Journal of Biotechnology Letters
ISSN: 0976-7045 & E-ISSN: 0976-7053, Volume 4, Issue 1, 2013, pp.-60-67.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000250

BIOLOGICAL TREATMENT OF TANNERY SYNTHETIC EFFLUENT

FAOUZI M., MERZOUKI M.*, EL FADEL H. AND BENLEMLIH M.

Faculty of Science, Dhar El Mahraz, P.O. Box-1796, Atlas-Fez, University Sidi Mohammed Ben Abdellah, Fez, Morocco.
*Corresponding Author: Email- merzoukimo@yahoo.fr

Received: December 13, 2012; Accepted: January 17, 2013

Abstract- The Fez city is an important industrial center of Morocco. Unfortunately, its economic development activities have been accompanied by a pollution caused mainly by oil mills and tanneries which effluents are discharged into the Sebou river. The most dangerous and toxic pollutant is chromium coming from tanneries, that causes biological and physico-chemical degradation of the aquatic ecosystem of this river. Faced to this situation, our laboratory has been engaged in looking for a simple method for reducing the pollution parameters of the synthetic sewage of tanneries by bioaugmentation, using bacterial strains isolated from effluents of Moroccan tanneries. This is the first study that tests the bacteria *Bacillus thuringiensis*, *Bacillus cereus* and *Bacillus pumilusses* for the elimination of total chromium (Cr_T) and Chemical oxygen demand (COD_T). The results obtained with bioaugmentation treatment show that the best yields are obtained when decreasing organic concentration of the synthetic effluent to a COD_T of 4 g l^{-1} and the concentrations of bacteria consortium to 4%. The process allowed a highly effective treatment of synthetic tannery effluent with high purification yields of 95% for COD_T and 98% for total chromium with the bacterium *Bacillus thuringiensis*.

Keywords- bioaugmentation; tannery effluent; chromium; chemical pollution.

Citation: Faouzi M., et al. (2013) Biological Treatment of Tannery Synthetic Effluent. Journal of Biotechnology Letters, ISSN: 0976-7045 & E-ISSN: 0976-7053, Volume 4, Issue 1, pp.-60-67.

Introduction

The Fez city developing industry does not escape the intense phenomena of pollution that results. Fez demographic and industrial growth faces a problematic of poor sanitation and limited or absence of infrastructure especially in areas of extension, what affects in alarming way the environment of the city. Industrial effluents are discharged directly into the Sebou river without any treatment, causing degradation of the aquatic ecosystem which is the most important watershed of the Morocco with a debit of 6.6 billion m^3 per year, a surface of 40,000 km^2 and it deserves a 191,000 ha of farmland [1]. An annual volume of 120 million m^3 of waste water is discharged into the river system with a pollution load of 68 tons / year in Cr_T [2]. If one assumes a rate of chromium precipitation of 75%, this means that 325 kg of chromium from the medina end each day in Fez rivers and flow directly into the Sebou river [1]. Conventional methods for removing chromium (VI) ions from wastewater include; chemical reduction, electrochemical treatment, ion exchange, and evaporative recovery. Nevertheless, certain drawbacks of these methods have been noticed, such as high cost, low efficiency, operational complexity, and other difficulties [3,4] which has urged for an alternative process.

The use of biological materials, including living and non-living micro -organisms, in the removal and possibly recovery of toxic or precious metals from industrial wastes, has gained important credibility during recent years, because of the good performance and low cost of these materials. The natural affinity of biological compounds for metallic elements could contribute to the purification of metal-loaded wastewater, a fact which has been already proved in many cases and by many researchers [5,6]. However, despite the extensive literature available on metal–microbial interactions and the production of *Bacillus thuringiensis* based biopesticides but little information exists on treatment of total chromium by bioaugmentation, by the use of bacteria *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus.* Biological process is a promising technique for removal of molecules with different physicochemical properties, since it uses specific microorganisms potentially capable of biodegradation. It is a procedure that improves the yield of treatment in a purification plant with direct introduction of selected microorganisms, either wild strains or genetically modified [7].

The principle of the biological treatment is based on the degradation of organic compounds and metals present in the effluent by microorganisms (aerobic and / or anaerobic). Those microorganisms exert on the other hand, a physical effect of organic pollution retention by gathering it in films or flakes. In bibliography, the treatment of tannery effluent by bacteria *Thiobacillus ferrooxidans, Staphylococcus aureus* and *Vibrio sp* allows a COD removal of 69%, 80%, and 87.5% and for the disposal of Cr_T bacteria *Pseudomonas aeruginosa*, *Acinetobacter sp* and *hirsutella sp* gives an eliminition of 87%, 90% and 70%, respectively [8]. *Bacillus sp*. JDM-2-1 and *Staphylococcus capitis* could reduce 85% and 81% of

hexavalent chromium from the industrial effluent [9]. In the policy of environmental performance of industry, bioaugmentation has proved effective in reducing sludge production, energy saving for ventilation and improving the quality of the discharge with reduced costs [10,11]. The aim of this work is to establish a biological process to reduce pollution of synthetic tanneries effluent by bioaugmentation, using bacterial strains isolated from effluents of tanneries capable of degrading organic matter and mineral matter in terms of chromium.

Material and Methods

Purification of the Strains

The strains of bacteria were isolated from effluents of Moroccan tanneries. The colonies are large (2-7 mm), matt or granular, and whose form is variable. Once isolated, the strains were purified by five successive subcultures on the isolation medium (nutrient agar). The dishes were incubated at 37°C for 24 hours. We have isolated three strains and identification of purified bacterial strains was performed by means of molecular biology.

