The Malachite Green Biodegradation in Bioreactors on Various *p*H Domains

VIOREL GHEORGHE[1], CATALINA GABRIELA GHEORGHE[1]*, ANDREEA BONDAREV[1], VASILE MATEI[1], MIHAELA BOMBOS[2]

[1]Petroleum-Gas University of Ploiesti, 39 Bucuresti Blvd., 100680 Ploiesti, Romania

[2]National Institute for Research Development for Chemistry and Petrochemistry- ICECHIM-Bucuresti, 202 Splaiul Independentei, 060021, Bucharest, Romania

In the experimental study was studied the malachite green colorant biodegradation in biological sludge with biological activity. The biodegradability tests were carried out in laboratory bioreactors, on aqueous solutions of green malachite contacted with microorganisms in which the dominant species is Paramecium caudatum, in a pH range between 8 and 12, temperatures in the ranges 25-35^{0}C, using pH neutralizing substances and biomass growth promoters. The colorant initial concentrations and those obtained after biological degradation depending on the contact time, at certain pH values, were established through UV-Vis spectrometry. The studies have shown the measure of possible biological degradation of some organic substances with extended uses, with largely aromatic structure, resistance to biodegradation of microorganisms, commonly used in wastewater treatment plants.

Keywords: malachite green colorant, wastewater, biodegradation, Paramecium caudatum

Industrial activity generates negative effects on the environment by contaminating the biotype (soil, water, air, flora and fauna) with pollutants of a chemical, physical or biological nature [1-5]. Industrial activity contaminates the environment through the processes of exploitation, processing and storage of industrial waste [6-12]. To act properly in order to eliminate contaminants it is necessary to know the sources of pollution and their effects over time[13-16]. In the scientific literature, there are various studies that have successfully used ciliary microorganism called Paramecium caudatum to reduce the concentration of some toxic pollutants from aquatic environments [11-14]. Malachite green is is a derived of triphenyl methane, resistant to biodegradation and with toxic action (fig. 1),

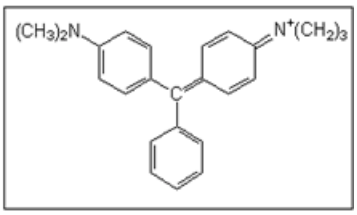

Fig.1. Structure of the dye malachite green

water-soluble with great retentive effect. Thus at less than 1 ppm of such dye produces a significant coloration [17-19].

This dye affects the photosynthesis process of the aquatic flora due to diminishing light diffusion and generates accumulation and persistence in the fish body. For the elimination of green malachite present in fish waters, there are studies in the literature that used Desmodesmus and Cosmarium green algae, microorganisms able to degrade 96% of colorant. These studies presents the treatment of polluted waters with the above mentioned microorganisms under certain temperature and *p*H conditions. The dependence of the discoloring speed on the dye concentration is described by the Michaelis-Menten type relationship.

Feng researcher [20] have been applied the methods of biodegradation of dyes with aromatic nucleus structure by using the bacterium Pseudomonas sp. in a polluted medium with malachite green and purple crystal. Following the experiments, Feng also highlighted the toxic effects of malachite verdens. The pollutant could be removed by a biodegradation process. Thus, in solutions with initial concentrations of 1000 mg/L were eliminated up to 90% of malachite green using bacterial suspensions at temperatures of 25, 30, 37°C and *p*H between 6 and 8 [20-21]. In the researches of Ramezani[22] cultures of the bacterium Klebsiella Terrigenapt *cc.* were used in the presence of malachite medium contaminated at 30°C and *p*H 6 using lactose and ammonium nitrate like nutrition sources. In the work of Li [23], Micrococcus sp. has proven effective in discoloring malachite green using some fungal species (Aspergillus flavus and Alternaria solan). Both species were able to biodegrade the green malachite in 6 days with a biodegradation yield of 96%.

The purpose of the paper was to study the biodegradation of green malachite dye at various *p*H values under a biological material action which contains the Paramecium caudatum microorganism as a predominant component.

Experimental part

Biological material for metabolism substance tested was obtained by infusing hay into water in the presence of growth factors favoring the development of the ciliary Paramecium caudatum. Tests on various pollutants in the presence of microorganisms have demonstrated the ability to use these pollutants as a carbon source for metabolic processes [24-26].

The chemical reagents used in the study were $FeSO_4$, $Ca(OH)_2$, Na_3PO_4, H_2SO_4 and had the main role to correct the initial *p*H. To improve the viability of the microorganism used in testing, a nutrient complex containing nitrogen, phosphorus and potassium has been used.

