Systematic review and meta-analysis of the application of microorganisms for the Cr(VI) removal from tannery effluents

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Abstract*– The application of microorganisms as bioadsorbents for the treatment of industrial effluents is shown to be an efficient and environmentally viable technology, since it does not generate toxic products. In the present systematic review and meta-analysis, information was gathered about the capacity of microorganisms to remove hexavalent chromium (Cr(VI)) from tannery effluents. In addition, the different variables of the bioadsorption process such as pH, microorganism dose, incubation time, initial metal concentration and final metal concentration were investigated. These parameters were considered according to each species studied in the included investigations. For the meta-analysis, Review Manager 4.0 (RevMan) software was used through the fixed effect model with a 95% confidence interval. The results revealed that the included investigations showed a considerable statistical heterogeneity (I^2 = 87%) and that the application of microorganisms for the Cr(VI) removal from tannery effluents showed greater efficiency at lower initial concentrations of the metal. It was also observed that the microorganisms used in the included investigations showed percentages of Cr(VI) removal higher than 70% for the treatment of tannery effluents.

***Keywords*— systematic review, meta-analysis, microorganisms, hexavalent chromium, tannery effluents.**

I. INTRODUCTION

The tanning industries develop among their activities a stage called tanning, in which a chemical treatment is carried out on the skins, being its main inputs sulfuric acid and chromium. These industries generate about 600,000 tons of chrome waste with concentrations that vary from 2000 to 5000 mg/L, and that finally end up contaminating the environment [1], [2]. Cr(VI) is a highly harmful compound found most frequently in effluents from industrial activities such as metallurgy, tanneries, textiles, mining and others [3]. Such actions result in wastewater loaded with Cr(VI) and other heavy metals that are discharged into water bodies such as rivers, posing a threat to the health of people and other living organisms. Therefore, it is important that industrial effluents be treated appropriately before discharge, so that their physicochemical parameters meet the permissible limits acceptable to human health and the environment [4].

The environmental impact produced by hexavalent chromium is varied and it can be found in the air, soil and water due to different industrial activities such as leather tanning, steel manufacturing, etc. [5]. Processes to treat industrial wastewater with high concentrations of Cr(VI) have been a top priority because this contaminant produces extreme biological toxicity and has high mobility in natural water systems [6]. Chromium exists mainly in trivalent and hexavalent forms, being Cr(VI) the one that has attracted integral attention due to its high solubility, mobility and high toxicity [7]. Meanwhile, Cr(III) is an essential element in human metabolism and plays an important role in the metabolism of sugar and lipids. Therefore, the reduction of Cr(VI) to Cr(III) from industrial effluents is important before disposal [8]. The hexavalent form of chromium is well known to be mutagenic, carcinogenic and toxic [9]. Studies show that Cr(VI) in drinking water can cause an increased risk of stomach cancer and reproductive harm, and breathing this contaminant at high levels over a long period of time increases the risk of lung cancer and nasal cancers [10]. Given the disadvantages of the presence of Cr(VI) in the environment and its effects on human health, the elimination, removal or reduction of this contaminant is necessary.

Several methods have been developed to reduce or eliminate Cr(VI) from industrial effluents, generally through physicochemical processes, which are usually expensive, complicated and generate secondary environmental pollution at the end of their process [11]. Among the variety of techniques used, biological treatments are presented as a promising technique that allows *in situ* remediation of environments contaminated with heavy metals, since they are viable, economical and environmentally friendly treatments [12]. Environmental biotechnology states that the interaction between microorganisms and metals are primordial factors for the biogeochemical cycle due to the implementation of methodologies to remove, recover or detoxify heavy metals [13]. Therefore, the application of microorganisms is considered an innovative alternative that does not generate

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impact on the environment, and is also viable and efficient to provide solutions to various environmental problems [14].

Recently, bioremediation has been observed as an alternative to physical-chemical methods because it is a safe and cost-effective option. Bioremediation techniques have been developed to take advantage of the potential that certain microorganisms have to degrade and detoxify specific contaminants [15]. Microorganisms play an important role in the detoxification and elimination of Cr(VI) from contaminated environments [16]. The microbial strains that can reduce Cr(VI) are called chromium reducing bacteria (CRB). Gram-positive bacteria show a high tolerance to the toxicity of high concentrations of Cr(VI), while Gram-negative bacteria are less tolerant of Cr(VI) [17]. It is known that several bacterial species have the ability to reduce Cr(VI) to Cr(III), among them are the *Pseudomonas fluorescens* LB300, *Bacillus sp.*, *Enterobacter cloacae* HO1, *Enterobacter aerogenes* and others [18].