Identification of Isolates by Molecular Approach

Isolates were identified by molecular biology techniques. After DNA extraction and amplification by PCR (Polymerase Chain Reaction), The tubes containing the reaction mixture are given in the thermocycler. the nucleotide sequence was determined by sequencing at the National Institute of Hygiene in Rabat-Morocco. The molecular identification of 16S ribosomal DNA (16S-rDNA) was used extensively in bacterial phylogeny [12] by using universal primers for bacteria [13], additional to amplified and constant sequences of variable regions specific to the species. The three bacterial strains L2, LK1 and B4 were identified after amplification and sequencing of a fragment of rDNA 16 S. A fragment of a size of 440 bp corresponding to the C-terminal portion of the 16S rDNA was amplified using the primer pair:

91E (5'-TCAAAK [T / G] GAATTGACGGGGGC-3 ').
13B (5'-GCCCGGAACGTATTCAC-3 ').

The amplified products were separated by electrophoresis (1 hour at 7 V cm^{-1}) in 12 l of amplification mixture on an agarose gel on 2% and visualized after staining with ethidium bromide. The agarose gel electrophoresis gives sufficient resolution. The two strands of DNA amplified by PCR from different bacteria are sequenced by sequencing at the National Institute of Hygiene in Rabat using the same primers used in PCR amplification.

Medium Power Reactor

The reactor was powered by a 500 ml of sterile synthetic wastewater, and incubated in continuous mode, ventilated with a mini-compressor and continuously stirred by a magnetic bar in which 2 ml of trace elements are added to one liter of synthetic medium with 2% and 4% of the 4 g l^{-1} and 8 g l^{-1} of COD_T [Table-1].

Then we aseptically added 10 ml of pure young bacterial culture of 24h in nutrient broth of each strain isolated from the effluent of tanneries at 2%. A second feeding of the fermenter was carried out with 20 ml of bacterial culture at 4% [Fig-1]. A control test was performed without adding strain on synthetic medium to evaluate the effect of aeration and agitation on the degradation of organic substance. The physico-chemical and bacteriological parameters were determined every 2, 4, 6, 8 and 24 hours. After preliminary essays, we chose the treatment by 24 hours due to the best performance of treatment. The pH of synthetic medium was adjusted to 7 at the beginning of essays by adding NaOH (0.5 N) or H_2SO_4 (5 N) to meet the conditions of neutrality.

Table 1- Composition of synthetic tannery effluent

Composition of Synthetic	Tannery Effluent	Composition of Trace Elements	
Glucose	3.64 g l^{-1}	$FeCl_3,H_2O$	1.5 g l^{-1}
$(NH_4)_2SO_4$	0.91 g l^{-1}	H_3BO_3	0.15 g l^{-1}
$MgSO_4,7H_2O$	0.025 g l^{-1}	KI	0.03 g l^{-1}
$FeSO_4,7H_2O$	0.02 g l^{-1}	$MnCl_2,4H_2O$	0.12 g l^{-1}
KH_2PO_4	0.088 g l^{-1}	Na_2MO_4,H_2O	0.06 g l^{-1}
K_2HPO_4	0.09 g l^{-1}	$ZnSO_4,7H_2O$	0.12 g l^{-1}
Na_2CO_3	0.066 g l^{-1}	$CaCl_2,6H_2O$	0.15 g l^{-1}
$NaHCO_3$	0.105 g l^{-1}	$CuSO_4,5H_2O$	0.03 g l^{-1}
$CaCl_2$	0.03 g l^{-1}	-	-
KCrO4	1.86 g l^{-1}at a concentration of 500 mg l^{-1} total chromium	-	-

Fig. 1- Photo of the reactor at laboratory scale

Physicochemical and Microbiological Analysis

The synthetic wastewater of tanneries was conducted by three kinetic strains for each question for each kinetic study in fact physicochemical and microbiological analyzes that are based primarily on the determination of total chromium and COD_T and bacterial growth. The samples were taken during treatment every 2, 4, 6, 8 and 24 hours.

Chemical Oxygen Demand (COD_T)

The determination of Total Chemical Oxygen Demand COD_T was performed by the method of potassium dichromate. The optical density of the sample was obtained by a spectrophotometer at a wavelength of 585 nm. COD_T values are measured using a spectrophotometer type UV/ Visible brand Jenway 6405 [14].

Determination of Total Chromium

Elemental analysis of samples of total chromium was carried out by plasma emission spectrometry inductively (ICP-AES).

Microbiological Analyzes

These analyzes are based mainly on the determination of microbial growth before and after the launch of the reactor in every 2, 4, 6.8 and 24 hours.

Statistical Analysis

Due to the partition of observations into classes, we used Factor Analysis (CVA) which is a descriptive and explanatory statistical technique, akin to principal component analysis (PCA). This is a linear analysis which leads to a generalized principal component analysis on the individual class-centroids [15]. We used the software XLStat-Pro Addinsoft leads to a graphical representation

where, as in PCA, the correlation circle represents the factorial design with initial observations and other spraying discriminant classes in the system of factor axes to visualize the quality of discrimination. The study of Correlation Between biomass and the concentration of total chromium and COD_T and directed by excel 2007.

Results and Discussion

Molecular Identification of Isolated Strains

To determine the taxonomic position of some bacteria isolated from effluents of tanneries, we amplified and sequenced 16S rDNA. These sequences were compared to sequences of database. The program used is BLASTN 2.2.14 of the National Center for Biotechnology Information (NCBI). The results are as a percentage of identity [Table-2]. The molecular definition of gender states is that the homology of the 16S rDNA should be greater than or equal to 97%.

Table 2- Strains identification and percentage of homology

Isolates	Species	% of homologye
BT	Bacillus thuringiensis	99%
BP	Bacillus pumilus	98%
BC	Bacillus cereus	98%

Study of Changes in Bacterial Growth

The effluent to be treated is a synthetic wastewater simulating that of the tanneries of Fez, whose chemical composition has a minimum concentration of COD_T of 4 g l^{-1}.

Survival of Strains in Synthetic Effluent at 4 g l^{-1} of COD_T

We determined the evolution of bacterial growth in the bioreactor over time. The results found are shown in [Fig-2].