The reaction medium was obtained by infusing dry grass (100 g/L) in distilled water at 25°C and keeping under sterile conditions for 60 days. After the incubation, development and optimization period, in the reaction medium has developed predominantly the microorganism Paremecium sp.

email: ghe.catalina@yahoo.com, Phone: 0723636651

In order to achieve study were used a BINOCULAR B-500BPL type microscope (to observe the microorganism), pH-meter, oxygen-meter, Vortex shaker, incubator and bioreactors micropilot for testing. For determination the concentration of malachite green was used the spectrophotometric method using a UV-Vis spectrophotometer CECIL CE 1011 type.

In each bioreactor, were introduced solutions of 5% (v/v) $Ca(OH)_2$ and H_2SO_4 1M to correct the *p*H to the desired values. The dosing of the reagents in each bioreactor was performed so as to obtain 4 different initial *p*H values: at the bioreactor 1 the initial *p*H was 6.5, at the bioreactor 2 the initial *p*H was 4.2, the bioreactor 3 the *p*H was initially 8.5 and in the bioreactor 4 the initial *p*H was 10. Likewise, in each reactor were introduced the necessary nutrients for microorganisms: 10 g complex N: P: K (nitrogen: phosphorus: potassium), 50 mL $FeSO_4$ (100 ppm), 10 mL Na3PO4 (10 ppm) [27-31]. The biological medium and the malachite green solution in each reactor were introduced after stabilization of the initial pH values. The standard solution of the greenish malachite dye had a concentration of 1 mg/L. The resultant mixture was in contact with a continuous stream of air to support the growth of ciliary microorganisms. The introduced air also contributes at homogenizing the mixture of the biological medium with the aqueous pollutant solution by bubbling.

The reaction medium of the four reactors was conditioned corresponding to pollutant biodegradation conditions at room temperature. During the experiments there was a variation of the *p*H with the process duration. During the experiments there was a *p*H variation.

The removal of the malachite green dye was followed by laboratory analyzes to determine its concentration after contact with the reaction medium in which the desired microbiological flora was developed. The variation in dye concentration and *p*H value of the 4 reactors was followed at certain time intervals up to 360 h.

In order to determine the absorption maxima of the malachite green dye the absorption spectrum in the UV-Vis domain was drawn (fig.2). Based on the spectrum, the wavelength of the absorption wave was selected to determine the pollutant concentration (ie λ was 620nm). Thus, dye concentrations were established based on the calibration curves plotted for certain values of the dye concentration found in the range of variation in this study.

Results and discussions

The biological material obtained after incubation for 60 days, was examined under the optical microscope at 900x. In the field of view, was observed the formation of the biological sludge flocs in which Paramecium ciliated microorganisms have grown as a dominant species as well as agglomerations of bacteria, cocci and bacilli, and filamentous bacteria Nostoc. In the 1 cm^2 visual field of biological sludge, approximately 20 units of Paramecium active ciliate cells were identified. (fig.3)

Effectiveness of biological media based on Paramecium caudatum on the green malachite pollutant was revealed by determining the concentration of the dye and the *p*H of the biological medium at different exposure times. The experimental results of the malachite green dye concentration with the time in the biological media of the 4 bioreactors are shown in Figures 4-7.

It is observed from Figure 4 that for an initial *p*H of the reaction medium of 6.5 there is a rapid decrease of the dye concentration in the first 24 h, after which the decrease of the concentration of the pollutant takes place with a slow slope so that after 360 h the concentration of the green malachite decreases to about half of the value at 24 h.

The biological medium from the second bioreactor (fig. 5) with an initial *p*H more acidic (4.5) than the first reactor (6.5), favors the decrease of the malachite green dye concentration after an almost linear curve over a 288 h, but with a slower slope than in the first reactor, and at over

