently possible, since all were performed in suspended not attached growth systems. Panousi et al. [16] investigated the efficiency of biological groundwater treatment for Cr(VI) removal under anoxic conditions in suspended growth reactors using cheese whey as the carbon source. Since Cr(VI) content in groundwater can be as high as 300 µg L⁻¹, they studied low Cr(VI) concentration (up to 200 µg L⁻¹). Low substrate concentrations varying between 100 and 200 mgCOD L⁻¹ were also tested. Panousi et al. [16] found that, when added to groundwater at a concentration of 200 µg L⁻¹, complex fermentable substrates, such as cheese whey (at organic loadings above 120 mg COD L⁻¹), can support complete microbial Cr(VI) removal (over 99%). Orozco et al. [27] also revealed (with an initial Cr(VI) concentration of 25 mg L⁻¹) that significant specific Cr(VI) removal rates can be attained with fermentable substrates, such as cheese whey and lactose (0.12 mgCr(VI) gTSS⁻¹ h⁻¹, TSS: Total suspended solids, for initial biomass concentration 3000 ± 200 mgTSS⁻¹ L⁻¹). Conversely, with non-fermentable substrates, such as citrate and acetate, lower specific Cr(VI) removal rates were obtained (below 0.08 mgCr(VI) gTSS⁻¹ h⁻¹, for initial biomass concentration 3000 ± 200 mgTSS⁻¹ L⁻¹). In their experiments, the concentration of the carbonaceous substrate was just 5000 mg L⁻¹. Contreras et al. [28,29] tested higher initial Cr(VI) concentrations (up to 300 mg L⁻¹) using dehydrated cheese whey of 5000 mg d-COD L⁻¹. With regard to the effect of initial Cr(VI), a threshold inhibitory concentration of Cr(VI) within the range of 100 to 300 mg L⁻¹ was obtained, while for initial Cr(VI) concentrations that are below 100 mg L⁻¹ percentage reduction rates did not exceed 70% (after about 100 h). The toxic effect of high initial Cr(VI) concentration was also illustrated in the present study. Specifically, for the two highest initial d-COD concentrations of 21,000 and 25,000 mg L⁻¹, Cr(VI) reduction rates increased as the initial Cr(VI) concentration rose to 60 mg Cr(VI) L⁻¹, achieving maximum Cr(VI) reduction rates of 35.99 and 23.29 mg L⁻¹ h⁻¹, respectively (Figure 2a). However, higher initial Cr(VI) concentrations presented the toxic effect of chromium and lowered Cr(VI) reduction rates considerably (~5 mg L⁻¹ h⁻¹).

To our knowledge, winery effluent has not been used as a carbon source for hexavalent chromium in either suspended or attached growth systems. An attempt to compare the Cr(VI) reduction rates achieved in this study with similar research works indicates that the Cr(VI) reduction rates observed in this study are among the highest reported in the literature compared to those obtained in attached mixed growth systems using other carbon sources, such as sugar (2 mg L⁻¹ h⁻¹) for initial Cr(VI) of 100 mg L⁻¹ [44], glucose (6.95 mg L⁻¹ h⁻¹ for 15 mg Cr(VI) L⁻¹) [45], sodium acetate (13 mg L⁻¹ h⁻¹ for 10 mg Cr(VI) L⁻¹) [46], and molasses (6.6 mg L⁻¹ h⁻¹ for 10 mg Cr(VI) L⁻¹) [47]. The results

of various studies have been summarized in a Table in a previous paper (Table 2) by our research group [25], and show that the biological system used in this study is a very effective method of treating Cr(VI) with SCW, as it can achieve very high Cr(VI) reduction rates (up to 35.99 and 43.01 mg L⁻¹ h⁻¹, using SCW and WE, respectively) even with high initial Cr(VI) concentrations.

Higher recirculation rates of 1.0 L min⁻¹ and 2.0 L min⁻¹ were also tested. However, when the recirculation rate was increased to 1.0 L min⁻¹, the Cr-SCW-filter and Cr-WE-filter (Tables 3/ Figure 2b and 4/ Figure 3b, respectively) exhibited lower Cr(VI) removal rates as well as incomplete Cr(VI) removal in some combinations of initial Cr(VI) and d-COD concentrations. With these recirculation rates, the Cr-SCW-filter and Cr-WE-filter could fully treat only significantly lower feed concentrations of Cr(VI). Specifically, only the initial d-COD concentration of 13,000 mg L⁻¹ was able to safely remove Cr(VI) concentrations up to 100 mg L⁻¹, leading to final Cr(VI) concentrations below the maximum permitted limit of 0.05 mg L⁻¹ [7,8]. On the contrary, all of the other initial d-COD concentrations (21,000 and 25,000 mg d-COD L⁻¹) were able to completely remove Cr(VI) up to 30 mg L⁻¹ and 10 mg L⁻¹, for Cr-SCW-filter and Cr-WE-filter, respectively.