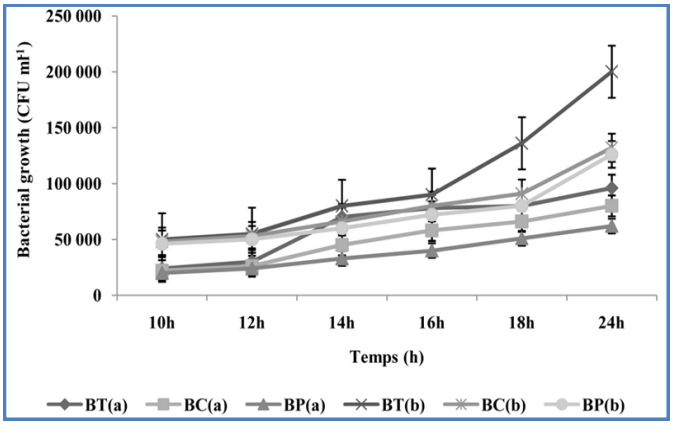

Fig. 2- Change in bacterial growth at 4 g l^{-1} of COD_T to 2% (a) and 4% (b) of the bacterial inoculum

The growth of microorganisms in the tannery synthetic effluent was monitored for 24 hours. The growth of microorganisms is maximum at T = 24 h, especially in the middle with a concentration of 4 g l^{-1} of COD_T and 4% of the bacterial strain [Fig-2]. Overall, the inoculation of *Bacillus thuringiensis* is the most effective for. The number of cells at a concentration of 4 g $l^{-1}$of COD_T and 2% of the bacterial strain at T_0 was 24×10^4 CFU ml^{-1}, 22×10^4 CFU ml^{-1} and 20×10^4 CFU ml^{-1}, respectively, for *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus* . While at T = 24 h, abundances reached 96×10^5 CFU ml^{-1} for *Bacillus thuringiensis* higher than that of *Bacillus cereus* 80×10^5 CFU ml^{-1} and that of *Bacillus pumilus* 62×10^5 CFU ml^{-1}. For treatment of synthetic effluent with a concentration of 4 g l^{-1} of COD_T and 4% of the bacterial strain [Fig. 2], bacterial density at T_0 was 50×10^4 CFU ml^{-1} for *Bacillus thuringiensis*, CFU 48×10^4 ml^{-1} for *Bacillus cereus* and 46×10^4 CFU ml^{-1} for *Bacillus pumilus*. After 24 hours, the growth increased to 200×10^5 CFU ml^{-1}, 132×10^5 CFU ml^{-1} and 126×10^5 CFU ml^{-1} respectively for *Bacillus thuringiensis*, *Bacillus cereus* and *Bacillus pumilus*.

The growth of these three bacteria in synthetic effluents may be due to chromate resistance by a chromosomal mutation of the transport system of sulfate [16]. CrO_4^{2-} ions can penetrate into cells by the same carrier enzyme as the SO_4^{2-} ions: sulfate permease. When the sulfate transport system is mutated, the sulfate ions cannot penetrate, nor chromate ions which can not damage the cell. there will be in this case a resistant phenotype. This resistance mechanism has been described in *Salmonella typhimurium* [16] and *Streptomyces coelicolor* [17].

Survival of Strains in Synthetic Effluent at 8 g l^{-1} of COD_T

The change in bacterial growth throughout the reactor operation during 24 hours shows that the growth of bacteria in a synthetic effluent concentration of 8 g l^{-1} of COD_T and 2% of the bacterial strain after 24 hours for *Bacillus thuringiensis* was 110×10^5 CFU ml^{-1}, 96×10^5 CFU ml^{-1} for *Bacillus cereus* and 84×10^5 CFU ml^{-1} for *Bacillus pumilus* [Fig-3].

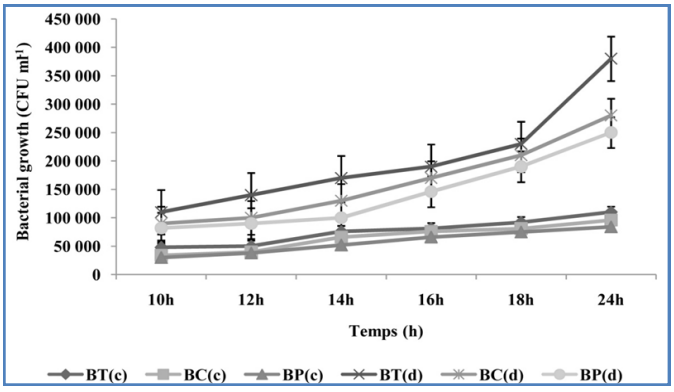

Fig. 3- Variation de la croissance bactérienne à 4 g l^{-1} de la DCO_T à 2% (c) et 4% (d) de la souche bactérienne

While the number of bacteria to 8 g l^{-1} of COD_T and 4% of the bacterial strain increases after 24 hours [Fig-3], it is of 380×10^5 CFU ml^{-1}, 280×10^5 CFU ml^{-1} and 250×10^5 CFU ml^{-1} respectively for *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus*. When inoculating a fresh culture medium, the cells do not multiply immediately. This period represents the lag phase. During this phase, bacteria carry out the synthesis of new cellular components (enzymes, cofactors, ribosomes beginning of DNA replication, etc.).

The duration of this period is variable and depends on several factors such as age of the inoculums temperature or the composition of the new environment. Following this period, the bacteria begin to multiply. They fall within the exponential phase. During this phase, the rate of bacterial growth is maximal. The bacteria split at a speed and at a regular interval. This phase has some features for the study of microbial species. Indeed, the population is homogeneous and each cell has almost the same chemical physiological and properties. The population then reached the stationary phase, where growth is slower [18]. We can explain this growth to increased organic matter (8 g l^{-1} of COD_T) and the bacterial concentration (4% of the bacterial strain) that contribute to a significant reduction of chromium (VI). Thank to their power and property chelating redox, organic matter is a redox active (AHox / AHred E0 = 0.7 mV) [19-21].

Organic Matter Elimination

Study of the Variation in the Concentration of COD_T at an Initial Organic Matter Load of 4 g l^{-1}

The evolution of COD_T depending on the concentration of bacterial inoculum is shown in [Fig-4].

