SUMMARY AND CONCLUSION

Three types of small laboratory-scale algal biofilters were built up using *Spirulina platensis, Chlorella ellipsoida, Scenedesmus quadricauda var. longispina* and *Nitzschia palea* on sponge and cotton carriers and capsulated in Ca-alginate beads. These biofiltres were used to treat the toxicity of four of the most toxic industrial effluents in Egypt (Talkha chemical fertilizers, Sandoub oils and soap, Mahalla dyes and Kafr-Ezzayyat salt and soda) through the bioremoval of their toxic heavy metal ions (Ni, Cd, Pb and Hg).

The use of this biological technologies are less expensive eco-friendly than traditional wastewater treatment methods.

It is convenient to briefly summarize this research work under the titles of its main goals.

1- Optimum contact time for metal ion bioremoval from synthetic solutions by immobilized test algae:

Five time periods (1 min., 5 min., 15 min., 30 min. and 60 min.) were checked for the optimum time at which maximum efficiency of metal ion bioremoval of alginate immobilized test algae. In all cases the initial metal ion concentration was 10 mgl^{-1} for the metal ions Ni(II), Cd(II) and Pb(II) and 1.0 mgl^{-1} for Hg(II).

The results revealed that, the efficiency of metal ion bioremoval increases as contact time increases. Although, in some cases their was a slight increase in metal ion concentration removed beyond 15 minutes, but such an increase was statistically non significant. Consequently, 15 minutes was selected as an optimum contact time for metal ion bioremoval.

2- Optimum pH value for metal ion bioremoval from synthetic solutions by immobilized test algae:

As bioremoval of heavy metal ions by algae is certainly pH dependant, eight pH values (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) were checked to select the optimum pH at which maximum metal ion bioremoval was attained. Results indicated a significant increase in metal ion bioremoval efficiency with the increase of pH until pH 5.0, at pH 6.0 it was more or less stable and then gradually decreased as pH increased further.

In some cases, there was slight increase in metal ion bioremoval beyond pH 6.0, however, though it was statistically non-significant. Therefore, pH 6.0 was selected as the optimum pH for metal ion bioremoval and adjusted for all subsequent treatments.

3- Optimum pH for metal ion elution from algae-alginate beads:

After loading heavy metal ions on algal beads by bioremoval, acidic solution of 0.1 M thiourea (acidified with HCl) was used to elute metal ions from the algal beads so that the algal biofilter could be reused. To select the optimum

pH of metal ion elution, the metal-laden algal beads were soaked in thiourea solution at different pH values of 2.0, 3.0, 4.0, 5.0 and 6.0.

Experimental results showed that the % of metal ions eluted by 0.1 M thiourea decreased as pH increased with highest and lowest elution capacity being recorded at pH 2.0 and pH 6.0, respectively. So, pH 3.0 was selected as an optimum for the elution of metal ions tested based on the fact that difference in metal ion elution efficiency at pH 2.0 and pH 3.0 were statistically non significant, but the pH 3.0 is supposed to be relatively less harmful to the viability and/or vitality of the test algae than pH 2.0.

4- Optimum time for metal ion elution from algae-alginate beads:

To select the optimum time of metal ion elution, the metal-laden beads were transferred to acidic 0.1 M thiourea at the optimum pH 3.0 and left to stand for different time periods (1.0, 2.0, 5.0 and 10.0 minutes), to check the optimum elution time.

Results revealed that the elution capacity of acidic (pH 3.0) 0.1 M thiourea increased significantly as contact time increased from 1.0 to 5.0 minutes and then non significantly from 5.0 to 10.0 minutes. The contact time of 5.0 minutes was selected as the optimum time for metal ions elution. Lowering the algae-elutant exposure time, minimizes the risks of acidity and toxicity of thiourea on biological performance of the test algae.

5- Consistency of metal ion bioremoval from synthetic solutions along successive removal-elution cycles:

The efficiency of individually immobilized test algae (one algal species capsulated inside the algal beads) and composite algae-alginate beads to remove different heavy metal ions prepared either singly or in one mixture in synthetic solutions was assessed through five successive bioremoval-elution cycles. The contact time of metal ion bioremoval was fixed at 15 minutes (optimum bioremoval time) in all treatments and pH of all metal test solutions was adjusted at pH 6.0 (optimum bioremoval pH). In each cycle, acidified 0.1 M thiourea solution, at pH 3.0 (optimum eluting pH), was used to elute metal ions loaded on algal beads.

This investigation seems practically important to validate the applicability of our test algae to design specific biofilters for metal ion bioremediation of industrial wastewaters.

The work with successive bioremoval-elution cycles revealed the following conclusions:

a- The efficiency of test algae to remove metal ions tested was slightly decreased with the increase of bioremoval-elution cycles with highest and lowest values being recorded at the first and fifth cycle respectively. However, even after five successive metal bioremoval elution cycles, all test algae maintained outstanding efficiency as the % metal ion removal never fallen below 75%.

b- The efficiency of test algae to remove different metal ions prepared singly was higher than that achieved when metal ions prepared in mixture solutions, due to the competition of metal ions over binding sites on algal cell surface.

c- One way analysis of variance (ANOVA) revealed non-significant differences in efficiencies of different test algae to remove metal ions either from the single metal or mixture metal solutions. However, the efficiency of composite beads (including more than one algal species) to remove metal ions from a mixture metal solution was significantly higher compared to the individually tested algae.

d- The efficiency of thiourea to elute Ni, Cd and Pb from different algal beads was higher than that achieved for Hg-laden beads. This may be due to that most algal strains prefer to remove Hg by volatilization instead of accumulation.

e- One way analysis of variance (ANOVA) showed that, the efficiency of thiourea to elute metal ions from composite algal beads was significantly higher compared to individual algal beads. This result may be explained by the higher capabilities of various binding sites of composite algal beads in removing different heavy metal ions than that of single algal beads.