Table 3. Cr(VI) reduction and d-COD removal rates for the different initial Cr(VI) and d-COD concentrations tested in the Cr-SCW-filter operating under SBR with a recirculation rate of 1.0 L min⁻¹.

Initial Cr(VI) (mg L ⁻¹)	Initial d-COD (mg L ⁻¹)	Cr(VI) Reduction Rate (mg L ⁻¹ h ⁻¹)	Cr(VI) Reduction Rate (g m ⁻² d ⁻¹)	d-COD Removal (Cr-SCW-Filter) (%)
5.01 ± 0.03	987 ± 10.21	6.68 ± 0.29	151.19 ± 6.56	24.52 ± 0.78
10.04 ± 0.25	996 ± 21.99	5.02 ± 0.14	113.68 ± 3.17	26.27 ± 0.99
5.01 ± 0.06	5123 ± 132	12.02 ± 0.14	272.12 ± 3.17	28.06 ± 1.12
9.79 ± 0.58	5058 ± 99	16.79 ± 0.31	380.24 ± 7.02	32.28 ± 1.07
29.96 ± 0.22	4988 ± 138	4.13 ± 0.11	93.60 ± 2.49	36.54 ± 0.93
72.59 ± 0.66	5029 ± 103	1.68 ± 0.03	38.03 ± 0.68	38.35 ± 1.11
5.05 ± 0.05	13120 ± 322	20.18 ± 0.34	457.08 ± 7.07	33.88 ± 1.27
30.09 ± 0.04	12995 ± 205	12.04 ± 0.29	272.61 ± 6.57	43.75 ± 1.38
60.94 ± 0.44	12800 ± 259	7.62 ± 0.25	172.51 ± 5.66	47.34 ± 1.94
101.69 ± 1.50	12715 ± 223	4.42 ± 0.09	100.13 ± 2.04	49.67 ± 2.02
4.96 ± 0.14	20760 ± 823	19.85 ± 0.28	449.46 ± 6.34	34.06 ± 1.14
20.09 ± 0.29	21400 ± 742	30.21 ± 0.58	684.26 ± 10.87	38.08 ± 0.96
29.94 ± 0.62	21200 ± 906	19.96 ± 0.31	452.00 ± 7.02	40.09 ± 1.21
* 60.09 ± 0.12	21420 ± 759	3.98 ± 0.03	90.08 ± 0.68	42.44 ± 1.04
* 100.27 ± 1.68	21400 ± 1014	3.57 ± 0.06	80.80 ± 1.36	43.78 ± 1.19
4.92 ± 0.09	25488 ± 1017	19.69 ± 0.19	445.94 ± 4.30	16.28 ± 0.34
30.00 ± 0.06	25475 ± 898	20.00 ± 0.24	452.88 ± 4.43	29.32 ± 0.59
* 60.61 ± 0.74	24760 ± 988	5.04 ± 0.08	114.07 ± 1.81	31.07 ± 0.81
* 102.41 ± 1.79	25123 ± 1003	4.26 ± 0.11	96.42 ± 2.48	32.06 ± 0.74

* Concentrations not able to completely remove Cr(VI).

Table 4. Cr(VI) reduction and d-COD removal rates for the different initial Cr(VI) and d-COD concentrations tested in the Cr-WE-filter operating under SBR with a recirculation rate of 1.0 L min⁻¹.