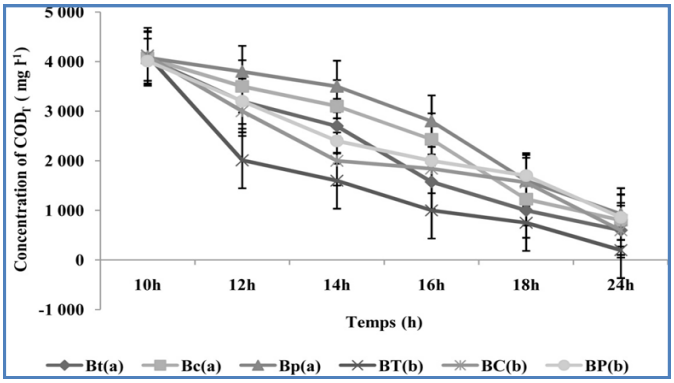

Fig. 4- Variation of the concentration of COD_T to 4 g l^{-1} of COD_T to 2% (a) and 4% (b) of the bacterial strain

The results obtained in the case of an inoculum of 2% showed the weakest performance since the concentration of COD_T after 24 hours of treatment was 600 mg l^{-1} for *Bacillus thuringiensis*, 800 mg l^{-1} for *Bacillus cereus* and 930 mg l^{-1} for *Bacillus pumilus*. Treatment with 4% of inoculum showed a strong reduction in COD_T to 200 mg l^{-1}, 600 mg l^{-1} and 830 mg l^{-1} respectively for *Bacillus thuringiensis*, *Bacillus cereus* and *Bacillus pumilus*. From these results, we can conclude that increasing the concentration of the bacterial strain promotes the elimination of carbon pollution and that the reduction of COD_T highly depends on the concentration of the bacterial strain. Indeed, *Bacillus thuringiensis* was the most performant when the inoculum doubled, followed by *Bacillus cereus* and *Bacillus pumilus.*. Therefore, sufficient time is needed for the degradation and assimilation of organic matter by micro-organisms in aerobic conditions [22,23].

Study of the Variation in the Concentration of COD_T at an Initial Organic Matter Load of 8 g l^{-1}

Two concentrations of bacteria were tested 2% and 4% at an initial concentration of COD_T of 8 g l^{-1} to study the performances of the three bacteria on the reduction of the concentration of tanneries synthetic sewage COD_T. The results are shown in [Fig-5].

By increasing the concentration of COD_T to 8g l^{-1}, the elimination of COD_T decreased. The concentration of synthetic sewage COD_T of tanneries to 8 g l^{-1} and 2% strain showed a decrease in COD_T during 24 hours of treatment with bioaugmentation of 8101 mg l^{-1} to 1400 mg l^{-1} for *Bacillus thuringiensis* with an elimination rate of 82.71% from 8056 mg l^{-1} to 2000 mg l^{-1} for *Bacillus cereus* with a removal rate of 75.17%, and from 8102 to 2400 mg l^{-1} with a rate of elimination of 70.37% for *Bacillus pumilus*. By contrast, treatment with a rate of 4% bacteria gave a reduction of COD_T of 8010 mg l^{-1} to 1200 mg l^{-1} with a removal rate of 85% for *Bacillus thuringiensis*, to 8000 mg l^{-1} to 1600 mg l^{-1} with a removal rate of 80% for *Bacillus cereus* and 8011-1900 mg l^{-1} with a removal rate of 76.28% for *Bacillus pumilus*. We can explain these results COD_T elimination of these bacteria by the fact that in the reactor, the organic substance present in the synthetic sewage is oxidized by bacteria. Breathing the oxygen produce energy necessary for bacteria that regenerate stocks of organic substance and grow. The biological removal of organic substance is related to reabsorption of this substance more important than the release [24].

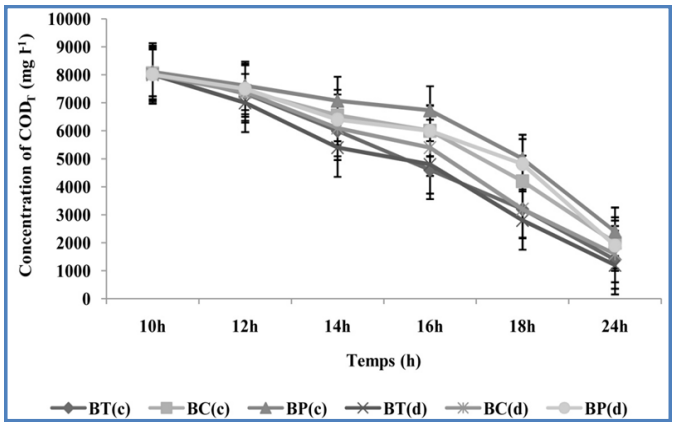

Fig. 5- Variation of the concentration of COD_T to 8 g l^{-1} of COD_T to 2% (c) and 4% (d) of the bacterial strain

Comparative Study of the Variation in Removal Rates of COD_T

The quality of effluent leaving the reactor after treatment with bio-augmentation in terms of COD_T is summarized in [Fig-6].

