Algerian Journal of Environmental Science and Technology
December edition. Vol .4 Nº3 (2018)
ISSN : 2437-1114
www.aljest.org

The degradation of methylene blue by bacterial strains isolated from the peel of Red Beet

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ARTICLE INFO

Article History :

Received ***: 02/09/2018***
Accepted ***: 25/11/2018***

***Key Words*:**

Dyes;
Methylene blue;
Red beet;
Identification of bacteria;
Biodegradation.

ABSTRACT/RESUME

Abstract: *The textile industry generates half of the industrial pollution flows, these effluents can be very colorful and difficult to treat. The treatment of these effluents has become a priority in the world, the development of methods and the optimization of existing processes, are the subject of a large number of works.*

In the present work the methylene blue was degraded by two bacterial strains BP1 and BP2 isolated from the peel of red beet, identified as Acinetobacter Johnson II BP1 and BP2 as Bacillus weihenstephanensis. The effect of the initial concentration, pH, temperature, and addition of the carbon source and nitrogen source on the biodegradation of the dye was determined.

I. Introduction

Synthetic dyes are used in several industries such as the pharmaceutical, cosmetic, textile and leather industries[1]. The effluents from the textile industry are the most polluting because of the volume and his composition [2], these effluents have a wide variety of dyes and high stability to light, to temperature and to microbial attack [3] almost 15% of the dyes used in textile industry are released into the environment[4, 5]. According to the report published in 2002 by RAC / CP, Algeria has 39 companies in the textile sector, and consumes 40.12 tons of dyes and pigments per year and a quantity of water exceeding 4.8 t / year. This generates voluminous and charged rejects. However, 70% of these industries release their effluents into the environment [6] . That cause serious problems of water pollution (surface waters and groundwater) in addition some synthetic dyes are carcinogenic or mutagenic [7] , Methylene blue (MB) is widely used dyes, he have been reported for his negative impact on living cells and organisms. The oral median lethal dose (LD50) of methylene blue in rats has been estimated as 1180 [8]. It was also found that at low and moderate doses of MB arterial blood pressure increased, where as at high doses it will worsen systemic hypotension, mycocardardial depression and hypertension after endotoxemia [9].

To avoid the dangers accumulated by dyes in the environment, several techniques are used to eliminate them such as coagulation flocculation, [10, 11], reverse osmosis[12], oxidation or chemical reduction[13-16] photocatalytic degradation[17-20].

The physico-chemical methods are expensive and complicated processes that are why we need to develop economical and efficient methods for the total elimination of dyes in effluents such as biological treatment. Biological treatment is an economical treatment that offers a better alternative to the analysis and control of the environment[21].

In recent years, several studies have focused to the degradation of dyes by microorganisms such as bacteria, fungi and algae. The use of bacteria for the elimination of synthetic dyes offers considerable advantages, the process is relatively inexpensive, running costs are low and the end products of the complete mineralization are not toxic[22].

There are several bacteria that have the ability to absorb or degrade dyes [23-25], these bacteria can be isolated from soil, water and from food.

The objective of this work is to isolate and identify bacterial strains with a strong ability to degrade methylene blue from waste of red beet.

II. Materials and Methods :

II .1. Microorganisms and the culture medium:

The bacterial strains used in this study were isolated from the peel of red beet cultivated in the region of Boumerdes in Algeria by the serial dilution method [26] . A 5 g sample of the peel of beet was suspended in 100 ml of physiological saline (water + NaCl at 0.85%). 1 ml of the suspension obtained was serially diluted in distilled water (10 times). A volume of 0.1 ml of the diluted suspension was spread onto nutrient agar plates added with methylene blue and incubated at 30 ° C for 24 h.

The selected isolate was identified by Maldi-Tof mass spectrometry bruker biotyper (Matrix Assisted Laser Desorption Ionisation - Time of Flight) by analysis of their ribosomal and membrane proteins.

The bacteria able of degrading methylene blue MB was cultivated in a nutrient broth consisting of water, peptone, yeast extract, meat extract and NaCl during 24h at a temperature of 37 ° C.

II .2 .The Dye

The dye used in this study is a cationic dye which is methylene blue C.I (52015) The Methylene Blue was obtained from panreac Ltd.

