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Heavy Metal Removal by Microalgae

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Industry development has created an important number and volumes of waste waters coming from the new manufacturing processes. Some of them contain pesticides, heavy metals and other toxic substances. In some cases, the toxic substances could be degraded by biological treatment, but in the case of heavy metals, they remain in the sediments and are slowly released into the water body (De la Noue, de Pawn 1988).

In the specific case of microalgae as living organism, traces of heavy metals are necessary as co-factors of enzymatic reactions, but high levels of heavy metals could result extremely toxic to them and, metabolic reactions can be inhibited (EPA 1976., Rai, Gaur, Kumar 1981, SEDUE-CINVESTAV 1989, Díaz Barriga 1991).

When heavy metals are released to the environment, they can create serious damage to the aquatic life. There are some aquatic organisms that can accumulate heavy metals into their protoplasmic structure without toxic effects. For example ***Euglena gracilis*** could accumulate Zn ions until 5 mg/g dry weight (Fukami M et al,1988). In other cases, toxic effects could inhibit the enzymatic system affecting the biochemical and physiological processes (Costa & Leite, 1990, Costa A.C.A. & Leite, 1991, Ting, Lawson & Prince, 1989). There are several chemicals and physical processes for heavy metals removal from wastewater, but scarce reports exist dealing with biological processes (Travieso 1979, Travieso 1980, Travieso 1983)

Some authors have studying the pH influence on the toxicity of heavy metals for microalgae growth (Darnall et al. 1986, Sakaguchi, Nakajima, Horikoshi, 1981) Microalgae show certain attraction for polyvalent ions resulting on the removal of heavy metals from the environment. This aspect is very important from the environmental protection point of view.

The objectives of this work were to study the effect of three heavy metals (Cd, Zn and Cr) on the microalgae growth using two different microalgae free cell's suspended culture (***Scenedesmus acutus*** and ***Chlorella vulgaris***) and to study the immobilization of microalgae cells (using kappa-carrageenan and polyurethane foam as supports) for heavy metals removal (Cd, Zn and Cr).

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MATERIALS AND METHODS

Two strains of microalgae were used: ***Chlorella vulgaris*** LAM-C30 and ***Scenedesmus acutus.*** The first one was supplied by the Microbial Culture Collection of CINVESTAV-IPN (Martínez-Cruz 1991). The second one was supplied by the Microalgae Collection of DECA-CNIC (Valiente, Travieso 1992). The culture media were modified Bristol for ***Scenedesmus acutus*** and C-30 with 2 % w/v agar addition for ***Chlorella vulgaris*** (Cañizares-Villanueva, Travieso 1991, Cañizares-Villanueva, Travieso 1992).

For the purification of the cultures, different dilution, antibiotic treatment and colonization were tested. The inoculum suspension was prepared with solid media, growing for 3 days. After this 1 ml of inoculum was mixed with 10 ml of the culture media.. After 2 days, 100 ml Erlenmeyer's were inoculated with this material. The incubation was made with continuous aeration and illumination of 2000 lux, 25 ± 3 °C and 1 vvm (air volume per effective volume of reactor). The studies for the growth kinetics were followed by absorbance determinations with a spectrophotometer Bausch & Lomb, Spectronic 20, each 24 hour until 7 days of culture.

Microscopy showed a satisfactory physiological condition for both strains. The microalgae growth was followed by chlorophyll determinations using a spectrophotometer Bauch & Lomb, Spectronic 20 (APHA 1993)

For the experiments of heavy metals removal, two materials were tested for the selection of the optimal matrix for microalgae immobilization: Kappa-carrageenan and polyurethane foam. In the first case the technique of Chevalier and De la Noüe, for the pellets formation was employed, the spheres had 5 mm diameter. In the second case, polyurethane foam white cubes of 5 mm width were used. The cubes were washed with distilled water and dried after this process.

For immobilization experiments, the reactors consist on Plexiglas cylinders (columns) of 1 liter volume, 7 cm diameter and 34 cm total height. For the experiments with Kappa-carrageenan pellets, the columns were filled with 100 ml volume of pellets (1538 pellets). The support was expanded to 1 liter (10 %). Fluidization effect was reached using up-flow air, oil free and saturated. The total transfer area was 960 cm^2 and the HRT was 18 hours.

In the experiments with polyurethane foam, the columns were packed with 400 cubes of this material with a total transfer area of 2100 cm^2 and HRT of 3 days (72 hours). The temperature in all the experiments was 25 °C ± 3 °C and the illumination was 2000 lux provided by 40 watt fluorescent lamps.

The experiments for heavy metals tolerance determination for Zn, Cr and Cd were carried on with free cell's systems of both Microalgae, growing on 500 ml flat

flask. The incubation was done at 25 ± 3 °C, shaking it at 100 rpm and with a continuo's illumination of 3 000 lux. The experiments were carried out during 7 days. The absorbance was determined each 24 hour. The experiments were triplicate and in all cases, the variability coefficient (VC) was below 6 %, determined by the correspondent statistical analysis. The control consisted on the culture media without metal.