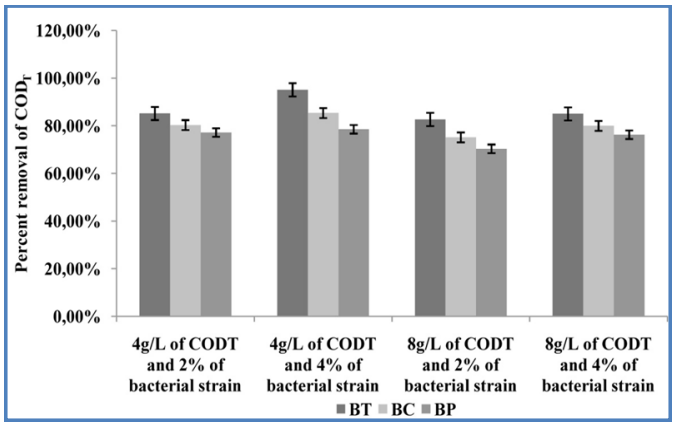

Fig. 6- Comparative study of the variation in removal rates of COD_T after 24h of treatment

These results show that the synthetic effluent treated with initial loads of 8 g l^{-1} of COD_T and 2% of bacterial inoculum has an average performance, because the turnover rate in this case did not exceed respectively 82.7%, 75.17% and 70.37% for *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus*. However, for treatment with initial loads of 8 g l^{-1} of COD_T and 4% of bacterial inoculum, the rate of reduction of COD_T increased significantly and respectively of 85.02%, 80% and 76.28% for *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus*. With culture conditions of 4 g l^{-1} of COD_T and 2% of bacterial inoculum, the rate of elimination for *Bacillus thuringiensis* is 85.2%, 80.34% for *Bacillus cereus* and 77.2% for *Bacillus pumilus*. The highest removal rates in the COD_T are recorded in the treatment of a synthetic sewage of 4 g l^{-1} of COD_T and 4% of the bacterial strain. They are 95.14%, 85.4% and 78.55% for *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus*. Since the effluent treatment at 4 g l^{-1} of COD_T and 4% of the bacterial strain is marked by a higher reduction of the COD_T compared to 8 g l^{-1}. The introduction of a high concentration of COD_T does not improve the reduction. The increase in COD_T can cause high bacterial growth which can lead to competition between micro-organisms that can lead to decreased activity of the population reducing chromium.

From these results, we can conclude that the increase of bacterial inoculum, the decrease of the tanneries synthetic sewage COD_T and the duration of the treatment promote the elimination of carbon pollution, in other words, these data show that abatement of COD_T is highly dependent on the concentration of bacteria, organic substance (COD_T) as well as processing time. In fact, enough time is needed for the degradation and assimilation of organic matter by micro-organisms in aerobic conditions [22,23].

Study of the Variation in the Concentration of Total Chromium

Study of the Change in Total Chromium Concentration for a Load of 4 g l^{-1} of COD_T

In the reactor inlet, the concentration of total chromium is 1000 mg l^{-1} for the concentration of tanneries effluent of 4 g l^{-1} of COD_T, 2% and 4% of the strains. At the exit of the reactor, the concentration of synthetic sewage at a concentration of 4 g l^{-1} of COD_T and 2% of bacterial inoculum is 150 mg l^{-1}, 178 mg l^{-1} and 200 mg l^{-1} respectively for *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus*, as shown in [Fig-7].

After the increase of the bacterial inoculum to 4%, the concentration of total chromium in the reactor outlet decreased and reached 120 mg l^{-1}, 160 mg l^{-1} and 190 mg l^{-1} respectively for *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus* as shown in [Fig-7].

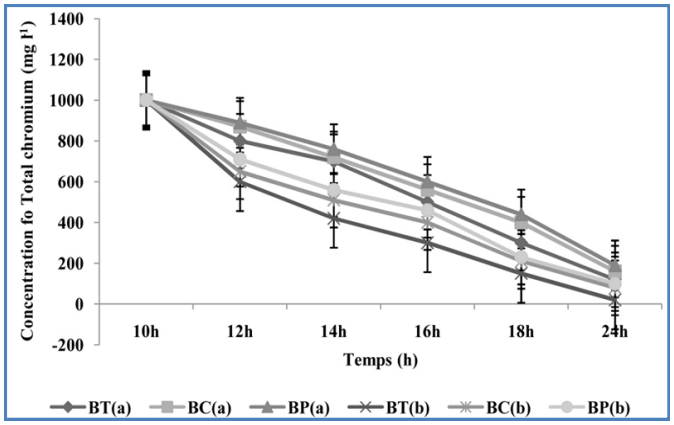

Fig. 7- Variation of the concentration of total chromium to 4 g l^{-1} of COD_T 2% (a) and 4% (b) of the bacterial strain

According to the obtained results, increasing concentrations of bacteria led to a decrease in the concentration of total chromium in tannery synthetic effluent. This can be explained by the fact that increasing concentrations of bacteria increases the rate of fixation of total chromium of synthetic sewage by reactor's microorganisms. Some authors suggest that tolerance to a high concentration of metals in solution have certain microorganisms is associated with the presence of extrachromosomal DNA or plasmid [25]. The metal binding properties of Gram-positive bacteria, are largely due to the existence of specific anionic polymers in the cell wall structure, consisting mainly of peptidoglycan teichoic or teichuronic acids [26-28].

Study Variation in the Concentration of Total Chromium for a Load of 8 g l^{-1} of COD_T

The [Fig-8] shows the evolution of the concentration of total chromium in the synthetic effluents of tanneries depending on time.

For the three bacterial strains tested, we found that the removal of total chromium decreases when reducing the concentration of bacteria to 2% after 24 hours of treatment concentrations of 100 mg l^{-1}, 120 mg l^{-1} and 150 mg l^{-1} respectively for *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus*. Treatment with 8 g l^{-1} of COD_T and 4% of bacterial inoculum showed a slight increase in treatment efficiency. The concentrations of total chromium after 24 hours of treatment were 80 mg l^{-1}, 100 mg l^{-1} and 140 mg l^{-1} respectively for *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus*. We can explain these findings by the fact that these bacteria possess proteins that have a high affinity for chromium metal. These proteins bind specifically to the ionic form of the metal, hence, trapping it inside the cell. So to withstand Cr (VI), there are three main strategies: prevent Cr (VI) to enter the cell (mutation of the transport system of sulfate), remove it out of the cell or reduce it Cr (III) [29]. Among all the tested strains, *Bacillus thuringiensis* seemed to have the highest tolerance against Cr(III), followed by Pb(II), Cu(II) and Cd(II) [30].