Table 1*: properties and characteristics of the MB[27]*

Generic name	Methylene blue
Chemical name	3,7-bis(Dimethylamino)-phenazathionium chloride tetramethylthionine chloride
Chemical formula	C16H18ClN3S·3H2O
Molecular weight (g / mol)	373.90
Molecular volume (cm^3 / mol)	241.9
λ max (nm)	668

The chemical structure of methylene blue is represented in the Figure 1

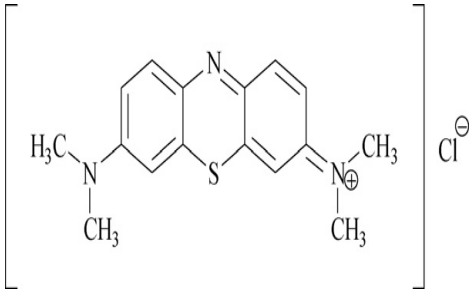

Figure 1: *Structure of Methylene Blue*

II.3. Degradation of the methylene blue

About 0.1 ml of bacterial culture was inoculated in test tube containing 20 ml nutrient broth media and incubated at 37 °C for 24 h; the precultures obtained are added to 200 ml of methylene blue at different concentrations in 250 ml Erlenmeyer flasks.

The degradation of dye was ascertained by measuring the absorbance of culture taken at different time intervals at the wavelength of MB (664 nm) using the shimadzu spectrophotometer UV-1800.

The percentage of elimination is calculated by the following equation Eq. (1)[28]:

$$\%\ \text{of elimination} = \frac{\text{initial OD} - \text{final OD}}{\text{initial OD}} \times 100$$

III.Results and Discussion

III.1. Isolation and identification of bacteria

The dilution suspensions method yielded a large number of isolates with nutrient agar supplemented with methylene blue, the follow-up of the decolorization of the medium by the bacterial strains isolated from the peel of the red beet during 24h has allowed to choose two strains BP1 and BP2.

The identification of these bacteria by Maldi-Tof mass spectrometry identified BP1 as coccobacilli , non-sporulating gram-negative bacteria Acinetobacter Johnson II and BP2 as rod-shaped bacillus Gram-positive, sporulating bacteria, Bacillus weihenstephanensis.

III.2. Influence of the initial concentration on the degradation of the methylene blue

The results obtained are presented in Figures 2 and 3, they showed that the two strains have a great ability to degrade MB, which is a hardly biodegradable dye, the BP2 bacterium have better results than the bacterium BP1 (90% of elimination for the concentration of 5 mg / l during 50h of incubation by the bacterium BP2 compared to 80% of elimination by the bacterium BP1).

The results showed that more the initial concentration of the MB increases the percentage of elimination decreases , 80% and 90% of elimination

by BP1and BP2 respectively for an initial MB concentration of 5 mg / l and by against there is 60% by BP1 and 70% by PB2 for an initial concentration of 100 mg / l. these results are similar to those found by Shah 2012[29], BANDARY and al 2016[30] and by Kilany 2017[31], after this results it can be said that the concentration of the dye affect the microbial activity by a combination of factors including the toxicity of the dye at high concentrations (the antiseptic effect of MB)[29]

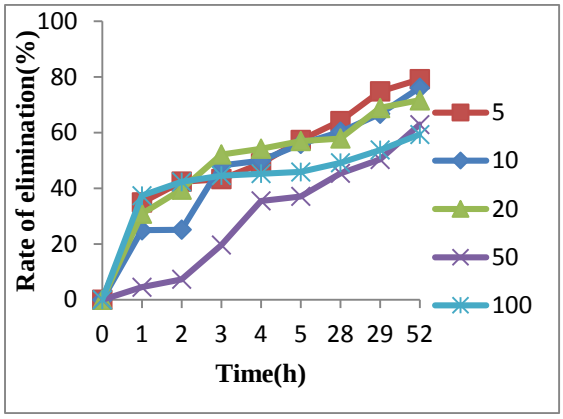

Figure 2: *The rate of degradation of the methylene blue as a function of the time and the initial concentration of the dye by the bacterium Acinetobacter Johnson II.*

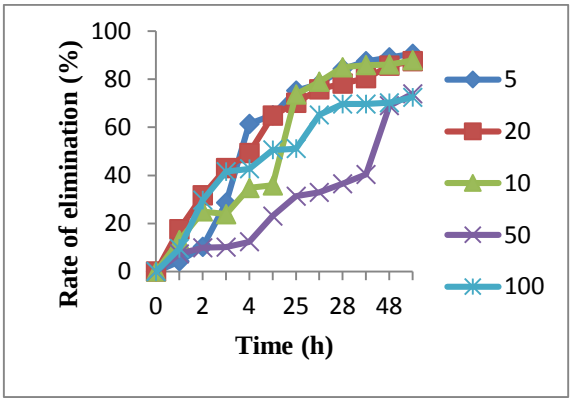

Figure 3: *The rate of degradation of the methylene blue as a function of the time and the initial concentration of the dye by the bacterium Bacillus weihenstephanensis.*

