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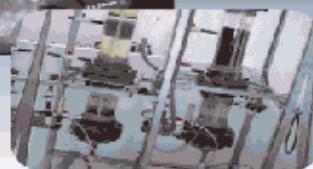
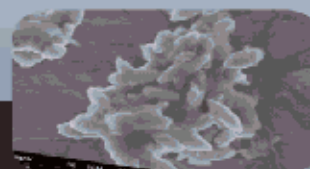
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# “BIOINGENIERÍA AMBIENTAL”

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## ÍNDICE

### LA PARTICIPACIÓN DE LA UNIVERSIDAD PÚBLICA EN EL DESARROLLO COMUNITARIO

Felipe Macías Gloria, Patricia Campos Rodríguez y Eloy Juárez Sandoval..... 1

### BIOSORPTION OF CU (II) AND PB (II) IN AQUEOUS SOLUTIONS USING PACKED COLUMNS WITH BIOSOLIDS (B) AND PYROLYSIS DERIVED BIOCHAR (BC)

Ortiz-Prieto Jorge A.<sup>1,2</sup>, Acosta-Slane Damaris<sup>1</sup>, Lozoya-Márquez Luis A.<sup>1</sup>, Gómez-Vargas  
Ramón<sup>1</sup>, González-Sánchez Guillermo<sup>1</sup>..... 14

### CARACTERIZACIÓN DE UN DESECHO AGROINDUSTRIAL MEXICANO PARA SU EMPLEO COMO MATERIAL PUZOLÁNICO

Víctor Jiménez-Quero<sup>□</sup>, Pedro Montes-García..... 23

### DESARROLLO DE UN BIOPROCESO ANAEROBIO PARA EL TRATAMIENTO DE AGUAS RESIDUALES DE LA INDUSTRIA LÁCTEA Y LA GENERACIÓN DE BIOGÁS

Luz Brenda Montserrat Crespo, Arodí Bernal Martínez y Germán Cuevas Rodríguez ..... 30

### REMOCIÓN DE HIERRO DISUELTU EN AGUA UTILIZANDO PET MODIFICADO QUÍMICAMENTE COMO AGENTE ADSORBENTE

T. V. Cervantes Melesio<sup>3</sup>, F. A. Horta Rangel<sup>3</sup>, M. A. Ramírez Morales<sup>1</sup>, G. Cruz Jiménez<sup>1</sup>,  
R. Navarro Mendoza<sup>2</sup> U. Morales Álvarez<sup>3</sup> <sup>□</sup>..... 37

### SECADO DE BIOMASA ALGAL EN SECADOR SOLAR

Moreno Funes José Saul, Davalos Navarrete Siikmine, Valle Moreno Andrés, Cervantes  
Torre-Marín Gemma\* ..... 44

### ESTIMACIÓN RESPIROMÉTRICA DEL RENDIMIENTO HETERÓTROFO DEL MODELO ASM1 PARA UNA PTAR EN CHIAPAS

Valeria Zuarth Coutiño<sup>1</sup>, Cristina Blanco González<sup>1</sup>, Josué Chanona Soto<sup>1</sup> y Gustavo Yáñez  
Ocampo<sup>1</sup>..... 52

### CARACTERIZACIÓN DE CEPAS BACTERIANAS NATIVAS DE UN RESIDUO INDUSTRIAL HIPERSALINO E HIPERALCALINO, CON ALTO CONTENIDO DE CROMO Y OTROS METALES

Jesús Fernando López Vázquez<sup>1</sup>, Pamela Romo Rodríguez<sup>1</sup>, J. Felix Gutiérrez Corona<sup>1</sup> ... 57

### MODELADO MATEMÁTICO DE UN PROCESO DE HIDRÓLISIS ENZIMÁTICA PARA LA PRODUCCIÓN DE BIOETANOL

Javier Ulises Hernández-Beltrán<sup>1</sup>, Ivette Michelle Navarro-Gutierrez<sup>1</sup>, Karla Cervantes-  
Quintero<sup>1</sup>, Héctor Hernández-Escoto<sup>1</sup> <sup>□</sup>..... 63

ELABORACIÓN DE CELDAS SOLARES TIPO GRÄTZEL EMPLEANDO  
SENSIBILIZADORES DE DIFERENTE PROCEDENCIA

Mónica Cedillo Alaniz<sup>1</sup>□, Juan Carlos Baltazar Vera<sup>2</sup>, Rosalba Fuentes Ramírez<sup>3</sup> ..... 69