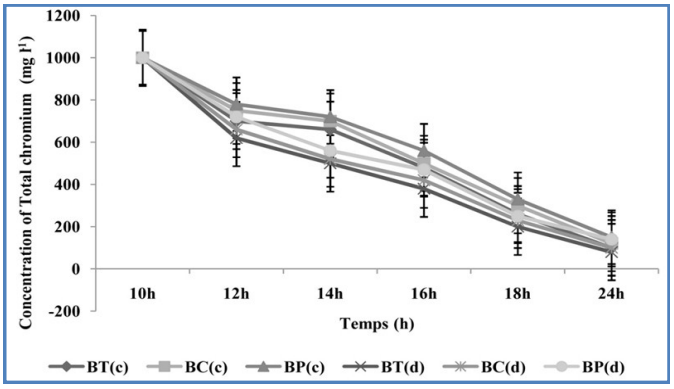

Fig. 8- Variation of the concentration of total chromium to 8 g l^{-1} of COD_T to 2% (c) and 4% (d) of the bacterial strain

Comparative Study of the Variation in Removal Rates of Total Chromium

The [Fig-9] shows the rate of removal of total chromium concentrations according to the COD_T and strains.

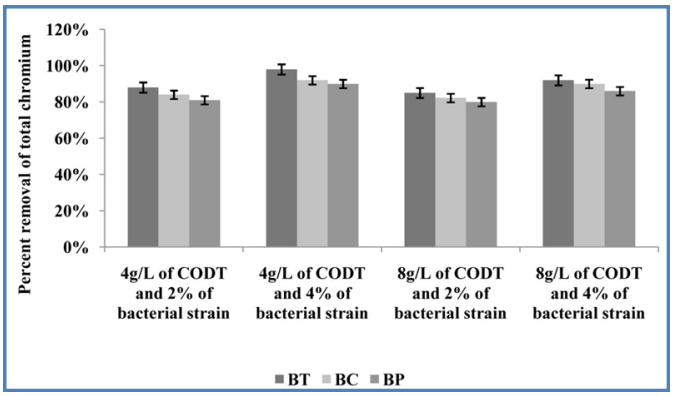

Fig. 9- Study of the variation in the rate of total chromium removal

The results obtained in the case of effluent concentration of 8 g l^{-1} of COD_T and 2% of bacterial inoculum show the weakest performance since the average rate of reduction of total chromium did not exceed 85%, 82.2% and 80% for *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus,* respectively. Effluent treatment with a concentration of 8 g l^{-1} of COD_T and 4% of bacterial inoculum showed a slight increase in treatment efficiency to achieve reduction rates of chromium of 92%, 90% and 86% for *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus,* respectively. By reducing the concentration of COD_T to 4 g l^{-1} and increasing the rate of bacterial inocu-

lum to 4%, the removal of total chromium was of 98% for *Bacillus thuringiensis,* 92% for *Bacillus cereus* and 90% for *Bacillus pumilus*. At a concentration of COD_T of 4 g l^{-1} and 2% of the bacterial inoculum, the rate of reduction of chromium is 88%, 84% and 81% for *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus,* respectively.

From these results, we can conclude that increasing the concentration of bacteria and reducing the COD_T, promote the elimination of pollution of chromium which increases the number of proteins able to bind chromium on bacteria. In literature, several mechanisms have been reported concerning resistance of microorganisms to heavy metals. For example, metals can bind to the cell wall without penetrating inside the cell. Transport across the plasma membrane can be reduced and a system of active transport of metals into the interior of the cell may appear. Heavy metals may be trapped in vacuoles, or they can be sequestered by complexation due to the action of proteins such as metallothionein [31,32].

Elimination Kinetics

Correlation Between Biomass and the Concentration of Total Chromium and COD_T

The results show a correlation between bacterial biomass and the concentration of total chromium and COD_T [Fig-10], [Fig-11] and [Fig-12]. The application of the first order kinetic equation aX + b, showed that the removal of total chromium and COD_T follows this model to all concentrations with coefficients of determination R^2 acceptable from 0.84 to 0.98 [Fig-10], [Fig-11] and [Fig-12]. The microbiological results also revealed that there is a clear inverse correlation between bacterial growth and the concentration of total chromium and COD_T, showing that treatment with bioaugmentation is performant.