III.3. Influence of PH on the degradation of the methylene blue:

Figures 5 and 6 show the degradation of methylene blue by the bacteria Acinetobacter Johnson II BP1 and Bacillus weihenstephanensis BP2 as a function of time and the pH , these results show that the rate of degradation is higher at a pH of 7 (50.5%) for the bacterium BP1, the same finding is obtained by BANDARY et al 2016[30] unlike Kilany 2017[31] which obtained a better elimination at a pH of 5, for against for the bacterium BP2 the degradation is much better in the high pH for example for a pH 9 it was an elimination of 59.54%. The rate of elimination was higher at only optimum pH but tends to decrease rapidly at strongly acid or strongly alkaline pH,This results may explained by the fact that at low pH, protons in solution compete with the cationic dye for the binding sites on the surface of bacteria. At higher pH, hydroxyl groups in solution complex with cationic dye preventing their adsorption by microbial sorbents [31].

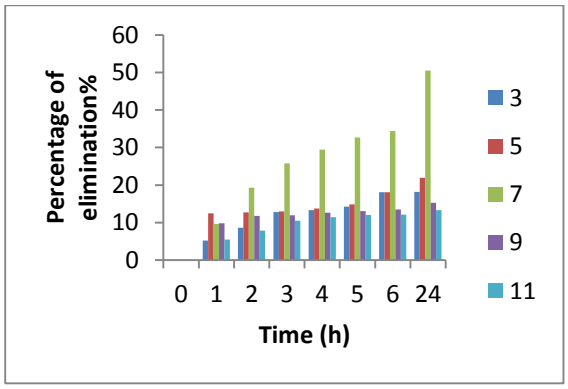

Figure 4: *The rate of degradation of the methylene blue as a function of time and the pH by the bacterium BP1.*

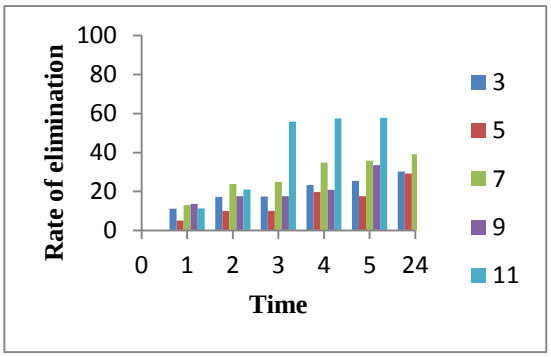

Figure 5: *The rate of degradation of the methylene blue as a function of time and the pH by the bacterium BP2.*

III.4. Influence of the temperature on the degradation of the methylene blue:

Microbial decolorization process is directly influenced by temperature because different groups of microorganisms need a defined range of temperature to perform their activity efficiently.

The results presented in figures 6 and 7 show that the degradation rate is much better at a temperature of 37 ° C in both cases for the bacterium BP1 (the percentage of elimination is 59% during 5 hours of incubation) as well as for the bacterium BP2 (a removal rate is 67% during 5 hours of incubation), the further away from 37 ° C the elimination rate decreases , The same finding is obtained by Tripathi and al 2011[22]; Shah 2012[29] and BANDARY et al 2016[30] , by contrast Kilany 2017[31] achieved the best degradation at a temperature of 30 ° C.

The degradation rate decreases at a higher temperature and this result must be due to the loss of cell viability or to the denaturation of the enzyme azo reductase [32]. At low temperatures the elimination rate is low because the growth rate, the biomass yield and the reaction mechanism require an optimum temperature for maximum efficiency [33].

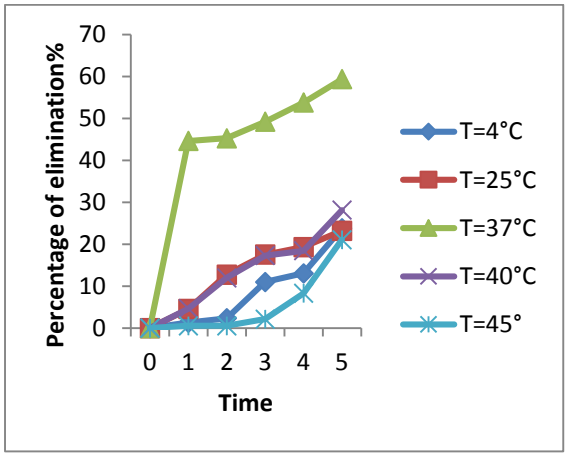

Figure 6: *The rate of degradation of the methylene blue as a function of time and temperature by the bacterium Acinetobacter Johnson II.*