Consequently, taking into account the statements mentioned above, this research summarized and compiled studies about the application of various microorganisms for the removal of Cr(VI) from industrial effluents, mainly from tanneries. Therefore, the present study evaluated through systematic review and meta-analysis the efficiency of the application of microorganisms based on two different initial concentrations of Cr(VI) to determine the highest percentage of metal removal from tannery effluents. To achieve this purpose, the following objectives were followed: (a) to identify the investigations that developed the application of microorganisms for the removal of Cr(VI) from tannery wastewaters, (b) to identify the origin of the wastewaters, their physicochemical parameters and the concentration of Cr(VI), (c) to identify the operational conditions and concentration of the microorganisms to evaluate their influence on the physicochemical parameters of the effluents and (d) to carry out a meta-analysis to evaluate the efficiency of removal of the microorganisms using two different initial concentrations of Cr(VI).

II. MATERIALS AND METHODS

A. *Information sources and search strategy*

The systematic review of the present research followed the proposal of MOOSE (meta-analysis of observational studies in epidemiology). The scientific articles were extracted from reliable sources of information, such as ScienceDirect, Scopus, Scielo, Concytec and Cientifi-k. It was verified that all the included investigations do not exceed 5 years of antiquity from the current date (May, 2020). The search for information in the electronic databases was systematically carried out according to key words such as "microorganisms", "removal", "chromium", "tanning effluents" and "bioreduction", and in the English language. In addition, references from reviews on the subject were checked in order to explore the most relevant studies.

B. *Inclusion and exclusion criteria*

Initially, duplicate documents were removed according to the title. Then, the summary of potentially eligible articles was reviewed and irrelevant documents were excluded. Subsequently, all documents were downloaded for review according to the inclusion criteria. Finally, all selected documents were thoroughly evaluated according to the inclusion and exclusion criteria defined as follows:

1. All investigations showing the removal of other heavy metals or contaminants in wastewater were included as long as data for Cr(VI) was collected individually.
2. All investigations that worked with wastewater from various sources (domestic or industrial), as well as from various water bodies (lagoon, lakes, rivers, etc.) contaminated with Cr(VI) were included.
3. All research assessing the effectiveness of microorganisms compared to other Cr(VI) removal techniques was included.
4. All investigations that performed Cr(VI) removal in other media (soil, plants, sludge, etc.) were excluded.
5. All investigations conducted in controlled environments, whose Cr(VI) contamination did not exceed quality standards, were excluded.
6. All researches with insufficient data and opinion articles were excluded.

C. *Articles selection and data extraction*

After identifying the potentially eligible documents, the information of each studied article was reviewed, extracted and summarized individually according to the inclusion and exclusion criteria. The data extracted from each included research (using validated data collection instruments) consisted of the following items: (a) Name of author(s) and year, (b) origin of the effluent sample, (c) type of microorganism, (d) pH, (e) contact time, (f) temperature and (g) dosage, (h) initial concentration of Cr(VI), (i) final concentration of Cr(VI) and (k) percentage of Cr(VI) removal.

D. *Evaluation of the methodological quality*

The Newcastle-Ottawa Quality Assessment Scale for Cohort Studies checklist was used to assess the methodological quality of the included investigations. This checklist was adapted according to the interest of the present research, evaluating the quality of each research that refers to representativeness and exposure. The representativeness manifests if the sample truly represents the wastewater contaminated with Cr(VI) from anthropogenic activities, and the exposure evaluated if the physicochemical characteristics (concentration of Cr(VI), BOD, COD, TDS and pH) of the wastewater were described and if the application of the microorganisms was efficient to decrease or remove the concentration of the metal.

The results section measured the removal percentage (indicates if the initial concentration of Cr(VI) decreases after the application of the microorganisms) and the application period (shows the time in which the microorganisms manage to remove the concentrations of Cr(VI)). On the other hand, the time required by the microorganisms for the removal of Cr(VI) indicates the period in which they work more efficiently. Finally, each section (selection and results) was evaluated with the criteria of "Yes" and "No" to determine the quality standards of information between good, acceptable and bad.

E. Data meta-analysis

The Review Manager program (RevMan 5.4) was used for the analysis of the data. This is statistical software developed by the Cochrane Collaboration to carry out systematic reviews and generate meta-analyses. For the meta-analysis, dichotomous data were used, which were presented and compared with the odds ratio (OR). The effect estimates presented for the study were developed with 95% confidence intervals. The heterogeneity of the investigations was evaluated through visual analysis of the forest plot, in order to determine the overlap of the confidence intervals. Then, the chi-square (χ^2 or Chi^2) homogeneity test was performed and the degree of heterogeneity was quantified with the index I^2.