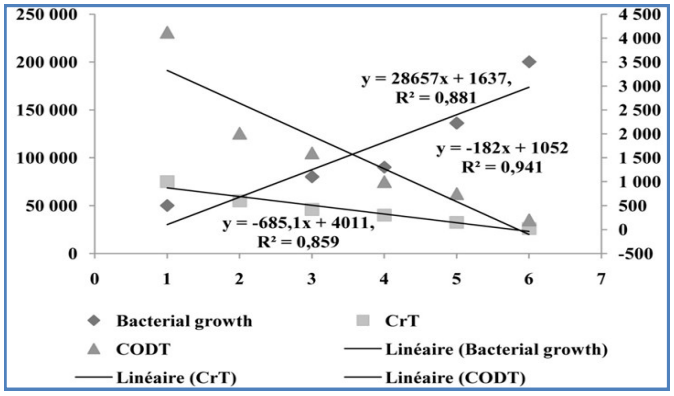

Fig. 10- Growth kinetics of bacteria elimination BT and Cr_T

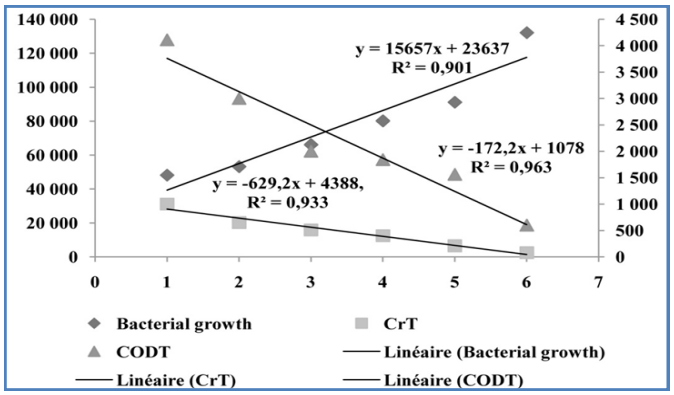

Fig. 11- Growth kinetics of bacteria elimination BC and Cr_T

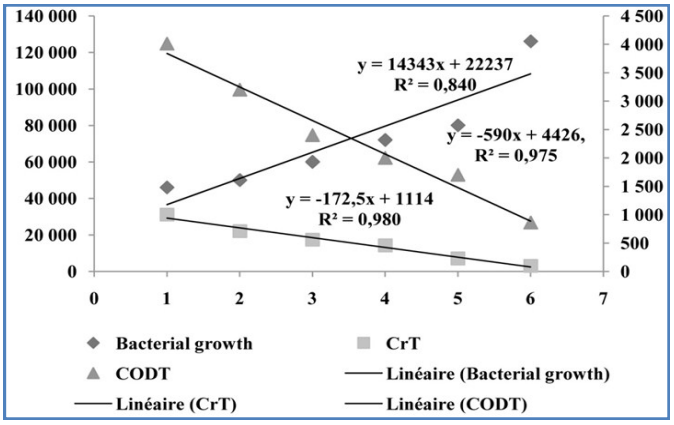

Fig. 12- Growth kinetics of bacteria elimination BP and Cr_T

Factor Analysis Discriminate of the Groups Depending on the Concentration of COD_T and Bacterial Strains

The data are quantitative variables, also called observations, measured for each physicochemical parameter, (bacterial growth, COD_T and Cr_T). These quantitative variables are divided into two classes, time and the concentration of organic matter of the synthetic sewage, which are qualitative variables. The discriminate factor analysis allowed to discriminate three large groups [Fig-13].

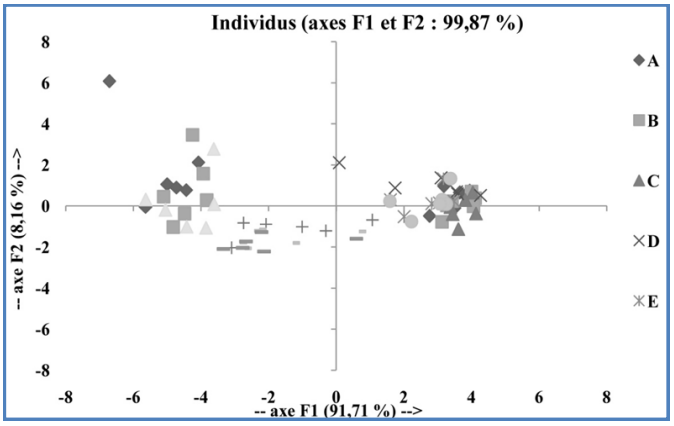

Fig. 13- Factor analysis discriminate of the groups depending on the concentration of COD_T and bacterial strains

i) composed of six sub-groups (A, B, C, D, E, F) which are characterized by maximum growth rates and removal performance of the Gr_T and COD_T especially for the three subgroups D F, G.

ii) composed of three sub-groups (G, H, I) characterized by the lower growth and removal performance of the Cr_T and COD_T is lower.

iii) composed of three sub-groups (J, K, L) characterized by bacterial growth more elves a sub-group (G, H, I) and in lower concentrations of Cr_T and COD_T

The bacterial strains BT, BC and BP have a relatively similar growth profile when incubated with low concentrations of COD_T (group I). The bacterial strains BT, BC, BP respond to the addition of COD_T by increasing their growth (group II).

The bacterial strains BT, BC, BP respond to the addition of COD_T concentrations and bacterial strains by increasing their growth (group III).

They all respond to the addition of COD_T concentrations and bacterial strains by increasing their growth for all groups I, II and III.

Factor Analysis Discriminate of the Groups According to Time

The discriminate factor analysis can well discriminate groups according to time and there is 5 main groups [Fig-14].

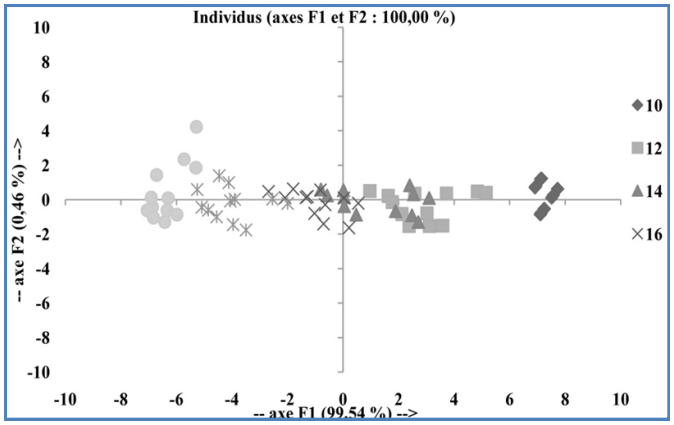

Fig. 14-Factor analysis discriminate of the groups according

Group 1- containing the subgroup (10) this confirms that at this time the different strains have little to take the same growth behavior whatever the concentration of COD_T and the bacterial strain.

Group 2- consists of two subgroups (12h and 14h). After 12 h all strains have the same growth behavior, but after 14h strains grade the same growth behavior that after 12h.

Group 3- two subgroups (14h, 16h). All strains change some behavior of the concentration of COD_T and the bacterial strain.

Group 4- Subgroup one (18h): strains of the elimination of exchange COD_T and growth of the bacterial strain.

Group 5- a single subgroup (24h): the strains change some behavior of the concentration of COD_T and the bacterial strain.

The addition of COD_T and the concentration of the bacterial strains accelerate bacterial growth for the three strains.