Different concentrations of the metal were used. Cr varies from 5 to 20 $mg.L^{-1}$ for *Scenedesmus acutus*. and from 5 to 48 $mg.L^{-1}$ for *Chlorella vulgaris.* The Zn concentration in the experiments varies from 100 to 600 $mg.L^{-1}$ for *Chlorella vulgaris,* and from 25 to 125 $mg.L^{-1}$ *for Scenedesmus acutus*. For *Chlorella vulgaris* Cd varies from 1 $mg.L^{-1}$ to 2 $mg.L^{-1}$ and for *Scenedesmus acutus* the same occur. For the obtention of the different concentrations different salts were used: Cl_2Cr, SO_4Zn and SO_4Cd.

The immobilization experiments were done adding to the culture media different concentrations of Zn, Cd and Cr. The selected concentrations were based on the results in the free cell's experiments. The experiments were triplicate.

For the columns with immobilized *Chlorella vulgaris* 1 mg/L^{-1} of Cr, 5 mg/L^{-1} of Cd and 300 mg/L^{-1} of Zn were added to the Bristol culture media. In the experiments for immobilized *Scenedesmus acutus,* 20 mg/L^{-1} Cr, 0.5 mg/L^{-1} Cd and 50 mg/L^{-1} Zn were added to the C-30 culture media. In both cases, packed and fluidized flux patterns were used.

All the analytical determinations were done according the standard methods for the examination of waters and wastewater (APHA 1993).

The tolerance to Zn,Cr and Cd was determined following the technique described by Cañizares-Villanueva and Travieso (1992). A spectrophotometer of Atomic Absorption was employed for the determination of metal concentration uptake in the microalgae suspension. The samples were previously centrifuged for cell removal. In the case of immobilized cells, chemical determination was employed using a Kit Aquaquant 14402 (Merck). The cells were previously removed by centrifugation.

RESULTS AND DISCUSSION

Tables 1, 2 and 3 present the kinetic behavior of *Chlorella vulgaris* and *Scenedesmus acutus* during the free cell's experiments. From these values were determined the exponential phase of the cultures for the inoculation in the immobilization experiments. It was determined that 96 hours was the minimum time for the inoculations.

The growth rates in the culture media were 0.015 h^{-1} for *Chlorella vulgaris* and 0.02 h^{-1} for *Scenedesmus acutus.* The generation times were 45 h 35 min and 37 h 53 min respectively.

The methodology employed for microalgae immobilization on kappa-carrageenan gel was satisfactory. The pellets had good stability and its utilization in the fluidized bed was satisfactory. No free cells were detected in the experiments. The methodology employed for the colonization of polyurethane foam was also adequate and the liquid media was colorless.

In the case of tolerance to Zn, Cr and Cd studies in the free cell's system, it was found that ***Chlorella vulgaris*** support zinc concentrations until 600 $mg.L^{-1}$, but some morphological variations, as gigantism and changes in the shape of the cellular walls, were detected in the cells, using traditional light microscopy. For concentrations values between 100 and 400 $mg.L^{-1}$ no variations occurred (Table1). The pH ranged 6.8-7.2.

Table 1. Effect of the Zn concentration on the growth rate of *Scenedesmus acutus and Chlorella vulgaris.*

Chlorella vulgaris			*Scenedesmus acutus*		
Concentration ($mg.L^{-1}$)	μ (h^{-1})	T_g (h)	Concentration ($mg.L^{-1}$)	μ (h^{-1})	T_g (h)
100	0.0104	66	25	0.0123	56
200	0.0111	62	30	0.0132	53
300	0.0104	66	45	0.0136	51
400	0.0096	72	50	0.0142	48
450	0.0078	88	60	0.0134	51
500	0.0079	87	75	0.0129	53
550	0.0092	74	100	0.0124	54
600	0.0000	n.d.	125	n.d.	n.d.
control	0.0106	65	control	0.0124	56

μ = microorganisms specific growth rate; Tg = generation time, n.d = no growth

Scenedesmus acutus resists zinc concentrations of 100 $mg.L^{-1}$ maximum, and cellular density diminishes when the concentration of zinc increase.

Concerning, cadmium, both strains presented morphological problems over concentrations of 2 $mg.L^{-1}$, but they remain with its metabolic characteristics and only the reproduction function was affected. The pH of the system range was 6.2 - 6.5.

The effect of Cr concentration is shown in Table 3. The maximum Cr concentration that was resisted by *Scenedesmus acutus* was 15 $mg.L^{-1}$. Over this concentration no growth was detected. A similar behavior occurs for *Chlorella vulgaris.* It only resists a maximum Cr concentration of 45 $mg.L^{-1}$.Over these concentrations, an abnormal growth of the cells occurs.

In the immobilized experiments, the packed columns, for both strains, had more efficient behavior than fluidized columns. This situation could be explained
because of the absence of fixation system in *Chlorella vulgaris* and, in *Scenedesmus acutus.*

The aeration effects in the columns promote the expulsion of the cells to the media. When Kappa-carrageenan was used, higher up-take of the metals were determined, compared with the polyuretane foam columns.