The meta-analysis was worked with the fixed effect model because the results of each research are directed to an environmental approach and the effluent treatments did not require an established population since different dosages of microorganisms were used to treat different concentrations of Cr(VI).

III. RESULTS AND DISCUSSION

A. Inclusion and exclusion of investigations

Figure 1 shows the selection flowchart of the investigations included for the meta-analysis. A total of 206 preliminary articles were identified through the search of information in the databases described above.

From Figure 1 it was observed that 17 investigations were included and 189 investigations were excluded. In the first phase, 76 researches were excluded after applying the inclusion criteria in both the title and the abstract. Already, in the second phase, 112 investigations were excluded after evaluating the full text. In this last exclusion phase, the following factors were considered: treatment in synthetic wastewater (n=26), insufficient data on the microorganism dosage (n=10), removal of Cr(VI) in soils (n=16), type of publication (narrative review or opinion articles) (n=30), research in controlled environments (n=16), application of adsorbents that were not microorganisms (plant species) (n=5) and removal of other heavy metals that did not include Cr(VI) (n=9).

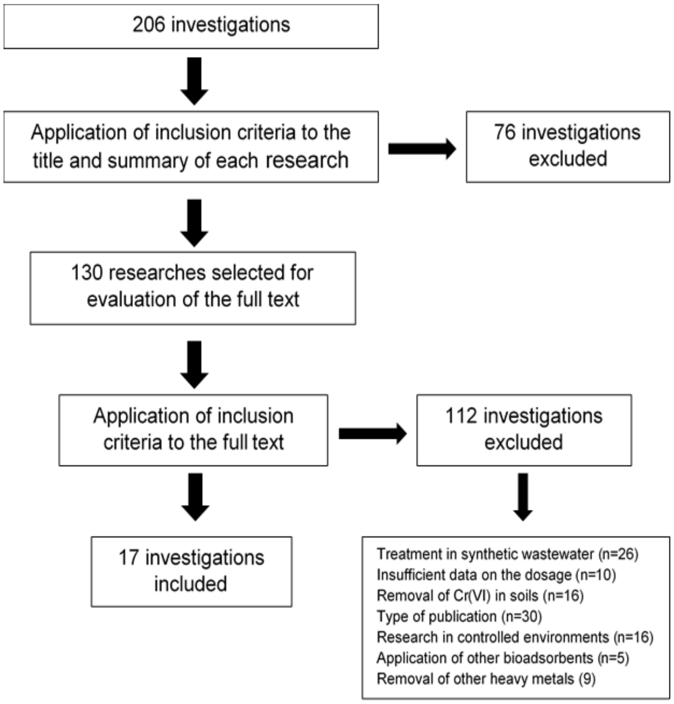


Fig. 1 Selection flow chart of included and excluded investigations.

B. Description of the investigations included

Table 1 shows the 17 researches reviewed that were published between 2009 and 2020. Each study used a different microorganism and the percentage of Cr(VI) removal varied from 63 to 100%, with an average efficiency of 81.5%. The investigations were carried out in the countries of India, China, Pakistan and Italy, and three of them used more than one microorganism to evaluate its capacity to remove Cr(VI). In addition, all the investigations included worked on the removal of Cr(VI) as a function of pH, temperature and contact time, and 06 investigations carried out statistical analyses.

The physicochemical characteristics of industrial effluents are shown in Table 2. In it, it is observed that all the included investigations evaluated the pH and evidenced a high concentration of Cr(VI) with values higher than 1.24 mg/l, which surpassed the standards of environmental quality. It is also observed that some investigations evaluated the chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solids (TDS) and color. On the other hand, microorganisms (n=18) used for Cr(VI) removal were isolated from samples of diverse origin such as aeration lagoons, deep water sediment, tannery effluents, intertidal zone sediments, salt lakes, coal mine soils, sludge and effluents from industrial wastewater treatment plants, electroplating effluents, hydrocarbon contaminated soils, algae cultivation and chromite mines. All of this indicates that microorganisms can be affected by many factors, including climate, type of effluent, type of crop, etc. [36], and their efficiency in the removal of Cr(VI) depends on various chemical-physiological and biological mechanisms [37]. For its identification, the authors carry out both molecular and phylogenetic analyses that consist mainly in the isolation of the 16S rRNA gene, which is later amplified and introduced to a database (GenBank, NCBI BLAST, etc.).

Table 1 Characteristics of the investigations selected for meta-analysis.