CÁLCULO DE EMISIONES DE GASES DE EFECTO INVERNADERO DE LA UNIDAD  
PROFESIONAL INTERDISCIPLINARIA DE BIOTECNOLOGÍA DEL IPN

Andrés Valle Moreno, Miguel Ángel Tapia Bustos, Cristina Ortega Nonoal, Gemma  
Cervantes Torre-Marín\* ..... 74

CONCRETE WITH RAW POLYETHYLENE TEREPHTHALATE

Luis Elias Chavez Valencia<sup>1</sup>, Claudia Hernandez Barriga<sup>2</sup>, Miguel Angel Manrique Ibarra<sup>3</sup>,  
Antonio Castro Lozano<sup>4</sup> ..... 83

BIOCOMPATIBILIDAD DE COMPOSITOS ÓSEOS - OSTEÓBLASTOS HUMANOS

M. Sabanero López<sup>1</sup>□, L. L. Flores Villavicencio<sup>1</sup>, Z. Miranda Rodríguez<sup>1</sup>, G. Barbosa  
Sabanero<sup>2</sup>, C. Piña Barba<sup>3</sup> ..... 86

COMPARACIÓN ENTRE UNA MEMBRANA PLANA Y UNA MEMBRANA DE FIBRA  
HUECA EN LA ELIMINACIÓN DE MACRONUTRIENTES PRESENTES EN AGUA  
RESIDUAL SINTÉTICA EN UN BIORREACTOR HÍBRIDO

Marco A. Silva, Germán Cuevas□ ..... 90

ALTERNATIVA PARA EL TRATAMIENTO DE AGUAS RESIDUALES DE LA INDUSTRIA  
TEXTIL EN LA REGIÓN SUR DEL ESTADO DE GUANAJUATO

J. Merced Martínez Rosales<sup>1</sup>, Miriam Rocío Contreras García<sup>1</sup>, Antonio Pérez Nieto<sup>2</sup> y  
Gabriela Arroyo Figueroa<sup>2</sup> ..... 97

PET MODIFICADO QUÍMICAMENTE COMO AGENTE ADSORBENTE DE MN(II) EN  
MEDIO ACUOSO

M. M. Marmolejo Lara<sup>2</sup>, L. Arroyo Álvarez<sup>1</sup>, F. A. Horta Rangel<sup>2</sup>, M. A. Ramírez Morales<sup>1</sup>, G.  
Cruz Jiménez<sup>1</sup>, U. Morales Álvarez<sup>2</sup>□ ..... 102

RECICLAJE DE CELULARES POR SOLVÓLISIS PARA RECUPERAR METALES

Lorena Eugenia Sánchez Cadena<sup>1</sup>\*, Zeferino Gamiño Arroyo<sup>2</sup>, Mario Alberto González Lara<sup>3</sup>,  
Demetrio Quiroz Q.<sup>4</sup>, Oscar Coreño A.<sup>5</sup> ..... 109

REMOCIÓN DE CR(VI) EN BAJAS CONCENTRACIONES PRESENTE EN AGUA  
MEDIANTE EL EMPLEO DE BIOMASA DE ORIGEN NATURAL

Pablo Carmona Medina<sup>1</sup>, Juan Jesús Serafín Muñoz<sup>1</sup>, Francisco Agustín Vidó García<sup>1</sup>,  
Francisco Javier Acevedo Aguilar<sup>2</sup>, Leticia López Martínez<sup>2</sup> ..... 115

CARACTERIZACIÓN DE CEPAS BACTERIANAS NATIVAS DE RESIDUOS  
INDUSTRIALES CON ALTO CONTENIDO DE METALES

Chávez Elías Amelia Fabiola<sup>1</sup>, Romo Rodríguez Pamela<sup>1</sup>, Gutiérrez Corona J. Félix<sup>1</sup>□ ..... 121

