



ISSN: 0067-2904

Bacterial Biodegradation of Congo Red Dye Using Local Bacterial Isolates

Saad Hussein Khudhair*, Duha Bahaa Mohammed Al-Fayaad
Al-Karkh University of Science, -College of Science, -Department of Microbiology

Received: 15/1/2022

Accepted: 20/4/2022

Published: 30/11/2022

Abstract:

The current study aims to get local bacterial isolates isolated from wastewater samples, with the highest ability to decolorize one selected Azo dye (Congo red as a model), and then test its ability to decompose Congo red dye, to obtain the most efficient bacterial isolate.

Four samples of wastewater collected from sewage transport pipes were used for the bacterial isolation. Forty-two bacterial isolates were obtained after inoculating these samples in the liquid MS medium, pH 7 with 1% glucose, and then on solid MS medium supplemented with 50 ppm of Congo red dye. Results from primary tests showed that only eighteen bacterial isolates own varying abilities to decolorize Congo red dye and the isolates WR7, WR18 and WR30 give high clear zones (20 mm).

The secondary screening was achieved to determine the most efficient isolates to degrade the Congo red dye. The results indicated that the isolates WR7, WR18, and WR30 appeared to have the highest ability to decolorize Congo red dye reaching 88.6%, 83.9%, and 92.8%, respectively.

The results from the optimum conditions experiment revealed that the isolates WR7, WR18 and WR30 appeared to have the highest ability to degrade dye reached 88.7%, 83.9%, and 92.9% respectively when 100 ppm of Congo red dye was used after 3 days of incubation as compared with other studied concentrations. While the second step in this experiment confirmed that the isolate WR30 has the highest percentage to degrade the Congo red dye during all incubation periods, which reached 14.6%, 42.7%, 92.9%, 92.4% and 91.8% after 1, 2, 3, 4, and 5 days of incubation when 100 ppm of Congo red dye was used respectively as compared with WR7 and WR18 isolates which showed lower values of degradation for all studied periods.

The three isolates which have the highest capability to decolorize Congo red dye were identified according to morphological, physiological and biochemical tests. The results indicated that the isolates WR7, WR18 and WR30 were *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Bacillus cereus* respectively.

Keywords: Congo red, Decolorization, Wastewater, *Bacillus* and *Pseudomonas*.

التفكك الحيوي البكتيري لصبغة الكونغو الحمراء باستخدام عزلات بكتيرية محلية

سعد حسين خضير* ، ضحى بهاء محمد الفياض

قسم الاحياء المجهرية، كلية العلوم، جامعة الكرخ للعلوم ، بغداد، العراق

*Email: saad_2019@kus.edu.iq

الخلاصة

تهدف الدراسة الحالية إلى الحصول على عزلات بكتيرية محلية معزولة من نماذج مياه الصرف الصحي، حيث تمتلك قدرة عالية على إزالة صبغة واحدة مختارة من نوع Azo (صبغة الكونغو الحمراء كموديل)، ومن ثم اختبار قدرتها على تحلل صبغة الكونغو الحمراء لغرض الحصول على أكثر عزلة بكتيرية كفوءة.

جمعت أربع عينات من مياه الصرف الصحي من انابيب نقل مياه المجاري واستخدمت للعزل البكتيري، تم الحصول على 42 عزلة بكتيرية بعد تلقيح هذه العينات في وسط الاملاح المعدنية السائل ذو الرقم الهيدروجيني 7 والحاوي على 1 % كلوكوز ومن ثم على وسط الاملاح المعدنية الصلب المدعم بواسطة 50 جزء من المليون من صبغة الكونغو الحمراء. أظهرت نتائج الاختبارات الأولية أن ثمانية عشر عزلة بكتيرية فقط تمتلك قدرات متفاوتة لإزالة لون صبغة الكونغو الحمراء وأن العزلات WR7 و WR18 و WR30 اعطت اعلى مناطق شفافة بلغت (20 ملم).

اجريت غرلة ثانوية لتحديد العزلات الاكثر كفاءة في تفكيك صبغة الكونغو الحمراء, اشارت النتائج إلى أن العزلات WR7 و WR18 و WR30 اظهرت القابلية الاعلى على إزالة لون صبغة الكونغو الحمراء بنسبة بلغت 88.6% و 83.9% و 92.8% على التوالي.