We conclude that the highest removal rates of COD_T are recorded after 24 hours of treatment of the synthetic sewage. The removal rates are of 95.14%, 85.4% and 78.55% for *Bacillus thuringiensis, Bacillus cereus* and *Bacillus pumilus,* respectively. The removal of total chromium was of 98% for *Bacillus thuringiensis,* 92% for *Bacillus cereus* and 90% for *Bacillus pumilus* after 24 hours of treatment while previous studies report that Chromium resistant bacteria of *Bacillus sp*. JDM2-1 and *Staphylococcus capitis* could reduce 85% and 81% of hexavalent chromium from the medium after 96 h and were also capable of reducing hexavalent chromium 86% and 89%, respectively, from the industrial effluents after 144 h. Cell free extracts of *Bacillus sp*. JDM-2-1 and *Staphylococcus capitis* showed reduction of 83% and 70% at concentration of 10 µg Cr(VI)/ml, respectively [9]. Therefore, treatment with bioaugmentation using three bacteria gives a removal rate very significant after only 24 h of treatment what shows the performance of this treatment method.

Conclusion

Biaugmentation treatment must be a solution to the treatment of tannery effluent. Indeed, this treatment was highly effective for treatment of tannery synthetic effluent with high purification yields. The results obtained after treatment with bioaugmentation show that the performance of the process significantly increases with decreasing the concentration of organic matter of the synthetic effluent to 4 g l^{-1} of COD_T and increasing concentrations of bacteria to 4%. Indeed, this process has shown a highly effective treatment of synthetic tannery effluent with very high treatment efficiency for the bacterium *Bacillus thuringiensis*. It was 95% for COD_T and 98% for total chromium. The removal rates for *Bacillus thuringiensis* at a concentration of COD_T of 4 g l^{-1} and 2% of the bacterial strain was of 88% and 85% for total chromium and COD_T, respectively. The results obtained in the case of effluent organic matter concentration of 8 g l^{-1} of COD_T show the weakest performance since the average turnover rate for *Bacillus thuringiensis* did not exceed 85% for total chromium and 82.7% for COD_T. Throughout the operation of the reactor for 24 hours, we noticed a gradual increase in the pH for the three bacteria over time. According to the microbiological study, especially in the middle with a concentration of 4 g l^{-1} of COD_T and 4% of the bacterial strain. Overall, *Bacillus thuringiensis* was the most performant strain.

Acknowledgement

We thank all those who contributed directly or indirectly in the development of this work in particular: Laboratory of Biotechnology, Faculty of Sciences Dhar El Mahraz, Fès, Morocco.

References

[1] Belkhiri M. (2007) *Revue HTE,* (137), 14.

[2] Lahlou A. (2011) *A.D-ISESCO,* Edition 1421H/2000, 100.

[3] Patterson J.W. (1985) *Butterworth Publishers, Stoneham, MA*, 512.

[4] Aksu Z., Kutsal T., Gun S., Haciosmanoglu N. and Gholaminejad M. (1991) *Environmental Technol*ogy., (9), 15-21.

[5] Volesky B. (1990) *Boca Raton, CRC press*, 3-5.

[6] Donmez G. and Aksu Z. (2001) *Water Research*, 35(6), 1425-1434.

[7] Stephenson T., Judd S., Jefferson B. and Brindle K. (2000) *IWA Publishing*, 1-176.

[8] Durai G. and Rajasimman M. (2011) *Journal of Environmental Sciences*, 4(1), 1-17.

[9] Zahoor A. and Abdul R. (2009) *Journal of Environmental Sciences,* (21), 814-820.

[10] Roane T.M., Josephson K.L. and Pepper I.L. (2001) *Applied and Environmental Microbiology*, 67(7), 3208-3215.

[11] Seyoum L., Fassil A. and Gunnel D. (2005) *Journal of Microbiology and Biotechnology*, 545-552.

[12] Woese C. R. And Kandler O. (1990) *Proceedings of the National Academy of Sciences*, (87), 4576-4579.

[13] Weisburg W.G., Barns S.M., Pelletier D.A. and Lane D.J. (1991) *Journal Bacteriology*, (173), 697-703.

[14] Rodier J., Bazin C., Broutin J.P., Chambon P., Champsaur H. and Rodi L. (1996) *8éme édition. Dunod*, 1383.

[15] Saporta G. (1990) *Celeux ed. INRIA*, 5-13.

[16] Ota N., Galsworthy P.R. and Paradee A.B. (1971) *Journal bacteriology*, 105, (3), 1053-1062.

[17] Lydiate D J, Mendez C., Kieser H.M. and Hopwood D.A. (1988) *Molecular and General Genetics*, (211), 415-423.

[18] Picher S. (2002) *Hydrometallurgy*, 65,(3),177-186.

[19] Bartlett R.J. and Kimble J.M. (1976) *Journal of. Environmental Quality*, (5), 383-386.

[20] Alloway B. J. (1995) *2nd edition, Blackie Academie et Professional*.

[21]Bruce R., John C., Rock J. and George R., (1995) *Environmental Science Technology*, *29* (9), 2377-2381.

[22]Yéo T. M. (2008) *European Journal of Scientific Research*, 24 (2), 187-196.

[23]Troyes C. (2002) *Action A10, EIER-ETSHER*, 2 (31).

[24]Deronzier G and Choubert J. M. (2004) *FNDAE, Edition Cemagref, Antony*, (29), 24.

[25]Leduc L. G. and Ferrom G. D. (1994) *FEMS Microbiological Reviews*., (14), 103-120.

[26]Hancock I. C. (1986) *journal of Chemical physics*. 84 (4).

[27]Hughes M. N. and Poole R. K. (1989) *Chapman and Hal*, 328-395.

[28]Yasemin S., Ayten O. (2005) *Process Biochemistry*, (40), 1895-1901.

[29]Desjardin V. (2002) *Waste Management*, (22), 195-200.

[30]Zhuang L., Zhou S., Wang Y., Liu Z. and Rongxian X. (2011) *Bioresource Technology*, (102), 4820-4826.

[31]Tuovinen O.H., Niemela S.I. and Gyllenberg H.G. (1971) *Antonie Van Leeuwenhoek*, (37), 489-496.

[32]Gimmler H., Dejesus J. and Greiser A. (2001) *Microbial. Ecology*, (42), 87-98.