DETERMINACIÓN DE PARÁMETROS DE DISEÑO EN SISTEMAS DE TRATAMIENTO DE AGUAS RESIDUALES	
Brett González <sup>1</sup> , Alejandra Cruz <sup>1</sup> .....	128
EVALUACIÓN DEL CRECIMIENTO DE <i>TRAMETES VERSICOLOR</i> EN PRESENCIA DE FURADAN®, BOSCALID (CANTUS)® Y QUINTACENO®	
Fátima Ojeda-Rodríguez <sup>1</sup> , Héctor G. Nuñez <sup>2</sup> , Blanca E. Gómez <sup>3</sup> , Noé Saldaña <sup>4</sup> , .....	
Graciela M. L. Ruiz-Aguilar <sup>1*</sup> .....	134
TRATAMIENTO VÍA FENTON DE AGUA RESIDUAL PROVENIENTE DE UNA INSTITUCIÓN EDUCATIVA	
Paola Abigail Martínez Aldape <sup>1</sup> , Carlos J. Escudero S. <sup>2</sup> .....	139
EFFECT OF THE WALNUT SHELL PECANERA IN GYPSUM	
Luis Elías Chávez Valencia <sup>1</sup> , Claudia Hernández Barriga <sup>2</sup> , Martín Alejandro Moreno Hernández <sup>3</sup> , Cesar Leonardo Ruiz Jaime <sup>4</sup> .....	144
ISOLATION OF SULFATE-REDUCING BACTERIA FOR POTENTIAL BIOREMEDIATION OF METAL-CONTAMINATED EFFLUENTS	
María Fernanda Pérez Bernal <sup>1*</sup> , Jéssica Jazmín Gómez Marmolejo <sup>1</sup> , Elcia M.S. Brito <sup>1</sup> , Germán Cuevas Rodríguez <sup>1</sup> .....	148
EVALUACIÓN DE UN CONSORCIO BACTERIANO Y UN EFLUENTE DE BIODIGESTOR ANAEROBIO PARA LA PRODUCCIÓN DE LOMBRICOMPOSTA	
Elsa A. Guerrero, Héctor G. Nuñez, Víctor Olalde-Portugal, Vicente J. Álvarez, Rafael Veloz Graciela M. L. Ruiz-Aguilar* .....	153
APLICACION OF MICROCULTURE FOR BACTERIAL ISOLATION FROM INDUSTRIAL RESIDUE CONTAMINATED BY HEXAVALENT CHROMIUM	
Mariana Pérez Medina <sup>1</sup> □, Carolina Alejandra Martínez Gutiérrez <sup>2</sup> □, Reyna Edith Padilla-Hernández <sup>3</sup> , Julio Cesar Valerdi Negreros <sup>1</sup> , Germán Cuevas Rodríguez <sup>4</sup> , Elcia M.S. Brito <sup>4</sup> □ .....	158
AISLAMIENTO DE BACTERIAS ANAEROBIAS DEL LAGO ALKALINO DEL CRÁTER DEL RINCÓN DE PARANGUEO	
Rivera Martínez, Laura Guadalupe <sup>1</sup> □, Cuevas-Rodríguez, Germán <sup>2</sup> , Malm Olaf <sup>3</sup> , Brito Elcia M. S. <sup>2</sup> .....	163
USING TILLANDSIA <i>USNEOIDES</i> AS BIOMARKER OF HEAVY METALS IN THE ATMOSPHERE: GUANAJUATO TUNELS	
Pedro Antonio Zárate-Santoyo <sup>1</sup> , Elcia M.S. Brito <sup>1</sup> , Adan Lino <sup>2</sup> , Rodrigo Meire <sup>2</sup> , Olaf Malm <sup>2</sup> , Joao P.M. Torres <sup>2</sup> , Germán Cuevas-Rodríguez <sup>1</sup> .....	169
ANAEROBIC BIOTRANSFORMATION OF HEXAVALENT CHROMIUM IN BATCH REACTORS	
Alba América Moreno González, Sergio Antonio Silva Muñoz, Elcia Souza Brito, Germán Cuevas Rodríguez, Arodí Bernal Martínez .....	175

## **ANAEROBIC BIOTRANSFORMATION OF HEXAVALENT CHROMIUM IN BATCH REACTORS**

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### **ABSTRACT**

Since its start, the chemical industry has generated residues which have been stockpiled in deposits and which behave as loci for contamination by toxic effluents and with a high content in heavy metals. The use of anaerobic bioreactors permits the biotransformation of Cr (VI) into Cr (III) from industrial leachate. In this type of systems one of the factors that play an important part in the reduction is the source of carbon (SC). In the present study, the effect of the SC was evaluated over the reduction in different concentrations (50, 100, 150, 200, and 300 mg Cr (VI)/L). Two types of SC were used, synthetic (SCS) and alternative (SCA). Since SCS were used (pyruvate, lactate, glycerol, acetate, and yeast extract) there were 100 percent reductions observed, for 50 mg Cr(VI)/L, in a time interval of 4 days, nonetheless these sources are expensive which is why an alternative was used using SCA a mixture of waste effluents: clarified from a waste sludge of a WWTP (CL) and waste lactose water (WLW), in a batch anaerobic system. For the mixture (CL+WLW and 50 mg Cr (VI)/L) 100% removal percentages were obtained in only 2 days.