6- Efficiency of immobilized algae; capsulated in alginate beads and on cotton and sponge flat-bed filters; to remove heavy metal ions from toxic industrial effluents:

Four industrial effluents chosen as the most toxic ones in egyptian industries (previous work of the authors), including chemical fertilizers, oil and soap , textile dyes and salt and soda were collected. After filtration; pH and heavy metal analysis; samples were adjusted at pH 6.0, that was found to be optimum for metal ions bioremoval working with synthetic solutions. The capacity of the four co-immobilized test algae either into alginate matrices or developed on flat-bed filters of cotton and sponge to remove Ni(Π), Cd(Π), Pb(Π) and Hg(Π) from industrial effluents were tested through **ten successive bioremoval-elution cycles.**

The work with **industrial effluents** and co-immobilized test algae revealed the following conclusions:

a- The highest and lowest capacity of different biofilters to remove all metal ions studied was recorded after the first and tenth bioremoval- elution cycles respectively.

b- Even after 10^{th} bioremoval-elution cycle, the efficiencies of algae-cotton filters, algae-sponge filters and algae-alginate beads were typically above 65%, 65% and 45% in removing the metal ions studied respectively.

c- Through ten bioremoval-elution cycles, the efficiencies of cotton and sponge filters to remove different heavy metal ions were generally higher than that achieved by algae-alginate beads.

d- Although, algae-cotton filters have slightly higher efficiencies to remove different heavy metal ions, the algae-sponge ones are more favorable for industrial wastewater treatment applications; since they are cheaper, more durable, non biodegradable, waste byproduct and liable for elastic design.

7- Checking the efficiency of our algal biofilters in treatment of industrial wastewater by toxicity assessments using standard algal bioassays:

Toxicity of raw (GF/C filtered) and pH adjusted effluent samples were assessed using a standard algal biotest procedure with the green Chlorococcalean alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) as a standard test alga. Standard algal bioassay was also employed to validate the efficiency of co-immobilized test algae to reduce the toxicity of the four toxic industrial effluents through ten successive bioremoval-elution cycles.

The results revealed that:

a- Talkha chemical fertilizer raw effluent was the most toxic one with EC_{50} as low as 1.0% (the lower the EC_{50} the higher toxicity

is). Adjustment of pH value of this effluent at pH 6.0 resulted in a significant decrease in its toxicity (EC_{50} = 21%).

b- Kafr-Ezzayyat salt and soda effluent was found to have EC_{50} 43% and 56% of raw and pH adjusted samples respectively, expressing lower toxicity than all other effluents .

c- Though still toxic the adjustment of effluent pH to pH 6.0 certainly reduced toxicity of raw effluent. So, the adjustment of extreme pH of raw effluents should be a primary step before using the algal filter technology for treating toxic industrial effluents.

d- Relatively higher algal growth stimulation on algal filters was recorded for all the biologically treated effluents particularly at the earlier bioremoval cycles (1^{st} and 4^{th} cycles), proving the success of algal filters in treating these effluents and reducing or removing their toxicity.

e- All algal biofilters showed high efficiencies to reduce the toxicity of all studied industrial effluents, specially, at the first and till fourth bioremoval-elution cycles. Although the efficiencies of algal biofilters decreased after the tenth cycle, their efficiencies throughout these cycles were still good enough to reduce the effluent toxicity.

8- Effect of toxic industrial effluents on viability of test algae grown within filters and alginate beads:

The abilities of the test algae of both mature sponge and cotton filters and alginate beads to tolerate and/or to grow at highly toxic doses (50% and 100%) of the four tested industrial wastewaters, were tested and compared with control biofilters throughout the incubation period that lasted for 10 days.

The results revealed that:

a- The effluent of Talkha chemical fertilizer adjusted at pH 6.0 stimulated significantly the growth of our algae compared to control. On the other hand the same concentration level of other industrial effluents were inhibitory for the growth of test algae.

b- *Chlorella ellipsoida* followed by *Spirulina platensis* were the most tolerant removable algae capable of growing within artificial carriers treated with 50% and 100% effluent concentrations. They represent the best efficient candidates for the bioremediation of toxic industrial effluents.

c- The biomass of *Chlorella ellipsoida* and *Spirulina platensis* formed approximately 90% of total immobilized algal biomass

calculated at the end of incubation period, assuring that these two species are very good candidates for treating industrial effluents.

d- *Scenedesmus quadricauda* was obviously unable to build up biomass within the artificial carriers when subjected to several cycles of toxic effluents. The pinnate diatom *Nitzschia palea* did not succeed to grow within either sponge or cotton filters as its growth was steadily declining throughout the incubation period.

e- This means that *Chlorella ellipsoida* and *Spirulina platensis* are the candidates for designing efficient, durable algal filters for treatment of toxic industrial effluents.

9- A general and collective conclusion is that immobilized algae certainly represent a great hope and an attractive candidate for treating toxic industrial wastewater. They are considered , so because of possessing various binding sites and mechanisms for heavy metal ion bioremoval, they are also cheap and liable for elastic design with sponge carriers. In this sense, the flat algal filters can be of great help in solving one of the greatest environmental problems allover the world.