No.	Microorganism	Operational conditions	Cr(VI) removal (%)	Statistical analysis	Country	Author
1	*Burkholderiales* and *Stenotrophomona sp.*	pH, temperature (°C) and contact time (h)	87.96	-	India	Chandra et al. [19]
2	*Lactobacillus paracasei*	pH, temperature (°C) and contact time (h)	63	-	China	Huang et al. [20]
3	*Paecilomyces lilacinus*	pH, temperature (°C) and contact time (h)	95.5	Correlation and linear regression analysis	India	Sharma and Adholeya [21]
4	*Sporosarcina saromensis*	pH, temperature (°C) and contact time (h)	82.5	ANOVA	China	Zhao et al. [22]
5	*Aeromonas Hydrophila*	pH, temperature (°C) and contact time (h)	93.71	-	China	Ji et al. [23]
6	*Dunaliella salina*	pH, temperature (°C) and contact time (h)	66.4	ANOVA	India	Vidyalaxmi et al. [24]
7	*Bacillus cereus*	pH, temperature (°C) and contact time (h)	92	-	India	Kumari et al. [25]
8	Bacterial consortium	pH, temperature (°C) and contact time (h)	72	-	Italy	Tammaro et al. [26]
9	*Pseudomonas brenneri*	pH, temperature (°C) and contact time (h)	96.3	-	India	Banerjee et al. [27]
10	*Bacillus sp.* and *Staphylococcus capitis*	pH, temperature (°C) and contact time (h)	87.5	Mean value and standard error	Pakistan	Zahoor and Rehman [28]
11	*Bacillus sp.*	pH, temperature (°C) and contact time (h)	93.71	ANOVA	China	Tan et al. [29]
12	*Chlorella vulgaris*	pH, temperature (°C) and contact time (h)	81.3	-	India	Sibi [30]
13	*Pseudomonas aeruginosa*	pH, temperature (°C) and contact time (h)	88	-	India	Shukla et al. [31]
14	*Chlorella Vulgaris*	pH, temperature (°C) and contact time (h)	100	ANOVA	India	Das et al. [32]
15	*Aspergillus*	pH, temperature (°C) and contact time (h)	96.3	-	India	Sivakumar [33]
16	*C. perangustum* and *Penicillium commune*	pH, temperature (°C) and contact time (h)	73.73	-	India	Sharma and Malaviya [34]
17	*Arthrobacter sp.*	pH, temperature (°C) and contact time (h)	90	-	India	Dey and Paul [35]

Table 2 Physicochemical characteristics of industrial effluents.

No.	pH	Cr(VI) concentration (mg/L)	COD (mg/L)	BOD (mg/L)	TDS (mg/L)	Colour	Author
1	8.5	38.90	-	2933	60180	-	Chandra et al. [19]
2	7.4	178	4200	-	73	Dark brown	Huang et al. [20]
3	8	1.24	520	325	-	-	Sharma and Adholeya [21]
4	8.5	50	-	-	-	-	Zhao et al. [22]
5	8	2.23	-	-	-	Greenish gray	Ji et al. [23]
6	8.6	25	-	-	-	-	Vidyalaxmi et al. [24]
7	8.4	2.41	1260	660	14000	Light yellow	Kumari et al. [25]
8	7.5	48.87	1186	211.1	758	-	Tammaro et al. [26]
9	6.6	60.60	1.12	1.42	-	Grayish	Banerjee et al. [27]
10	7.5	1.84	1.30	-	-	-	Zahoor and Rehman [28]
11	8	125	-	-	420	-	Tan et al. [29]
12	2.6	227	374	197	690	Yellowish brown	Sibi [30]
13	7.5	35	-	-	1.16	-	Shukla et al. [31]
14	8	30.22	4000	1350	1766	-	Das et al. [32]
15	4.3	290	-	-	1189	-	Sivakumar [33]
16	9.16	9.86	5776	-	17650	Dark gray	Sharma and Malaviya [34]
17	7.5	60	-	-	-	-	Dey and Paul [35]

C. Influence of operational conditions on Cr(VI) removal

Operational conditions such as pH, temperature, contact time, microorganism concentration and initial concentration of Cr(VI) (ver Table 3 and Table 4) can affect the effectiveness of the microorganism in the process of removing Cr(VI). Therefore, it should be considered the effects that cause these parameters in the treatment to optimize the process and maximize the rates of removal of Cr(VI) from industrial effluents [38], [39].

Table 3 Optimal values of pH, temperature, microorganism concentration and contact time in the removal of Cr(VI).