### **INTRODUCTION**

The development of chemical industry has generated a large quantity of residues that are difficult to manipulate and treat; previously, the lack of normativity in the disposal of these residues permitted their accumulation in open air deposits, which with time have become a problem because of the production of toxic leachate with a high content of heavy metals an environmental migration; such is the case of a chemical industry in San Francisco del Rincón, Guanajuato Mexico. Industry produces and distributes chromium salts for tanning. With more than 60 years on the market, it presents an accumulation of old residues that go above 300,000 ton [1] and which constantly are found producing chemical leachates with a high concentration of hexavalent chromium [Cr (VI)]. The physicochemical processes are an alternative treatment for these effluents, but they are expensive and they present a high generation of chemical sludges.

Currently there are alternative biological treatments that have been studied and generated good results in the reduction of Cr (VI) into Cr (III), either under aerobic or anaerobic conditions. Bioreactors are inoculated with isolated microbial populations isolated from industrial deposits, these are capable of metabolizing organic and inorganic substances, intervening in the solution or precipitation of metals [2]; the activity and the growth of these populations, many times is limited by the disposition of the SC [3]. The operation with synthetic SC generates control, but also disadvantages in terms of costs, nonetheless, it is worth mentioning that residual effluents can be found in nature with a high content of nutrients, which are disposed of in the environment as residues and can be taken advantage of as natural SC.

### **METHODOLOGY**

The sampling of leachates which are rich in Cr (VI) was performed in a chemical industry in Guanajuato, Mexico; leachates were characterized physic-chemically and the metal content was analyzed in a certified laboratory.

The biotransformation of Cr (VI) was batch evaluated in anaerobic systems, using as an SC: pyruvate (P), glycerol (G), acetate (A), lactate (L) and yeast extracts (YE). These tests were

performed on serological 100 mL bottles. Each system contained a volume of 50 mL of water coming from a sample site. Different compositions of SC were evaluated: 1) PGALYE, 2) GALYE, 3) ALYE, 4 )LYE, and 5) YE. The concentration of each nutrient was 100 mM. Each of these media was prepared and 50 mg/L of Cr (VI) was added, without microorganisms and SC. Two SCA waste effluents were used: "clarified" (CL) and waste lactose water of a local cheese business (WLW) for SC a 50:50 v/v mixture was used (CL: WLW). In said sources, the reduction process was evaluated at different concentrations of Cr (VI) (50,100,150,200,250,300 mg/L) which came from the matrix of industrial leachate. The test samples were inoculated with a microbial proliferation consortium, while the control samples were treated without microorganisms. The tests were performed in serological 120 mL bottles. The conditions for the operation were: room temperature ( $25\pm 2^\circ\text{C}$ ) and constant agitation at 120 rpm. These tests were monitored daily over a period of 15 days in which the parameters analyzed were the concentration of Cr (VI) by the colorimetric method of diphenylcarbazide [4] and pH. Later the culture was performed on the plaque over trypticase soy agar (TSA) with the intent of quantifying the microorganisms present in test samples once the biotransformation of metal was achieved.

## RESULTS AND DISCUSSION

The leachate had a pH of 14 and 6810 mg/L of Cr (VI). In figure 2, the biotransformation of the metal through time is illustrated, and the effect of lactate as a donator of potential electrons is observed and corroborated [3, 5, 6].

This permits the total biotransformation of metal. For all treatments a reduction in the concentration of Cr (VI) was observed. The composition of SCS which most quickly reduces the concentration of 50 mg/L of Cr (VI) was LYE (91.3%) in a period of three days, while with the PAGALYE medium (which contains all of the SC) a reduction of 83% was achieved in the same time interval. With all of the SC tried, reductions of 100% were achieved in a period of 4 days, except for the YE medium, which achieved highest efficiency at 10 days (89%). This is due to the potential that compounds have to donate electrons in this medium; lactate and acetate are two nutrients that have been proven to generate this effect [5, 7].