Initial Cr(VI) (mg L ⁻¹)	Initial d-COD (mg L ⁻¹)	Cr(VI) Reduction Rate (mg L ⁻¹ h ⁻¹)	Cr(VI) Reduction Rate (g m ⁻² d ⁻¹)	d-COD Removal (Cr-WE-Filter) (%)
5.32 ± 0.03	1120 ± 19	7.09 ± 0.19	160.47 ± 4.3	6.50 ± 0.12
10.12 ± 0.18	1080 ± 28	6.50 ± 0.11	147.12 ± 2.48	9.50 ± 0.24
5.27 ± 0.04	4980 ± 95	16.23 ± 0.32	367.36 ± 7.24	14.16 ± 0.37
10.13 ± 0.11	5132 ± 156	20.76 ± 0.52	469.89 ± 11.77	17.26 ± 0.51
30.25 ± 0.22	4990 ± 87	17.41 ± 0.59	394.06 ± 6.56	19.05 ± 0.43
68.96 ± 0.51	5110 ± 190	6.90 ± 0.19	156.17 ± 4.3	21.52 ± 0.57
5.15 ± 0.02	13030 ± 197	26.40 ± 0.55	597.54 ± 12.45	15.44 ± 0.38
30.15 ± 0.31	13100 ± 209	23.71 ± 0.41	536.66 ± 9.28	23.55 ± 0.58
59.84 ± 0.47	12900 ± 274	9.62 ± 0.21	341.77 ± 7.46	24.44 ± 0.67
100.09 ± 1.21	12855 ± 328	10.20 ± 0.19	230.87 ± 4.3	29.67 ± 0.76
5.06 ± 0.04	20520 ± 487	5.32 ± 0.09	120.41 ± 2.04	8.55 ± 0.23
10.22 ± 0.31	21100 ± 854	5.89 ± 0.12	133.31 ± 2.72	9.87 ± 0.19
* 21.19 ± 0.38	21150 ± 902	14.83 ± 0.34	335.66 ± 7.70	12.81 ± 0.47
* 30.04 ± 0.29	20800 ± 855	10.60 ± 0.22	239.92 ± 4.98	13.09 ± 0.32
* 61.19 ± 0.61	20950 ± 932	2.56 ± 0.03	57.94 ± 0.68	14.49 ± 0.42
* 101.17 ± 1.32	21100 ± 844	1.53 ± 0.02	34.63 ± 0.45	21.78 ± 0.62
5.13 ± 0.03	25400 ± 1009	3.22 ± 0.06	72.88 ± 1.35	5.32 ± 0.07
10.32 ± 0.27	24900 ± 954	3.54 ± 0.05	80.13 ± 1.13	8.05 ± 0.17
* 31.55 ± 0.37	25150 ± 784	1.71 ± 0.04	15.66 ± 0.09	12.02 ± 0.27
* 61.01 ± 0.54	25600 ± 1109	1.52 ± 0.02	34.40 ± 0.45	12.88 ± 0.35
* 100.55 ± 0.98	24980 ± 1005	1.26 ± 0.01	28.51 ± 0.22	15.09 ± 0.37

* Concentrations not able to completely remove Cr(VI).

Additionally, for all initial d-COD concentrations tested, Cr(VI) reduction rates dropped significantly when the initial Cr(VI) concentrations exceeded the value of 30 mg L⁻¹ (Table 3). This was due to the high flow velocities that were exerted across the filter with the recirculation rate of 1.0 L min⁻¹ that resulted in biomass detachment or in environmental conditions with less effective removal of Cr(VI). The maximum Cr(VI) removal rate achieved for the recirculation rate of 1.0 L min⁻¹ in the Cr-SCW-filter was 30.21 mg L⁻¹ h⁻¹ for initial Cr(VI) and d-COD concentrations of 20 and 21,000 mg L⁻¹, respectively (Figure 2b and Table 3), while the maximum rate for the Cr-WE-filter was 26.4 mg L⁻¹ h⁻¹ for initial Cr(VI) and d-COD concentrations of 5 and 13,000 mg L⁻¹, respectively (Figure 3b and Table 4). It must be mentioned that for both the recirculation rates of 0.5 and 1.0 L min⁻¹, the feed SCW concentration of d-COD 1000 mg L⁻¹ was inadequate (for Cr(VI) above 10 mg L⁻¹), and caused microbe growth limitation by carbon throughout the process.

For volumetric flow rate of 1.0 L min⁻¹, the initial d-COD concentration of SCW was found to have significant effects on Cr(VI) removal for many of the paired data of each initial Cr(VI) concentration tested. For each initial Cr(VI) concentration, the pairs in which significant differences were observed are: 5 mg Cr(VI) L⁻¹: (1000–5000; 5000–13,000), 10 mg Cr(VI) L⁻¹: (1000–5000; 5000–13,000; 13,000–21,000), 30 mg Cr(VI) L⁻¹: (5000–13,000; 13,000–21,000; 21,000–25,000), 60 mg Cr(VI) L⁻¹: (5000–13,000; 13,000–21,000; 21,000–25,000), and 100 mg Cr(VI) L⁻¹: (13,000–21,000; 21,000–25,000).