No.	Microorganism	pH	Temperature (ºC)	Concentration (mg/L)	Contact time (h)	Cr(VI) removal (%)	Author
1	*Burkholderiales* and *Stenotrophomona sp.*	7	25	22.5	24	87.96	Chandra et al. [19]
2	*Lactobacillus paracasei*	5	25	10	24	63	Huang et al. [20]
3	*Paecilomyces lilacinus*	5.5	30	2	360	95.5	Sharma and Adholeya [21]
4	*Sporosarcina saromensis*	8.5	35	10	168	82.5	Zhao et al. [22]
5	*Aeromonas Hydrophila*	7	30	10	24	93.71	Ji et al. [23]
6	*Dunaliella salina*	8.6	25	10	120	66.4	Vidyalaxmi et al. [24]
7	*Bacillus cereus*	8.4	35	25	48	92	Kumari et al. [25]
8	Bacterial consortium	7	26	10	72	72	Tammaro et al. [26]
9	*Pseudomonas brenneri*	6	30	45	360	96.3	Banerjee et al. [27]
10	*Bacillus sp.* and *Staphylococcus capitis*	6	37	13.3	48	87.5	Zahoor and Rehman [28]
11	*Bacillus sp.*	7	37	20	30	93.71	Tan et al. [29]
12	*Chlorella vulgaris*	5	25	100	24	81.3	Sibi [30]
13	*Pseudomonas aeruginosa*	7	32	20	24	88	Shukla et al. [31]
14	*Chlorella Vulgaris*	8	28	250	24	100	Das et al. [32]
15	*Aspergillus*	5	28	20	72	96.3	Sivakumar [33]
16	*C. perangustum* and *Penicillium commune*	4	28	50	24	73.73	Sharma and Malaviya [34]
17	*Arthrobacter sp.*	7	25	46.3	96	90	Dey and Paul [35]

1) pH: The pH is a very important factor in the removal process and its control favors the percentage of Cr(VI) removal from wastewater [40]. The rate of effectiveness and percentage of metal removal increases as the pH increases or it is oriented to a neutral state [41]. The maximum removal (≅100%) of Cr(VI) from wastewater was achieved with the microorganisms *Aspergillus niger* [33], *Pseudomonas brenneri* [27] and *Chlorella Vulgaris* [32] at pH 5, 6 and 8, respectively. Kumar and Dwivedi [42] and Zhang et al. [43] showed that in neutral to weakly alkaline conditions the reduction of Cr(VI) is favorable due to the fact that some microorganisms have better growth and as a consequence causes the increase of chromate reductase activity and biomass production. From Table 1 it was observed that most of the investigations included worked at pH values around 7 to achieve the maximum removal of Cr (VI) for each microorganism used. However, in the study of Nourbakhsh et al. [44], the maximum removal of Cr(VI) reached by the microorganisms *Chlorella vulgaris*, *Clodophara crispata*, *Zoogloea ramigera*, *Rhizopus arrhizus* and *Saccharomyces cerevisiae* was obtained in the range of pH 1 to 2 due to the fact that in acid medium chemical exchange takes place between the cells of each microorganism and the metal chromium. This shows that the parameter pH is important for each microorganism to achieve the maximum reduction of Cr(VI) efficiently [22].

2) Temperature: Temperature is also a crucial component for the biosorption of heavy metals by microorganisms, because if not worked under optimal conditions, it can destabilize the composition and configuration of the cell wall of the microorganism affecting the efficiency of the removal of Cr(VI) in the medium [45]. According to this, among the investigations included, *Paecilomyces lilacinus* [21], *Pseudomonas brenneri* [27] and *Aeromonas hydrophila* [23] reached the maximum capacity of Cr(VI) removal at temperatures of 30°C, while *Sporosarcina saromensis* [22] and *Bacillus cereus* [25] at 35 °C. This suggests that the mentioned microorganisms are mesophilic, since they have the capacity to grow at extreme temperatures while performing the elimination of Cr(VI) [46]. In the research of Qu et al. [47] and Sepehr et al. [48], using a magnetotactic bacteria and microorganisms such as *Aspergillus niger* and *Aspergillus oryzae* achieved efficiencies of removal of Cr(VI) higher than 76% at temperatures of 29 and 30°C, respectively. Most of the research included (Table 3) for the meta-analysis worked with temperatures between 28 and 30°C to have a higher percentage of Cr(VI) removal. This shows that temperature has a significant effect on the growth rate of the microorganism and indicates that the removal efficiency could decrease due to the reduced activity of its cellular metabolism at temperatures higher or lower than its optimal growth value [49], [50]. Elahi and Rehman [51] and Dey and Paul [35] worked at temperatures of 37°C with the microorganisms *Bacillus aerius* and *Arthrobacter sp.* - SUK1201, respectively. The same authors manifested that at such temperature the microorganisms grew in a favorable way, but these were affected when working in the presence of Cr(VI) and in acid or alkaline media. This suggests that extreme environmental conditions such as temperature and pH could inactivate the microorganisms, restricting the scope of their use, and consequently would require a previous variation of the physical conditions necessary for their application [52].