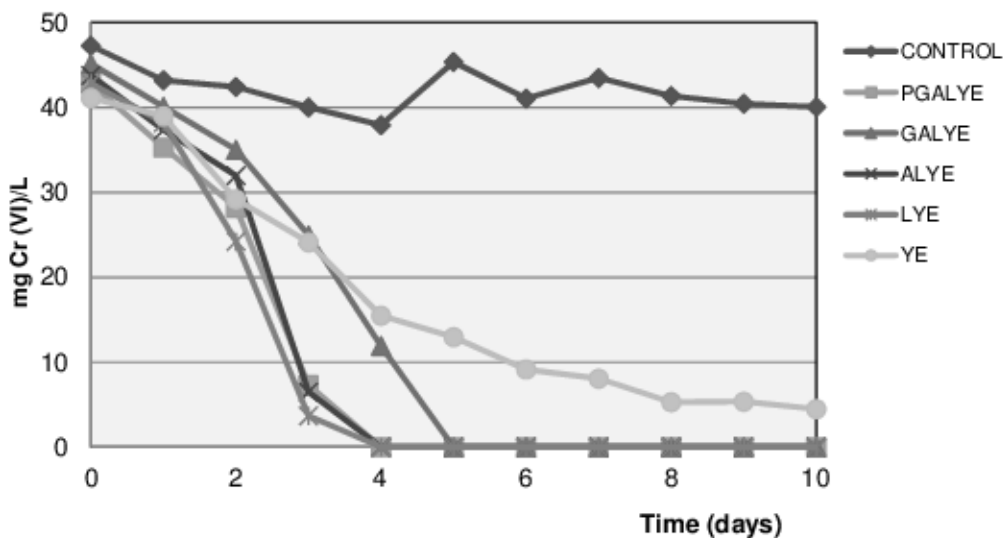


Figure 2. Behavior of the reduction of Cr (VI) in batch systems with different combinations of SC.



In this part it was concluded that lactate is an essential SC in systems for the reduction of Cr (VI), due to its high potential for the donation of electrons.

Figure 3 presents the behavior of the reduction of Cr (VI) in systems, the mixture of CL+WLW, for test samples presented a 99% reduction in 2 days for 50 mg Cr (VI)/L while for controls with the same concentration it presented only 1.2% reduction in a period of 14 days.