For WE, the initial d-COD concentration was also found to have significant effects on Cr(VI) removal for many of the paired data of each initial Cr(VI) concentration tested. For each initial Cr(VI) concentration the pairs in which significant differences were observed are: 5 mg Cr(VI) L⁻¹: (1000–5000; 5000–13,000; 13,000–21,000; 21,000–25,000), 10 mg Cr(VI) L⁻¹: (1000–5000; 21,000–25,000), 30 mg Cr(VI) L⁻¹: (5000–13,000; 13,000–21,000; 21,000–25,000), 60 mg Cr(VI) L⁻¹: (5000–13,000; 13,000–21,000; 21,000–25,000), and 100 mg Cr(VI) L⁻¹: (13,000–21,000; 21,000–25,000).

Finally, with a recirculation rate of 2 L min⁻¹, the filter was not able to operate sufficiently (for both type of wastewaters used, SCW and WE) due to the detachment of biofilm from the support material. Experiments under continuous operating mode with and without recirculation were also performed. Various Cr(VI) and d-COD feed concentrations were examined with different recirculation rates, however, very low Cr(VI) bioreduction rates were achieved in all the cases (data not shown).

Similar observations have also been reported in the literature where the use of continuous biological methods for Cr(VI) treatment presented difficulties due to the eventual loss of active biomass [25,48].

Taking all of the above results into account, it is concluded that SBR with a recirculation rate of 0.5 L min^{-1} proved to be a very effective operating mode since it ensured high Cr(VI) removal rates (35.99 and $43.01 \text{ mg L}^{-1} \text{ h}^{-1}$, for SCW and WE, respectively). Despite the high removal levels of Cr(VI) achieved in this work, the final effluent was still not suitable for discharge since the residual d-COD concentration of the treated wastewater was very high (Figure 4). For the recirculation rate of 0.5 L min^{-1} , the d-COD percentage removal ranged between 14 and 42.5% for SCW, and from 4 to 29% for WE, depending on initial Cr(VI) and d-COD feed concentrations (Figures 4a/Table 1 and 5a/Table 2, respectively). This can be explained as the process of Cr(VI) reduction took place over a very short time period (from 0.6 to 25 h and from 0.4 to 23 h for SCW and WE, respectively), thus not allowing for the further removal of d-COD. Contreras et al. have reported higher d-COD percentage removal (in suspended growth systems), but over longer periods of time (up to 100 h) [28,29].

For the recirculation rates of 0.5 and 1.0 L min^{-1} , it is noteworthy that the percentage of d-COD removal slightly increased with increasing initial Cr(VI) concentration (Figures 4 and 5). This is rather expected, as high Cr(VI) concentrations require greater amounts of carbon source. It was also observed that the percentages of d-COD removal increased to the maximum when the initial d-COD concentration was $13,000 \text{ mg L}^{-1}$. For the concentration of $13,000 \text{ mg d-COD L}^{-1}$, the microbial community structure and function appeared to tolerant to both metal and organic load contaminants [41].

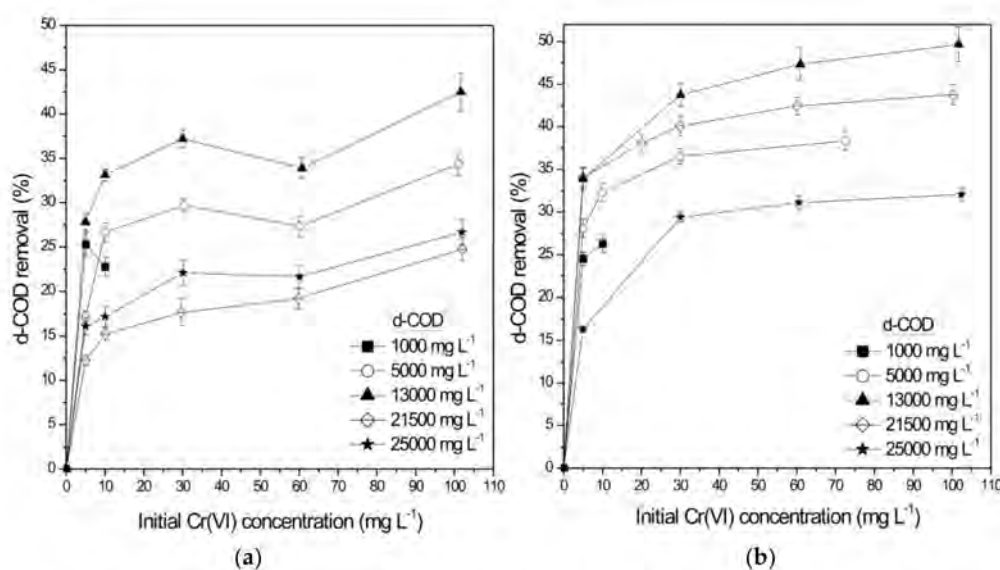


Figure 4. Percentage removal of d-COD in the Cr-SCW-filter for two different recirculation rates: (a) 0.5 L min^{-1} and (b) 1.0 L min^{-1} .