أظهرت النتائج من تجربة الظروف المثلى أن العزلات WR7 و WR18 و WR30 أبدت أعلى قدرة على تفكيك الصبغة بلغت 88.7% و 83.9% و 92.9% على التوالي عند استخدام 100 جزء من المليون من صبغة الكونغو الحمراء بعد 3 أيام من الحضانة مقارنة مع باقي التراكيز المدروسة، بينما أكدت الخطوة الثانية من هذه التجربة أن العزلة WR30 اعطت اعلى نسبة مئوية لتفكيك صبغة الكونغو الحمراء وخلال جميع فترات الحضانة، فقد بلغت 14.6% و 42.7% و 92.9% و 92.4% و 91.8% بعد 1 ، 2 ، 3 ، 4 و 5 يوم من الحضانة عند استخدام 100 جزء من المليون من صبغة الكونغو الحمراء وعلى التوالي مقارنة مع العزلات WR7 و WR18 والتي اظهرت قيم تفكيك أقل ولجميع الفترات المدروسة.

شخصت العزلات الثلاثة والتي ابدت القابلية الاعلى على إزالة لون صبغة الكونغو الحمراء وحسب الاختبارات المورفولوجية والفسولوجية والكيموحياتية، أشارت النتائج إلى أن العزلات WR7 و WR18 و WR30 تعود الى *Bacillus subtilis* و *Pseudomonas aeruginosa* و *Bacillus cereus* على التوالي.

Introduction:

The dyes were extracted and manufactured from vegetable sources at the start of their use. Thereafter with their increasing needs in industries, they were industrially produced to cover the increasing market demand [1]. The expansion of industries and consequently the increase in the types and quantities of dyes led to the creation of many environmental problems, especially for the aquatic environment as sewage water containing these industrial dyes, was released into the rivers and seas [2].

Bioremediation is the process that uses organisms such as bacteria, molds, yeasts, algae and others to clean up sites contaminated with pollutants. This process has been applied to degrade many pollutants in soil and wastewater. It is superior when compared to the chemical and physical methods because of its lower cost and good efficiency in biological treating units to remove contaminants [3]. All synthetic dyes own complex chemical structures and are highly resistant to natural degradation. Around 280,000 tons of textile dyes are discharged into the water bodies every year worldwide [4].

Azo are one of the textile dyes widely used in industries. These dyes contain one or more Azo bonds (-N=N-) in their structure, about 70% of dyestuff used in textile processing are Azo dyes because of their low price, good stability and availability of different colours as

compared to the other dyes, the textile industry uses around 70% Azo dyes. Therefore, about 10 – 15 % of them are discharged into the water bodies as effluent causing toxicity problems to the water ecosystem [5, 6].

Using microorganisms such as bacteria and fungi, many studies have successfully achieved to decolorize or degraded different types of Azo dyes. With the aim of developing wastewater treatment techniques by safe method, other researchers have also proven that the study of the biodegradation process of Azo dyes has many benefits [7,8,9]. Various microorganisms, including bacterial and fungal isolates, have been used to treat Azo dyes as their widespread use in the textile industry has badly affected the aquatic environment. Many researchers have used different species of bacteria such as *Bacillus*, *E. coli*, *Klebsiella*, *Enterobacter*, and *Pseudomonas* to decolorize Congo red dye [10- 15].

The present study focuses on obtaining local bacterial isolates capable of decolorizing Congo red dye as a model for the biodegradation process of Azo dyes, in order to select the most active isolate and study some factors affecting the biodegradation of this dye.

Materials and methods:

Samples collection:

Clean and autoclave containers were used to collect four samples of wastewater and sludge from different sites of pipelines that transport wastewater in Al-Ghazaliya city situated on the outskirts of Baghdad in Iraq.

Bacterial isolation and screening:

The medium used for bacterial isolation, characterization, and screening for dye biodegradation is solid mineral salts medium (MSM) with 1% glucose, pH 7 with some modification according to the purpose of the research.

Subsequently, 1 ml from each sample was added to flasks containing 50 ml sterilized liquid MS medium. The flasks were incubated at 37 °C for 2 days in a shaker incubator at 150 rpm, and thereafter 1 ml from each flask was spread on solid MS medium and incubated at 37 °C for 3 days. The bacterial colonies were purified by transferring the samples to new plates of solid MS medium.