3) Microorganism concentration: The concentration of microorganisms has a significant influence on the process of removing Cr(VI) from industrial effluents. Several researchers agree that an increase in the concentration of microorganisms also increases the efficiency of elimination, reduction or removal of Cr(VI), because it increases the bacterial mass and increases the number of available adsorption sites [22], [24], [53]–[55]. Table 3 showed that the different concentrations of microorganisms used in the included investigations did not exceed 50 mg/L. The microorganisms *Pseudomonas brenneri* [27] and *Bacillus cereus* [25] reached removal percentages of Cr(VI) higher than 92%, with concentrations of 30 mg/L and 215 mg/L, respectively. This shows that the optimal concentration of microorganisms for the removal of Cr(VI) can vary and depends on several factors such as initial concentration of Cr, pH, time and among others [47].

4) Contact time: Most of the researches included had a prolonged contact time, being the minimum of 2h and the maximum of 360h (see Table 3). The investigations of Shukla et al. [31], Ji et al. [23] and Das et al. [32] achieved Cr (VI) removal percentages of 88% 93.71% y ≅100%, respectively, with a contact time of 24 h. This shows that the reduction of Cr(VI) increases significantly with increasing exposure time [42], and reflects that microorganisms have better removal efficiency when they reach a stable growth level, that is, the stationary growth phase [21], [48]. Some microorganisms such as *Pseudomonas aeruginosa* [31], *Aeromonas hydrophila* [23] and *Chlorella vulgaris* [32] achieved Cr (VI) removal percentages of 88%, 93.71% and ≅100%, respectively, with a contact time of 24h. This indicates the practicality of these microorganisms to achieve high percentages of Cr(VI) removal in less time. On the other hand, the removal of Cr(VI) by some microorganisms is independent of the contact time [56], but its good results are associated with operational conditions such as pH, temperature and initial concentration of Cr(VI) in the medium to be treated.

5) Initial concentration of Cr(VI): The adsorption process performed by the microorganisms is influenced by the initial concentration of Cr(VI). High concentrations of Cr(VI) negatively influence the removal of the metal because the medium becomes more toxic and prevents the microorganisms from developing adequately to perform the task of elimination, reduction or removal of the chromate. Table 4 shows the microorganisms used in the included investigations and their efficiency in removing Cr(VI) from two different initial concentrations of the metal.

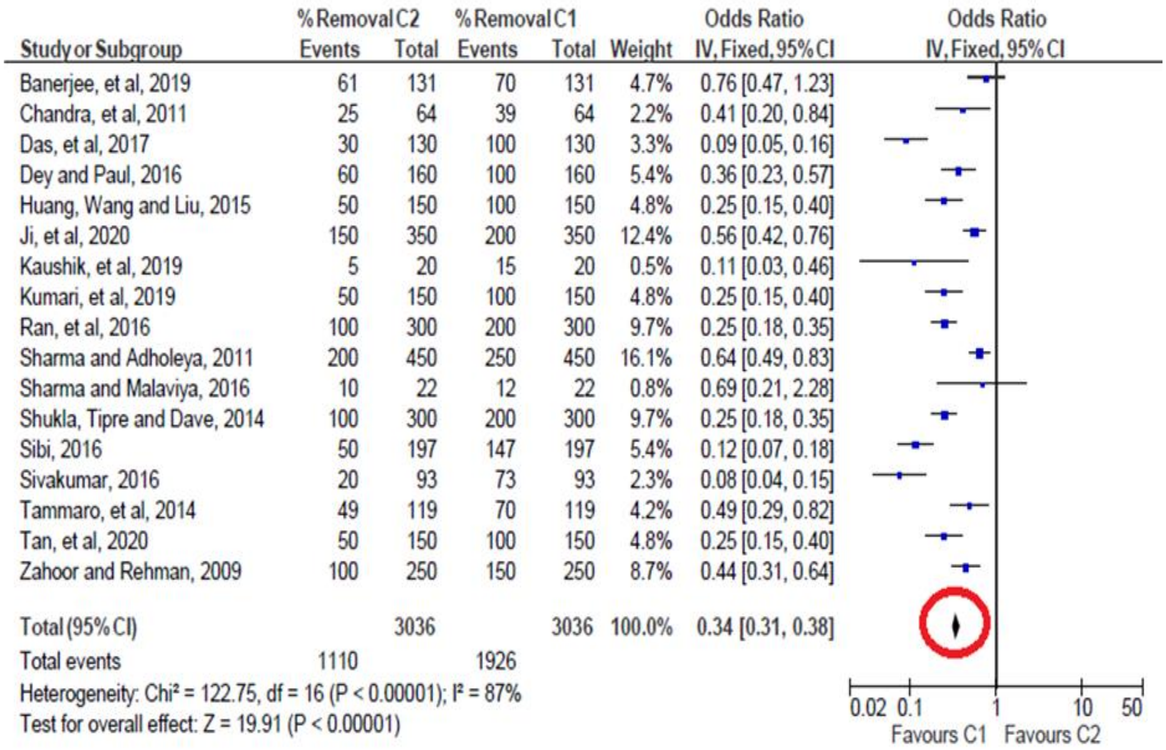
Table 4 Cr(VI) removal efficiency as a function of its initial concentrations.