For SCW and volumetric flow rate of 0.5 L min^{-1} , the following pairs were found to have significant differences for each initial d-COD concentration: $5000 \text{ mg d-COD L}^{-1}$: (5–10), $13,000 \text{ mg d-COD L}^{-1}$: (5–10), and $21,000 \text{ mg d-COD L}^{-1}$: (5–10; 10–30; 60–100). For the same wastewater and a volumetric flow rate of 1.0 L min^{-1} , significant differences were found only for the initial d-COD concentration of $13,000 \text{ mg d-COD L}^{-1}$: (5–10; 10–30). For WE and volumetric flow rate of 0.5 L min^{-1} , significant differences were found in the following pairs for each initial d-COD concentration: $1000 \text{ mg d-COD L}^{-1}$: (5–10), $5000 \text{ mg d-COD L}^{-1}$: (5–10; 30–60), $13,000 \text{ mg d-COD L}^{-1}$: (30–60), while for 1.0 L min^{-1} volumetric flow rate, the pairs: $1000 \text{ mg d-COD L}^{-1}$: (5–10), $13,000 \text{ mg d-COD L}^{-1}$: (5–30; 60–100) and $21,000 \text{ mg d-COD L}^{-1}$: (60–100) had significant differences.

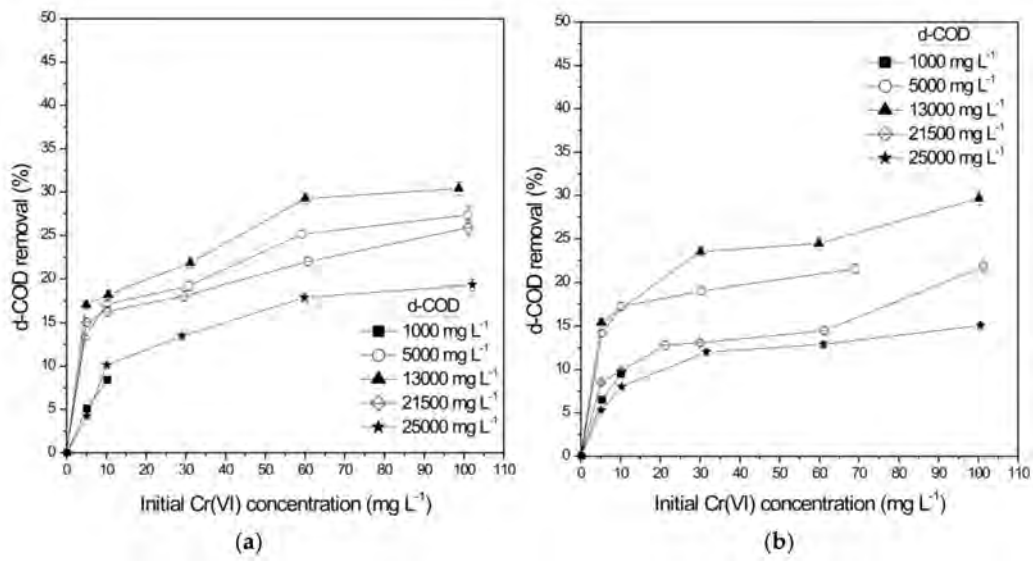


Figure 5. Percentage removal of d-COD in the Cr-WE-filter for two different recirculation rates: (a) 0.5 L min⁻¹ and (b) 1.0 L min⁻¹.

Figure 6a,b and Figure 7a,b help determine the best operating conditions for the highest viable Cr(VI) and d-COD removal rates in the Cr-SCW-filter and Cr-WE-filter, respectively. The Figure 6a,b and Figure 7a,b show that with low d-COD/Cr(VI) ratios, higher removal rates of both Cr(VI) and d-COD are achieved.