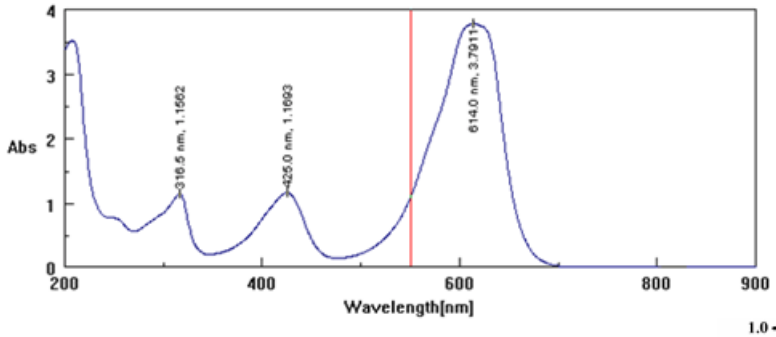

Fig.2. Absorption spectra of malachite green dye in the UV-Vis

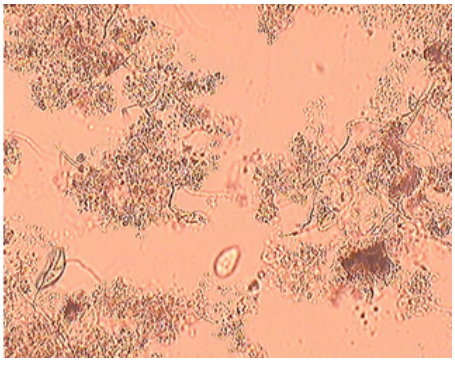

Fig.3. Microscopic image of the suspension in which has been developed the Paramecium caudatum microorganism

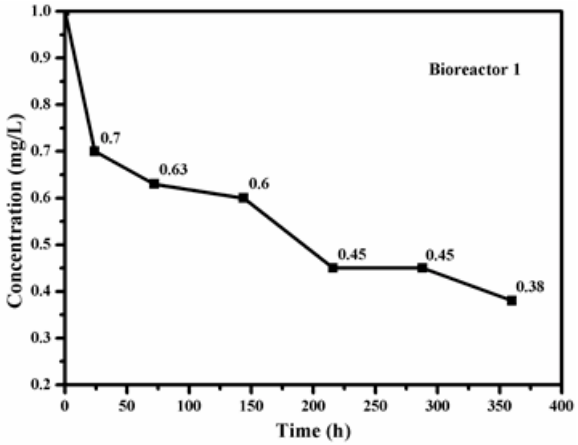

Fig.4. Influence of exposure period on green malachite pollutant concentration in the reactor with initial *p*H 6.5

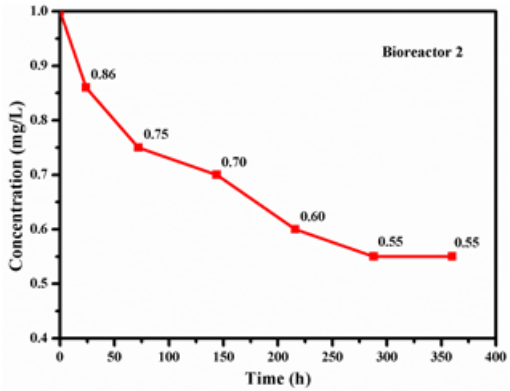

Fig.5. Influence of exposure period on green malachite pollutant concentration in the reactor with initial *p*H 4.2

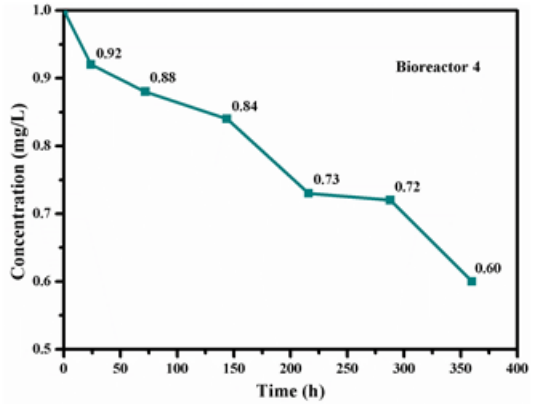

Fig.7. Influence of exposure period on green malachite pollutant concentration in the reactor with initial *p*H 10

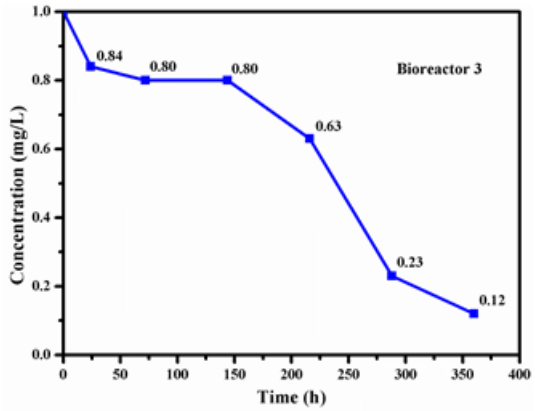

Fig.6. Influence of exposure period on green malachite pollutant concentration in the reactor with initial *p*H 8,5