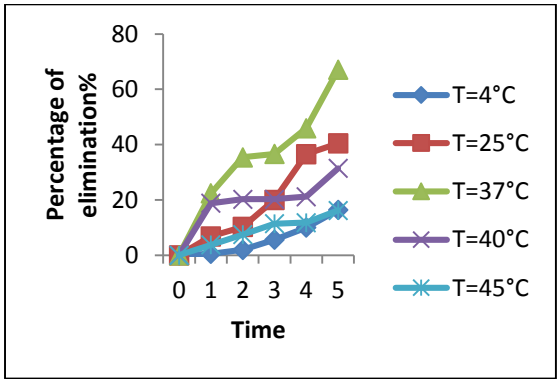

Figure 7: *The rate of degradation of the methylene blue as a function of time and temperature by the bacterium Bacillus weihenstephanensis.*

III.5. Influence of carbon source and source of nitrogen on the degradation of the methylene blue

The addition of a source of carbon and nitrogen in the medium has a negative effect on the degradation of the dye, for the two bacteria (Figures 8 and 9), a degradation of 48% was obtained in the presence of carbon source and 53% in the presence of a nitrogen source in 28 hours of incubation compared to 71% without the addition of the latter for the bacterium BP2 and a degradation of 19% in the presence of carbon source, 41% in the presence of nitrogen for the bacterium BP1 on the other hand there is a degradation of 75% of degradation without the addition of a source of carbon and nitrogen. From its results, it can be said that the two bacteria degrade methylene blue by taking more carbon than nitrogen.

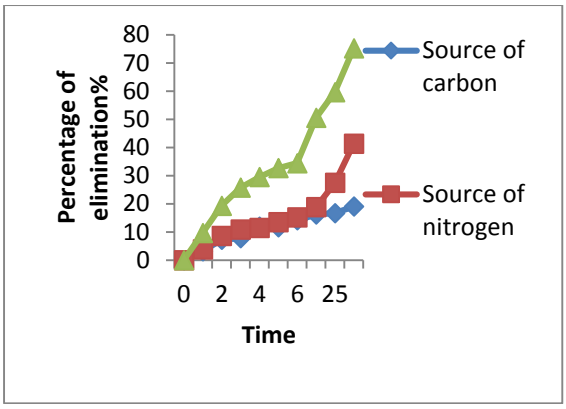

Figure 8: *The degradation of methylene blue by Acinetobacter Johnson II as a function of time and the source of carbon and nitrogen.*

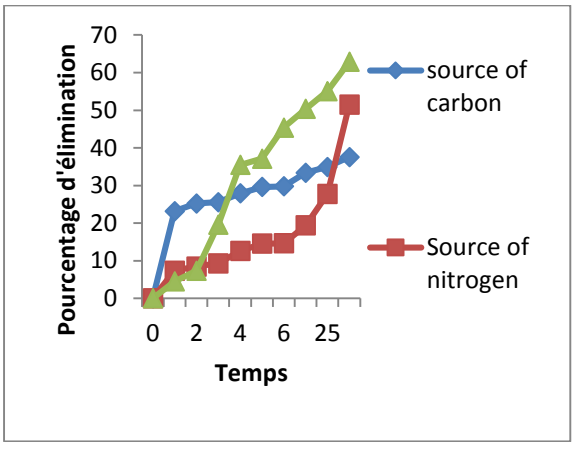

Figure 9: The degradation of methylene blue by *Bacillus weihenstephanensis* in function of the time and the source of carbon and nitrogen.

IV.Conclusion

The present work is a contribution to the study of the degradation of methylene blue by bacterial strains isolated from the peel of red beet; the two selected bacteria were identified as Acinetobacter Johnson II BP1, Bacillus weihenstephanensis BP2.

The degradation of the dye is affected by several parameters such as the initial concentration, the pH, the temperature and the addition of the carbon source and the nitrogen source.

The elimination rate of the dye decreases with the increase of the concentration as well it decreases with the addition of glucose and nitrogen in the medium.

The best biodegradation is obtained for a methylene blue concentration of 5 mg / l at a pH of 7 and a temperature of 37 °C by bacillus weihenstephanensis.

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Please cite this Article as:
Abai N., Abed F., Babakhouya N., Benrachdi K., The degradation of methylene blue by bacterial strains isolated from the peel of Red Beet , ***Algerian J. Env. Sc. Technology, 4*:3 (2018) 794-799**