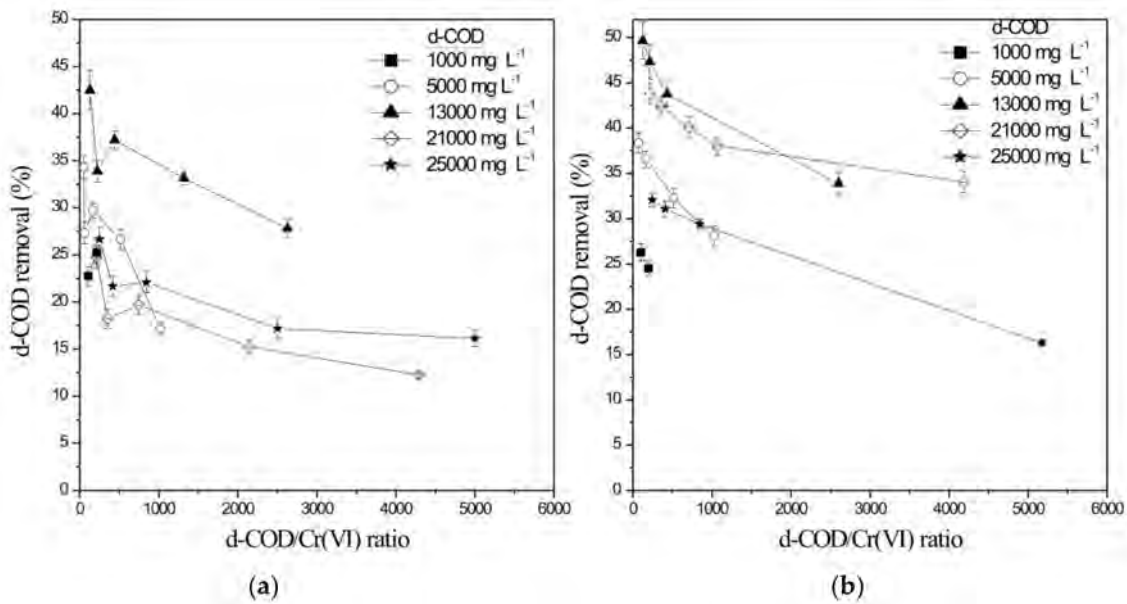


Figure 6. Effect of (initial d-COD)/(initial Cr(VI)) ratio on d-COD removal in the Cr-SCW-filter for various initial d-COD concentrations under SBR mode with recirculation rates of: (a) 0.5 L min⁻¹ and (b) 1.0 L min⁻¹.

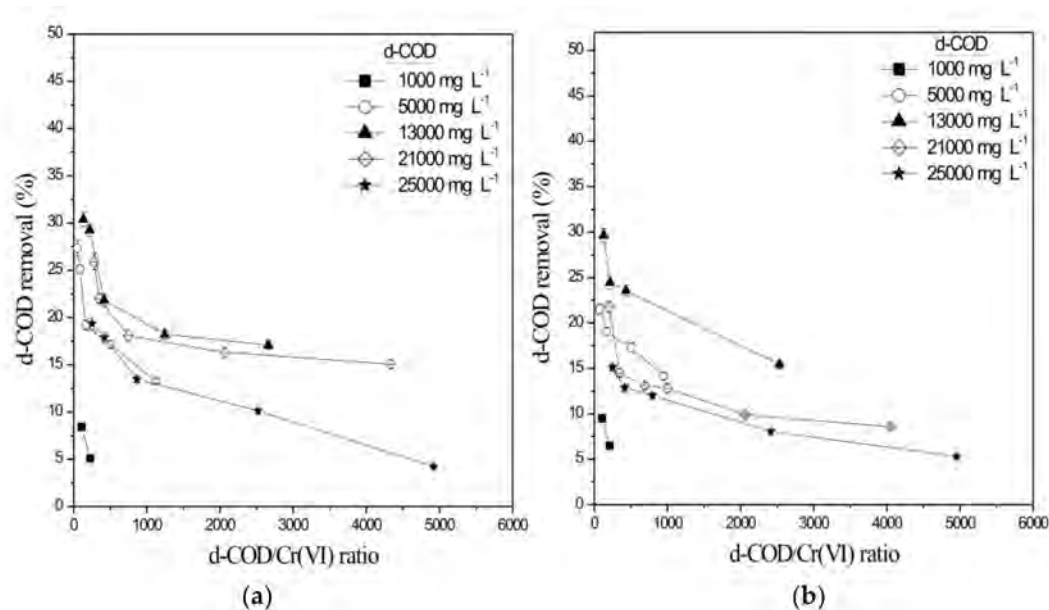


Figure 7. Effect of (initial d-COD)/(initial Cr(VI)) ratio on d-COD removal in the Cr-WE-filter for various initial d-COD concentrations under SBR mode with recirculation rates of: (a) 0.5 L min^{-1} and (b) 1.0 L min^{-1} .