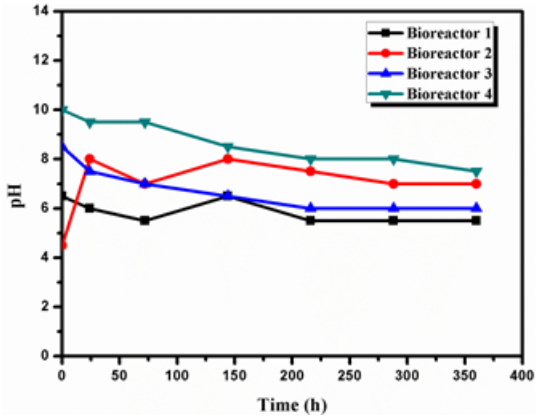

Fig.8. Variation of *p*H with time in the four bioreactors

288 hours the concentration stabilizes at 0.55 mg/L. Therefore, the increase in the acidity of the environment determines reduces the propagation rate of micro-organisms population, which results in a higher final concentration of dye.

The medium in the third bioreactor (fig. 6), having an initial *p*H of 8.5, favors the most advantageous evolution of the malachite green concentration with the reaction time. Thus, after a period of 24 h, the concentration of the pollutant decreases from 1 mg/L to 0.84 mg/L, and then it tends to stabilize at 0.8 mg/L for 120 h, after which a decrease with a high slope reaching 0.23 mg/L after 288 h of reaction and respectively, 0.12 mg/L after 360 h. This decrease in the pollutant concentration is explained by the positive influence of the initial pH of 8.5 on the growth of the microorganism population in the biological environment.

For fourth bioreactor with an initial *p*H of 10, the variation of the pollutant concentration with the reaction time is slow, almost linear, decreasing from the initial value of 1mg /L to 0.6 mg /L after 360 h. Therefore, an initially too high *p*H is not recommended for the removal of malachite green waters, due to the negative influence on the viability of microorganism.

Indifferent of the initial *p*H, its value changes with the increase of the reaction time. Thus, in figure 8 are presented the experimental data on the *p*H variation with the reaction time in the four bioreactors.

It was found that in the first bioreactor the *p*H had a sinusoidal variation in the first 216 h, after which it stabilized at 5.5.

The medium in the second bioreactor had a sinusoidal variation in the first 288 h. Thus, from the initial *p*H of 4.5, it increased in 72 h to 8.0, and after 216 h it decreased to 7 and was maintained at this value by the end of the test. Probably, in this case, in the initial period the micro-organisms act on the reagent that acidifies the environment and corrects the *p*H, bringing it to values close to neutral.

The medium in the third bioreactor was tested starting at an initial *p*H of 8.5, and over time its value evolved thus: decreased with an almost constant slope over 216 h to 6, and then kept constant until the end of the test.

The medium in the fourth bioreactor 4 was tested starting at an initial *p*H of 10. The *p*H value decreased over the entire experiment period with a variable slope to a final *p*H of 7.5.

From comparing data shown in figure 5 results that *p*H variation over time in the four bioreactors can not be explained only based on the nature of the dye used (which presents basic character). Thus, in the biologic environment of first and second bioreactors, pH variation over time occurs after a sinusoidal curve. For the first reactor, the *p*H increases from 4.5 to 7 at the end of the experiment, while in the other bioreactors the pH at the experiment end has lower values than at the initial one. Therefore, regardless of the initial *p*H values, at the end of the experiment the tendency is to reach a pH nearly to neutral (between 5.5 and 7.5). Thus, experimental data show that initial pH values almost to neutral are most favorable to the viability of microrganisms and implicitly to pollutant degradation.

Also, the effectiveness of the Paramecium sp microorganism is influenced by the initial *p*H value. Thus the third bioreactor, having an initial *p*H of 8.5, had the best biodegradation yield of the green malachite after 360 h of exposure (88%).

Conclusions

The removal of the green malachite toxic pollutant can be done using the suspension of microorganisms tested in the laboratory containing the Paramecium sp.

The presence of microorganisms led to the variation of the pollutant for concentrations for different initial *p*H values. Thus, under the action of the Paramecium sp. ciliary microorganism, present as the dominant species in biocenoses in the chemical industry, the *p*H of the reaction medium varies over time.

Initial *p*H values influence the action of microorganisms on the malachite green dye, an initial *p*H close to the neutral being most recommended. Thus the best conditions for biodegradability of the analyzed pollutant were made at the initial *p*H of 8.5.