To perform an initial examination and to determine the ability of bacterial isolates to breakdown the dye, the poured bacterial colonies were re-culturing in liquid MS medium with 1% glucose at 37 °C for 2 days., Then 1 ml from each flask was re-spread on solid MS medium supplemented with 50 ppm Congo red dye and the plates were incubated at 37 °C for 3 days. Subsequently, the colonies that caused a clear zone on the medium were selected [16].

The second step of screening to decolorize Congo red dye by bacterial isolates was achieved in liquid MS medium, pH 7 supplemented with 50 ppm Congo red dye, flasks were inoculated with 1 ml bacterial growth from each bacterial isolate (triplicate for each) and incubated at 37 °C for 3 days in a shaker incubator at 150 rpm. Later on 5 ml from each flask was withdrawn and was centrifuged at 8000 rpm for 10 min to get the supernatant of the free cells. The absorbance of test samples and control were measured at 490 nm using a UV-Visible spectrophotometer and the percentage of decolorization was determined using the below equation [17].

$$\text{Decolorization (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Optimum conditions to decolorize Congo red dye:

The effect of dye concentration on Congo red dye decolorization was evaluated in the liquid MS medium, pH 7, supplemented with different concentrations (25, 50, 100, 150, 200, and 250 ppm), flasks were inoculated with 1 ml of bacterial growth from the most active bacterial isolates (triplicate for each) and incubated at 37 °C for 3 days in a shaker incubator with 150 rpm, 5 ml from each flask was later withdrawn and centrifuged at 8000 rpm for 10 min to get the supernatant of the free cells. The absorbance of test samples and control, prepared as the sample test without bacterial inoculums, was measured at 490 nm using a UV-Visible spectrophotometer and the percentage of decolorization was determined using the above equation.

Also, the effect of incubation periods on Congo red decolorization was investigated in liquid MS medium, pH 7 supplemented with 100 ppm Congo red dye. Flasks were inoculated with 1 ml of bacterial growth from each isolate (triplicate for each) and incubated at 37 °C at different periods (1, 2, 3, 4 and 5 days) in a shaker incubator at 150 rpm. After each period, 5 ml from each flask was withdrawn and centrifuged at 8000 rpm for 10 min to get the supernatant of the free cells. The absorbance of test samples and control were measured at 490 nm using a UV-Visible spectrophotometer and the percentage of decolorization was determined using the same previous equation [17, 18].

Identification of decolorizing bacterial isolates:

The highest decolorized bacterial isolates were identified based upon macroscopic characteristics (such as the color, shape, texture, and transparency of colonies) and microscopic characteristics (Gram staining, capsule staining, and endospore staining), and biochemical tests (motility, catalase, oxidase, indole, and others). All the identification procedures were performed by methodologies reported in the Bergey's manual of determinative bacteriology [19].

Results and discussions:

Bacterial isolation and, screening of dye degradation:

Using mineral salts medium with 1% glucose, forty-two bacterial isolates were obtained from the four wastewater samples, as shown in the Table 1 below. The wastewater samples are considered a good source for obtaining different types of bacterial isolates that are capable of degradation or consuming many organic pollutants, including industrial dyes [20].

Table 1: Bacterial isolates obtained from four samples of wastewater from Al-Ghazaliya city, Baghdad

Symbol of sample	Number of isolates	Symbol of sample	Number of isolates
Samp.1	13	Samp.3	10
Samp.2	12	Samp.4	7

The primary results showed that only 18 isolates were able to degrade the Congo red dye when using the solid medium of mineral salts supplemented with 1% Congo red dye, which gave a clear zone ranging from 8-20 mm, Table 2. The biological processes to decolorize and degrade the industrial dyes are considered a friendly method for the environment and have shown many advantages when compared with chemical and physical methods [21]. Therefore, much research has been conducted to collect the bacterial isolates which decolorize the dyes from different environmental samples [22, 23]. When [18], the spread plate method was used

to obtain 14 bacterial isolates, results revealed that the AMS-XIII isolate exhibited maximum decolorization with 96.81% [24]. Results were qualified to isolate twenty-two bacterial isolates from soil samples contaminated with Azo dyes, where isolate SKB16 showed the highest ability to decolorize and degrade two types of Azo dyes reaching up to more than 91% [25].