It was easear to detect the morphological variations in *Scenedesmus acutus* than in *Chlorella vulgaris*. Also, *Scenedesmus acutus* showed better metal removal efficiencies than *Chlorella vulgaris* (Tables 4 and 5).

Table 2. Effect of the Cd concentration on the growth rate of *Scenedesmus acutus and Chlorella vulgaris.*

Chlorella vulgaris			*Scenedesmus acutus*		
Concent ($mg.L^{-1}$)	μ (h^{-1})	T_g (h)	Concent ($mg.L^{-1}$)	μ (h^{-1})	T_g (h)
1	0.03	214	1	0.02	181
2	0.028	194	2	0.017	154
3	n.d.	n.d.	3	n.d.	n.d.
4	n.d.	n.d.	4	n.d.	n.d.
control	0.03	216	control	0.02	182

μ = microorganisms specific growth rate; Tg = generation time n.d = no growth

It was demonstrated that microalgae *Scenedesmus acutus* and *Chlorella vulgaris* immobilized in polyurethane foam and Kappa-carrageenan gel, are tolerant to Cadmium, Chrome and Zinc concentrations over the normal concentration of these ions on industrial waters. This fact implies a great possibility for this type of waste water treatment using immobilized microalgae.

An economic analysis must be done for the selection of one of the immobilization matrix. The polyurethane foam is cheaper than Kappa-carrageenan, but the last one reached higher removal percentage of the metals.

For further studies *Scenedesmus acutus* must be used because this strain presents easier detectable variations in presence of Cd, Zn and Cr. The tolerance and uptake of Cd, Zn and Cr of *Scenedesmus acutus* is higher than *Chlorella vulgaris.*

Table 3. Effect of the Cr concentration on the growth rate of Scenedesmus *acutus and Chlorella vulgaris.*

Chlorella vulgaris			*Scenedesmus acutus*		
Concent. _(mg.L^{-1})	μ (h^{-1})	T_g (h)	Concent. (mg.L^{-1})	μ (h^{-1})	T_g (h)
5	0.0200	34	5	0.0073	94
10	0.0181	38	10	0.0026	266
15	0.0137	50	15	0.0038	182
20	0.0206	33	20	n.d.	n.d.
25	0.0197	35	25	n.d.	n.d.
30	0.0154	45	30	n.d.	n.d.
35	0.0148	46	35	n.d.	n.d.
36	0.0220	31	36	n.d.	n.d.
39	0.0207	33	39	n.d.	n.d.
42	0.0069	100	42	n.d.	n.d.
45	0.0024	27	45	n.d.	n.d.
48	n.d.	n.d.	48	n.d.	n.d.
control	0.0167	41	control	0.0092	75

μ = microorganisms specific growth rate; Tg = generation time; n.d = no growth

Table 4. Heavy metal removal using *Chlorella vulgaris* immobilized on Kappa-carrageenan (Fluidized bed) and polyurethane foam (Packed bed)

Time (h)	Kappa-carrageenan			Polyuretane foam		
	Cd (mg.L^{-1})	Zn (mg:l-1)	Cr (mg:l-1)	Cd (mg:l-1)	Zn (mg:l-1)	Cr (mg:l-1)
0	5.0	300.0	1.0	5.0	300.0	1.0
6	3.9	235.0	0.9	4.5	251.0	0.94
12	3.0	188.0	0.81	4.0	206.0	0.90
18	2.5	146.4	0.72	3.6	173.2	0.84
24	2.1	119.2	0.64	3.1	145.1	0.77
30	1.9	83.1	0.60	2.8	121.4	0.74
36	1.8	68.2	0.57	2.5	94.3	0.70
42	1.8	51.4	0.53	2.2	78.0	0.65
48	1.7	45.1	0.52	2.15	66.0	0.66
54	1.7	43.0	0.53	2.15	65.1	0.66
60	1.6	43.0	0.52	2.15	65.1	0.66
Maximun Removal %	66	85	48	57	78	34

Table 5. Heavy metal removal using *Scenedesmus acutus* immobilized on Kappa-carrageenan (Fluidized bed) and polyurethane foam (Packed bed)

Time (h)	Kappa-carrageenan			Polyuretane foam		
	Cd ($mg.L^{-1}$)	Zn (mg:l-1)	Cr (mg:l-1)	Cd (mg:l-1)	Zn (mg:l-1)	Cr (mg:l-1)
0	0.5	50.0	20.0	0.5	50	20.0
6	0.33	34.8	19.2	0.43	38.1	19.5
12	0.25	26.3	18.7	0.37	29.6	18.7
18	0.21	18.6	17.1	0.32	21.5	18.0
24	0.18	12.0	16.1	0.27	16.8	17.7
30	0.16	8.8	15.2	0.22	13.1	17.0
36	0.15	5.16	14.6	0.19	11.3	16.8
42	0.14	5.0	13.5	0.16	8.6	16.5
48	0.13	4.5	13.0	0.156	8.0	15.9
54	0.12	4.33	12.8	0.157	7.6	15.8
60	0.13	4.5	12.9	0.156	7.6	15.9
Maximun Removal %	73	91	36	69	84	31

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