3.2. Packed Bed Reactor Experiments in SCW-Filter and WE-Filter

In the previous section, the successful removal of Cr(VI) in attached growth systems was demonstrated with the use of SCW and WE. This is the first time that SCW and WE have been used as a carbon source for Cr(VI) reduction in biofilters (trickling filters). However, the residual organic load in the Cr-filters' effluent was found to be rather high, and is therefore requiring further treatment before being discharged into receiving waters.

According to Greek legislation, the permissible limit of d-COD for municipal and industrial effluents is 125 mg L^{-1} [49]. Therefore, a second filter (SCW-filter, WE-filter) was constructed, added to the system in series, and then operated as a post-biotreatment step using indigenous microorganisms originating from SCW or WE, respectively (Sections 2.2 and 2.3). The SCW-filter or WE-filter was loaded with the effluent of the Cr-SCW-filter or Cr-WE-filter, respectively (without the presence of Cr(VI)) and operated only under SBR with a recirculation rate of 0.5 L min^{-1} .

Kinetic experiments were performed in the SCW-filter or WE-filter, under SBR mode and with recirculation of 0.5 L min^{-1} , only for Cr-filter effluents that presented the highest Cr(VI) reduction rate for each initial d-COD concentration tested (5000, 13,000, 21,000, and 25,000 L min^{-1}). Tables 1 and 2 show that the total percentage d-COD removal (from both Cr-SCW and SCW-filters or Cr-WE and WE-filters) exceeds the value of 97.57% or 90.5% for SCW and WE, respectively, which is very high for aerobic biological systems. However, it must be pointed out that with high percentage removal rates for the high initial d-COD concentration of 25,000 L min^{-1} , the concentration of SCW-filter effluent was 325 mg L^{-1} , which was not below the maximum permitted limit of 125 mg L^{-1} (Figure 8a). Additionally, for all of initial d-COD concentrations that was examined in the WE-filter ((5000, 13,000, 21,000, and 25,000 L min^{-1}), the concentration of WE-filter effluent was always above the maximum permitted limit of 125 mg L^{-1} (445, 1028, 1753, and 2403 mg L^{-1} , respectively) (Figure 8b). Therefore, in these cases a suitable post-treatment step (e.g., a constructed wetland) should be applied to improve the quality of the final outflow [35]. Future research should include experiments in this direction in order to achieve an integrated wastewater treatment system even for high initial d-COD concentrations. SCW treatment has also been examined by our research group in a previous work using pilot-scale horizontal subsurface flow constructed wetlands [50]. Constructed wetlands could

successfully treat SCW and provide COD effluent concentrations below the EU legislation limits when hydraulic residence time is greater than two days and COD influent concentration ranges from 1200 to 3500 mg L⁻¹.