Table 2: The ability of bacterial isolates (Primary screening) to decolorize Congo red dye using mineral salts medium with 50 ppm of Congo red dye.

Symbol of sample	Symbol of isolate	Clear zone (mm)	Symbol of sample	Symbol of isolate	Clear zone (mm)
Samp. 1	WR 1	9	Samp. 3	WR 28	12
	WR 4	12		WR 30	20
	WR 7	20		WR 32	15
	WR 11	14		WR 33	9
Samp. 2	WR 14	8	Samp.4	WR 35	10
	WR 17	15		WR 36	9
	WR 18	20		WR 37	16
	WR 23	16		WR 39	12
	WR 25	9		WR 42	11

Also, the results from the second step of screening revealed that the best dissociation of the Congo red dye was obtained by isolates WR7, WR18, and WR30 respectively, where the percentage of decolorization values reached 88.6%, 83.9% and 92.8% respectively, as compared with the results of other studied isolates, Table 3.

Table 3: Secondary screening of bacterial isolates to degrade Congo red dye using mineral salts medium with 1% glucose

NO.	Symbol of isolate	Decolorization (%)	NO.	Symbol of isolate	Decolorization (%)
1	WR 1	44.9	10	WR 28	61.8
2	WR 4	65.6	11	WR 30	92.8
3	WR 7	88.6	12	WR 32	72.6
4	WR 11	71.5	13	WR 33	42.6
5	WR 14	25.3	14	WR 35	27.8
6	WR 17	72.9	15	WR 36	50.7
7	WR 18	83.9	16	WR 37	75.4
8	WR 23	77.1	17	WR 39	67.4
9	WR 25	28.4	18	WR 42	53.4

[17] Used several wastewater samples to obtain the bacterial isolates and studied their ability to decolorize the Congo red dye. The ability of 30 isolates to decolorize the Congo red dye was achieved using different steps of screening. Isolates MAM-B22, MAM-C9, MAM-29,

and MAM-B11 have shown maximum decolorization that reached 90.21%, 90.03%, 89.62%, and 85.84% respectively using 100 ppm of Congo red dye after 3 days of incubation at 37°C[26]. Also, [25] *Bacillus* sp. The isolate was used to decolorize Congo red dye by using a liquid medium with 25 ppm Congo red dye. The results indicated that the highest percentage of decolorization (83.12%) occurred after 5 days of incubation at 30 °C [27]. Aydin *et al.* (2021) used four different *Bacillus* spp. (*Bacillus* sp, *B. cereus*, *B. mycoides*, and *B. subtilis*) isolates to decolorize and degrade six different types of Azo dyes., The results obtained from this study indicated that all studied isolates were able to decolorize the six Azo dyes with high rates of decolorization that were higher than 98% [26].

Optimum conditions to decolorize Congo red dye:

The results of studying the effect of the concentration of Congo red dye on the efficiency of decolorization indicate that the studied isolates gave a clear variation in the decolorization susceptibility to all studied concentrations, where isolate WR30 showed the best decolorization results at all the studied concentrations, where the decolorization reached 96.8% and 92.8% at the concentrations 25 and 50 ppm respectively. While the isolates WR7 and WR18 gave 94.3% and 92.6% at 25 ppm respectively, and 88.6% and 83.9% at 50 ppm. The results also showed that the rate of decolorization decreases gradually with increasing the concentration of the dye to a concentration of 100 ppm and then the stability occurs at the rate of decolorization for other concentrations, Figure 1.