References

1. ATLAS, R.M., Microbiol. Rev., **45**, 1981, p.180
2. CHISTI, Y., Biotech. Adv., **6**, 2004, p. 431
3. PIRBAZARI, M., BADRIYHA, B.N., MILTNER, R.J., J. Environ. Eng., ASCE, **117**, 1991, p. 80
4. BONDAREV, A., PANTEA, O., MIHAI, S., CALIN, C., GHEORGHE, C.G., Rev.Chim. (Bucharest), **67**, no.4, 2016, p. 728
5. LUPU, F., GHEORGHE, C.G., PANTEA, O., CALIN, C., Rev.Chim. (Bucharest), **64**, no. 2, 2013, p.210
6. PEREZ-CANDELA, M., MARTIN-MARTINEZ, J.M., TORREGROSA-MACIA, R., Water Res., **29**, 1995, p. 2174
7. CURDS, C.R., Annual Review of Microbiology, **36**, 1982, p.27
8.*** U.N. Economic Commision for Europe. Protection of water resources and aquatic ecosystem, New York, 1993
9. FRIED, J., MAYR, G., BERGER, H., Water. Sci. Tech., **41**, 2000, p.309
10. TANDOI, V., JENKINS, D., WANNER, J., Water Sci. Technol., **39**, 1999, p.367
11. PAOLO, M., ROMEO, M.G., Environmental Pollution, **141**, 2006, p.1
12. PAOLO, M., Encyclopedia of Environmental Microbiology, John Wiley & Sons, New York, 2002, p. 2605
13. WITTHAUER, D.P., European Journal of Applied Microbiology and Biotechnology, **92**, 1980, p.151–163
14. VENKATESWARA RAO J., SRIKANTH, K., AREPALLI, S.K., GUNDA, V.G., Pesticide Biochemistry and Physiology, **86**(3), 2006, p.131
15. YANAGI, A., Jpn. J. Protozool., **43** (2), 2010,p.101
16. ALEXANDER, M., Biodegradation and Bioremediation, Academic Press, San Diego, CA, U.S.A., 1999, p. 306
17. DANESHVAR, N., KHATAEE, A.R., RASOULIFARD, M.H., POURHASSAN, M., Journal of Hazardous Materials, **143**, 2007, p.214
18. SUDOVA, E., MACHOVA, J., SVOBODOVA, Z., VESEL, T., VeterinarniMedicina, **52**(12), 2007, p. 527
19. RAMEZANI, S., POURBABAEE, A.A., JAVAHERI DANESHMAND H., J. Bioremed Biodeg., **4** (1), 2013, p.1
20. ALI, H., AHMAD, W., HA, T., African Journal of Biotechnology, **8** (8), 20, 2009, p. 1574
21. KOCH, A., L., Turbidity Measurements of Bacterial Cultures in Some Available Commercial Instruments., Anal Biochem., **38**, 1970, p.252
22. RAMEZANI, S., POURBABAEE, A.A., JAVAHERI DANESHMAND H., J. Bioremed Biodeg., **4** (1), 2013, p.1
23. WEN-HUA CHEN, YU-HUA ZHAO, International Biodeterioration & Biodegradation**, 78**, 2013, p. 108
24. GHEORGHE, C. G., PANTEA, O., MATEI, V., BOMBOS, D., BORCEA, A.F., Rev. Chim. (Bucharest), **62, no.** 5, 2011, p.582
25. GHEORGHE, C. G., PANTEA, O., MATEI, V., BOMBOS, D., BORCEA, A.F., Rev.Chim. (Bucharest), **62**, no. 6, 2011, p.655
26. MIHAI, S., MALAIESTEANU, M., Rev.Chim. (Bucharest), **64**, no. 1, 2013, p.105
27. *** STAS 8683-70 Ape de suprafata si ape uzate. Determinarea azotului amoniacal
28.*** STAS 8900/2-71 Ape de suprafata si ape uzate. Determinarea azotiþilor
29.*** STAS 8900/1-71 Ape de suprafata si ape uzate. Determinarea azotaþilor
30.*** STAS 10064-75 Ape de suprafata si ape uzate. Determinarea fosfaþilor
31. BOMBOS, D., GANEA, R., MATEI, V., MARINESCU, C., BONDAREV, A., MIHAI, S., NATU, T., TAMAS, I., Rev.Chim. (Bucharest), **65**, no. 8, 2014, p.976

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