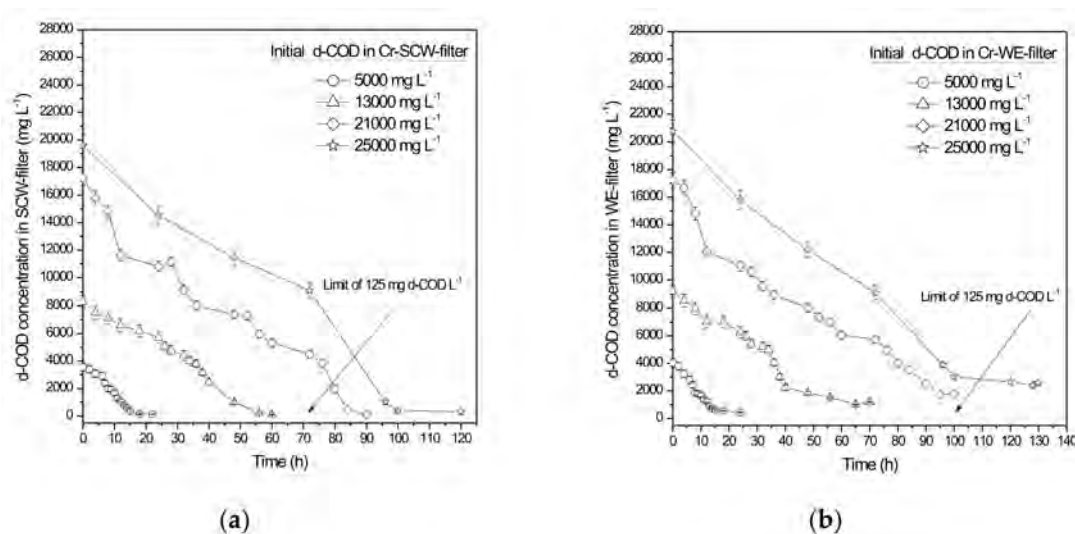


Figure 8. Percentage removal of d-COD in the: (a) SCW-filter or (b) WE-filter for various initial d-COD concentrations (depending on the effluent of the Cr-SCE-filter or Cr-WE-filter, respectively).

Experiments on SCW treatment using trickling filters have also been performed by our research team in a previous study without the presence of Cr(VI) [34]. Comparison of the results of that study with the results of the present work (in SCW-filter) indicates that the process of Cr(VI) removal in the Cr-filter negatively affects the bio-treatment of SCW. Specifically, in the case of SCW treatment following Cr(VI) removal, the duration of the operating cycles increased to 120 h for initial concentrations of about 21,000 mg L⁻¹, whereas in the case of SCW treatment without Cr(VI), the duration was significantly shorter (26 h) [34]. This may be attributed to the different biomass communities developing within the biofilters, as well as to the formation of compounds that prevent organic load degradation during the Cr(VI) reduction process.

4. Conclusions

The effect of SCW or WE on Cr(VI) reduction was examined in an attached growth system (Cr-SCW-biofilter or Cr-WE-biofilter) under various operating conditions. The post-treatment of the treated wastewater was also examined in a second biofilter that was connected in series (SCW-biofilter or WE-biofilter) to further reduce the organic load. The main conclusions derived from this research work are:

- High percentage biological Cr(VI) reduction can be achieved in an attached growth reactor (99.2–100%) by using an indigenous mixed population and SCW or WE (both very low-cost carbon sources) as the sole electron donor.
- Complete Cr(VI) reduction can be achieved in attached growth reactors operated in batch operation with recirculation of 0.5 L min⁻¹ for all initial Cr(VI) (5–110 mg L⁻¹) and d-COD (1000–25,000 mg L⁻¹) concentrations tested for both agro-industrial effluents (SCW or WE). The reduction rates that are accomplished (35.99 and 43.0 mg L⁻¹ h⁻¹ for SCW and WE, respectively) are the highest reported in the literature to date. With higher recirculation rates (1.0 L min⁻¹) the Cr-SCW-filter or Cr-WE-filter were unable to achieve complete Cr(VI) reduction for initial Cr(VI) concentrations above 30 or 10 mg L⁻¹, respectively, while for 2.0 L min⁻¹,

detachment of biofilm led to inadequate operation of the filter. Continuous operating mode with or without recirculation resulted in very low Cr(VI) bioreduction rates.

- Winery effluents presented slightly higher Cr(VI) reduction rates for initial d-COD concentrations up to 13,000 mg L⁻¹. The same was not observed for higher initial concentrations probably due to the presence of higher quantities of phenolic compounds that are associated with microbial toxicity.
- Initial d-COD concentration was found to effect Cr(VI) reduction rate. The feed SCW or WE concentration of 1000 mg d-COD L⁻¹ was limiting and caused microbial growth limitation by carbon during the process.
- Due to the high residual d-COD concentration of the treated wastewater, a post-treatment stage was required. The use of mixed indigenous microorganisms originating from SCW or WE provides high degradation rates (total d-COD removal above 97% and 90.5% for SCW and WE, respectively) and durability under various operating conditions. In cases where final d-COD concentrations of the second biofilters are still above the maximum permitted limit of 125 mg L⁻¹, a suitable post-treatment step (e.g., a constructed wetland) should be applied to improve the quality of the final outflow.

The combined treatment method presented here uses two biological treatment steps (biofilters) and proved to be very effective as high reduction rates of Cr(VI) and high d-COD removal rates were achieved. These results combined with the low construction and operating costs, suggest the feasibility of using these systems for the biological co-treatment of Cr(VI) (in high concentrations in the range of mg L⁻¹, present mainly in industrial wastewaters and not in contaminated groundwaters that usually contain much lower concentrations, in the range of few µg L⁻¹) and SCW or WE.

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