No.	Microorganism	1º Cr(VI) concentration (mg/L)	2º Cr(VI) concentration (mg/L)	1º Cr(VI) removal efficiency	2º Cr(VI) removal efficiency	Author
1	*Burkholderiales* and *Stenotrophomona sp.*	25	38.9	87.96	71.59	Chandra et al. [19]
2	*Lactobacillus paracasei*	50	100	99	63	Huang et al. [20]
3	*Paecilomyces lilacinus*	200	250	100	72	Sharma and Adholeya [21]
4	*Sporosarcina saromensis*	100	200	100	83	Zhao et al. [22]
5	*Aeromonas Hydrophila*	150	200	96.3	72.23	Ji et al. [23]
6	*Dunaliella salina*	5	15	72	54.7	Vidyalaxmi et al. [24]
7	*Bacillus cereus*	50	100	84	59	Kumari et al. [25]
8	Bacterial consortium	48.87	70	72	67	Tammaro et al. [26]
9	*Pseudomonas brenneri*	60.6	70	96.3	90	Banerjee et al. [27]
10	*Bacillus sp.* and *Staphylococcus capitis*	100	150	93	69.7	Zahoor and Rehman [28]
11	*Bacillus sp.*	50	100	100	97	Tan et al. [29]
12	*Chlorella vulgaris*	50	147	80.3	65	Sibi [30]
13	*Pseudomonas aeruginosa*	100	200	99	80	Shukla et al. [31]
14	*Chlorella Vulgaris*	30.22	100	100	85	Das et al. [32]
15	*Aspergillus*	20	72.5	96.3	88.2	Sivakumar [33]
16	*C. perangustum* and *Penicillium commune*	9.86	12.26	100	73	Sharma and Malaviya [34]
17	*Arthrobacter sp.*	60	100	90	70	Dey and Paul [35]

From Table 4, although a methodological diversity was evidenced among the included investigations, there is a growing trend of works that confirm the influence of the initial concentration of Cr(VI) on the efficiency of metal removal. The microorganisms as Bacillus sp. [28], Staphylococcus capitis [28], Bacillus sp. CRB-B1 [29], Chlorella vulgaris [30], Pseudomonas aeruginosa [31], Aspergillus niger [33], Cladosporium perangustum [34], Penicillium commune [34] and Arthrobacter sp. [35] achieved removal rates of Cr(VI) above 80%, with initial metal concentrations of 10 to 100 mg/L. The effect of the variation in the concentration of chromium, is possibly due to the enzymatic reactions of the microorganisms in the medium, and the speed of reduction of chromium decreases with the increase of the concentration of the metal [57]. In other investigations were carried out tests of removal of Cr(VI) with the microorganisms Trichoderma sp. [42], Pseudomonas aeruginosa [58], Trichoderma viride [59], Chlorella vulgaris [44] and Pannonibacter phragmitetus [60] using initial concentrations of the metal superior to 200 mg/L. The results showed that the decrease in the removal efficiency of Cr(VI) is due to the fact that many of the metal ions present in the solution were not adsorbed by the saturation of the binding or chemical exchange sites of the microorganism [61]. This indicates that the microorganisms reduce the concentration of chromium to a maximum amount that depends on the mutagenic and toxic effects of the metal [62], [63]

D. Meta-analysis

The forest plot (Figure 2) shows the 17 investigations included for the meta-analysis, which were worked with a 95% confidence interval. The Cr (VI) removal efficiency is represented by the polygon at the bottom of the graph, and its edges represent the limit of the confidence interval.