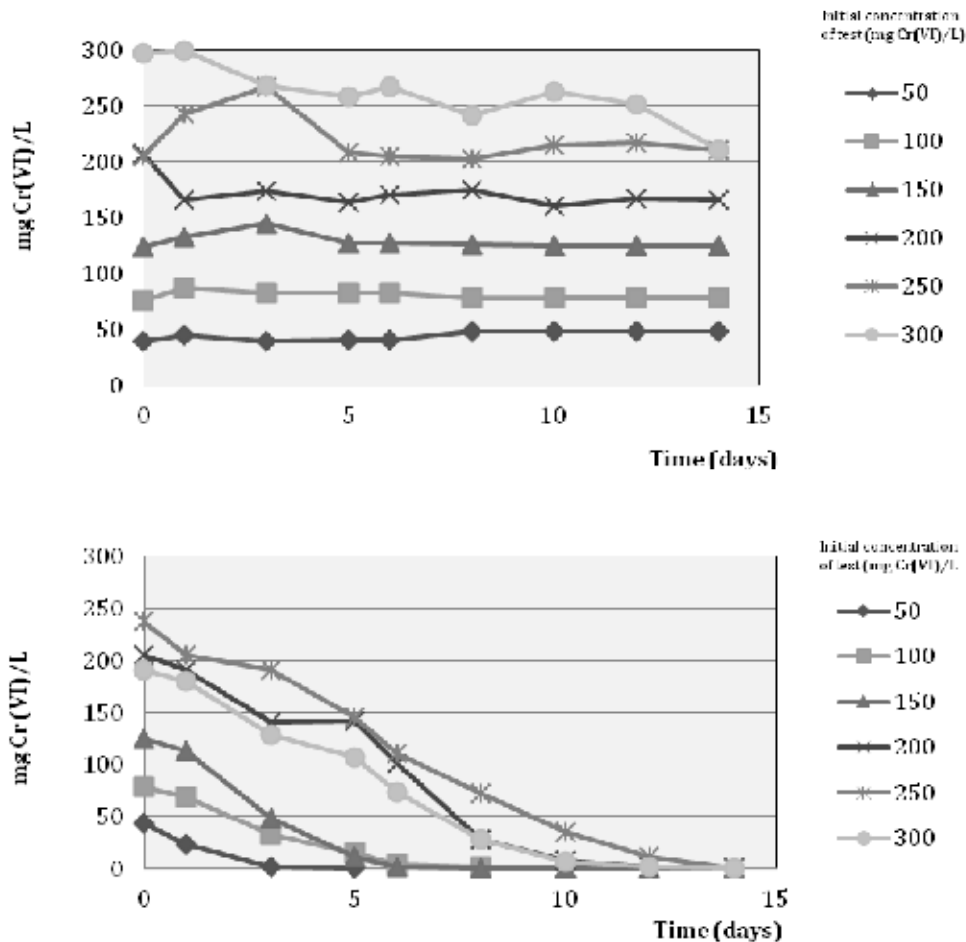


Figure 3. Effect of different doses of Cr (VI) using CL+WLW and a microbial consortium as SC. above (control samples), below (test samples).

For a mixture of CL+WLW, all test samples reached a 100% reduction of Cr (VI). The required time varied with the initial concentration of Cr in the system, while in control samples percentages of reduction were less than 3%.

From the previous results, SC is an important factor in the biotransformation of Cr (VI) such as is explained [3, 5, 7]. Lactate present in WLW presented a greater donation of electrons in the system without reducing the importance of biotransformation on the part of microorganisms. This can be observed clearly in tests with SC (CL+WLW at 300 mg Cr(VI)/L), while for the test samples, a 99% reduction was reached in 12 days, control samples only reached 29% over a period of 15 days.

With respect to the pH levels (table 1) it was observed that in systems with a combination of SC (CL+WLW) there is a considerable reduction in test samples that oscillates between 4 and 4.6 while in control samples of this same SC, the reduction in pH was not so evident. This can be because low pH levels (between 2-4) favor reactions of absorption/desorption of chromium ions and protonation/deprotonation of the cell walls. The increase of pH in the solution increases the negative charge on the cell surface because of the deprotonation of sites of metal unions [8].

Table 1. pH in the anaerobic systems for batch reduction of Cr (VI).

		Controls			Test samples		
		pH		Time	pH		Time
SC	mg Cr(VI)/L	Initial	Final	Days	Initial	Final	Days
CL+WLW	50	5.54	5.54	5	4.07	4.03	5
	100	6.36	6.4	8	5.9	4.2	8
	150	7.11	6.86	8	4.4	4.1	8
	200	7.4	7.31	12	7.4	4.4	12
	250	8.46	8.34	14	7.4	4.6	14
	300	9.0	8.65	14	9.5	4.6	14

Microbiological tests in plaques indicate that there was no reduction of growth, even though a slight reduction of CFU (colony forming unit) was observed in relation to the increment in the initial concentration of Cr (VI) in experimental systems (table 2). Gram stain of each test sample reveal that there are both gram<sup>+</sup> and gram<sup>-</sup> colonies.

The type of colonies presents are very similar and mostly present bacilli forms. On some occasions they join to allow diplobacilli and streptobacilli although they have also identified cocci, diplococci and coccobacillus.

Table 2. Biomass in the TSA plaques after reduction of Cr (VI) in the experimental samples.

mg Cr (VI)/L	50	100	150	200	250	300
CFU/mL	277X10 <sup>-3</sup>	156X10 <sup>-3</sup>	118X10 <sup>-3</sup>	61.3X10 <sup>-4</sup>	218X10 <sup>-4</sup>	151X10 <sup>-4</sup>

## 5. CONCLUSIONS

With this study we can conclude that the source of carbon is a very important factor in the biotransformation of Cr (VI) in anaerobic reactors.

Not all waste effluents present a reduction capacity (CL) and the availability of SC is another variable that should be considered (WLW); therefore it is considered convenient to use mixtures, since they present a higher percentage of reduction in batch systems (CL+WLW) for 300 mg Cr (VI)/L, reduction of 99% in 12 days while control samples only reached 29.2% in 14 days. The reduction time increased with the amount of heavy metals in the systems.

The pH in the system influences the development of the biomass and absorption and desorption reaction in cell walls, which permits a greater biotransformation of metal in systems. The most favorable pH are found between 4-7.

There is a selection of microbial populations by their resistance to extreme conditions and high concentrations of metal. The study and identification of these populations can be a great contribution for the development of economically viable alternatives for the reduction of Cr (VI), the studied industrial leachates and to avoid the contamination of the environment.

#### ACKNOWLEDGEMENTS

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