Study or Subgroup	% Removal C2 Events	% Removal C2 Total	% Removal C1 Events	% Removal C1 Total	Weight	Odds Ratio IV, Fixed, 95% CI
Banerjee, et al, 2019	61	131	70	131	4.7%	0.76 [0.47, 1.23]
Chandra, et al, 2011	25	64	39	64	2.2%	0.41 [0.20, 0.84]
Das, et al, 2017	30	130	100	130	3.3%	0.09 [0.05, 0.16]
Dey and Paul, 2016	60	160	100	160	5.4%	0.36 [0.23, 0.57]
Huang, Wang and Liu, 2015	50	150	100	150	4.8%	0.25 [0.15, 0.40]
Ji, et al, 2020	150	350	200	350	12.4%	0.56 [0.42, 0.76]
Kaushik, et al, 2019	5	20	15	20	0.5%	0.11 [0.03, 0.46]
Kumari, et al, 2019	50	150	100	150	4.8%	0.25 [0.15, 0.40]
Ran, et al, 2016	100	300	200	300	9.7%	0.25 [0.18, 0.35]
Sharma and Adholeya, 2011	200	450	250	450	16.1%	0.64 [0.49, 0.83]
Sharma and Malaviya, 2016	10	22	12	22	0.8%	0.69 [0.21, 2.28]
Shukla, Tipre and Dave, 2014	100	300	200	300	9.7%	0.25 [0.18, 0.35]
Sibi, 2016	50	197	147	197	5.4%	0.12 [0.07, 0.18]
Sivakumar, 2016	20	93	73	93	2.3%	0.08 [0.04, 0.15]
Tammaro, et al, 2014	49	119	70	119	4.2%	0.49 [0.29, 0.82]
Tan, et al, 2020	50	150	100	150	4.8%	0.25 [0.15, 0.40]
Zahoor and Rehman, 2009	100	250	150	250	8.7%	0.44 [0.31, 0.64]
Total (95% CI)		3036		3036	100.0%	0.34 [0.31, 0.38]
Total events	1110		1926			

Heterogeneity: Chi² = 122.75, df = 16 (P < 0.00001); I² = 87%

Test for overall effect: Z = 19.91 (P < 0.00001)

Fig. 2 Forest plot displaying the 17 investigations included for the meta-analysis on Cr(VI) removal.

From Figure 6, the Odds Ratio presented a value of 0.34, showing that the removal percentages for treatment 1 presented lower concentrations by 66%. It is also interpreted that the removal percentages for treatment 1 showed a 34% decrease in Cr(VI) concentrations compared to the removal percentages of treatment 2. That is, with lower initial concentrations of Cr(VI) in the medium, the removal of such contaminant increases favoring the removal percentages. On the other hand, the included researches showed a considerable statistical heterogeneity ($I^2 = 87\%$; $p<0.0001$). This indicates that the results of the investigations do not have a similarity among themselves and it is recommended to perform a subgroup analysis, using the articles that have more similarity among themselves for each subgroup under study [64]. Regarding the weight values, Ji et al. [23] and Sharma and Adholeya [21] presented percentages of 12.4% and 16.1%, respectively, indicating that these studies worked with higher values of initial Cr(VI) concentrations than the other studies included in the study.

Huang et al. [20] and Ji et al. [23] studied Cr(VI) removal using *Lactobacillus paracase* (CL1107) and *Aeromonas hydrophila* (LZ-MG14), respectively. In their studies, they highlighted that increasing the initial Cr(VI) concentration decreased the efficiency of the microorganisms, suggesting the importance of evaluating this parameter in the treatment of tannery effluents. Similarly, Kumari et al. [25] evidenced the same effect using *Bacillus cereus* (Cr1) because the strain exhibited a reduction of Cr(VI) depending on the initial concentration of this heavy metal. Thus, this research showed concretely that a microorganism can work efficiently in the removal of heavy metal concentrations, as long as the maximum tolerable concentration of the metal that can be treated is known because a medium with high Cr(VI) concentrations would affect the metabolism of the microorganism due to the mutagenic and toxic effects of the heavy metal.

IV. CONCLUSIONS

This systematic review and meta-analysis presented the various microorganisms used in the researches included according to their specific operational conditions to efficiently remove Cr(VI) from tannery effluents. According to this, there is enough evidence in the included investigations about the efficiency that the microorganisms have to achieve high percentages of Cr(VI) removal. The results of the meta-analysis showed that 69% of the included investigations, when experimenting with different initial concentrations of the metal, chose to use lower initial concentrations of Cr(VI) (between 5 and 100 mg/L generally) to treat industrial effluents. At lower initial concentrations of Cr(VI), the removal rate efficiency was higher because microorganisms can metabolize the contaminant chemicals without being harmed by the toxicity of the medium. While in high concentrations, the active metabolism of the microorganism's biomass is affected, as well as its bioaccumulation capacity and enzymatic activities, which would result in a smaller absorption surface for the metal bonding, that is, the microorganism would lose its capacity to efficiently remove Cr(VI) from the medium.

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