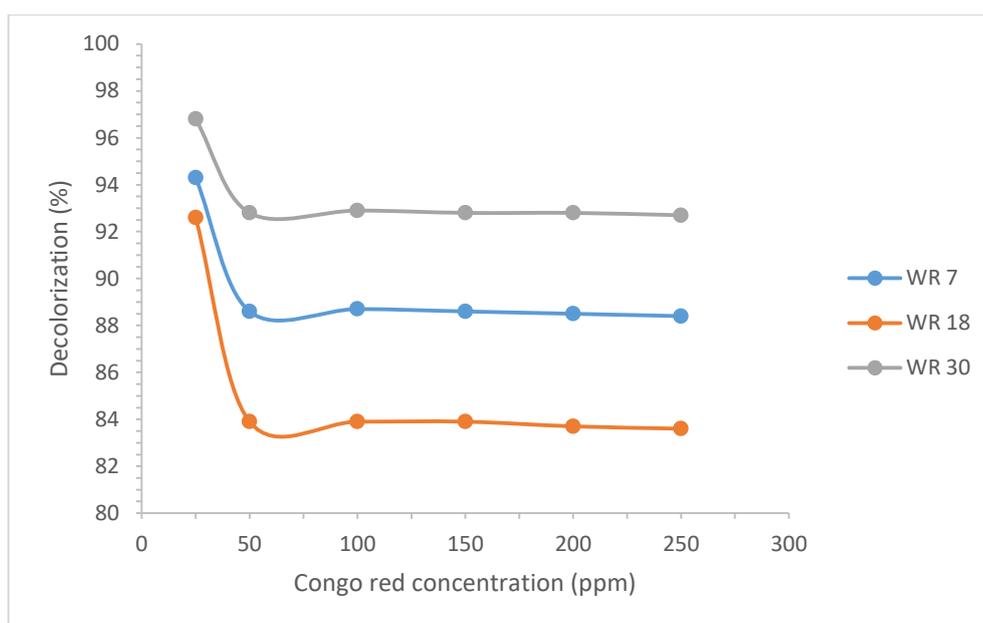


Figure 1: Effect of Congo red concentration on the efficiency of Congo red dye decolorization.

The results in Figure 2 indicate that the decolorization rate of Congo red dye was low on the first and second day of incubation by all studied isolates. The best decolorization rate was obtained on the third day of incubation for all isolates, Whereas incubation. Whereas, upon the other incubation periods (after 4 and 5 days), the rate of decolorization was similar to the third day in all of the studied isolates. Furthermore, the WR30 isolate gave the highest decolorization rate that reached 92.9% as compared to isolates WR7 and WR18, which gave 88.7% and 83.9% respectively.

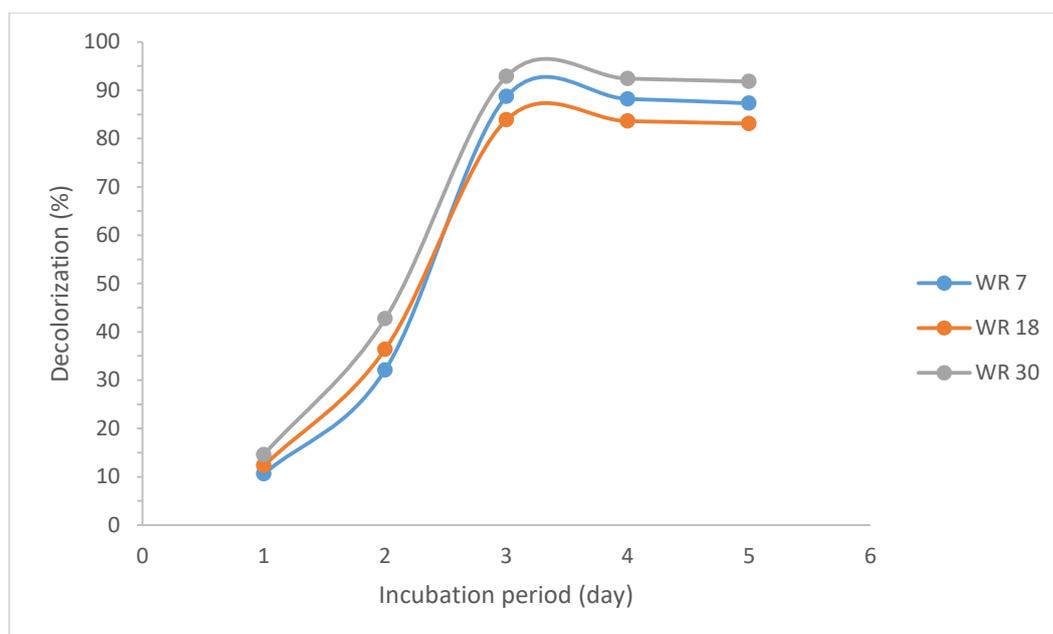


Figure 2: Effect of incubation periods on the efficiency of Congo red dye decolorization.

The rate of decolorization and degradation of dyes can be influenced by many factors such as temperature, pH, time and dye concentration. The type of bacteria also influences the rate of degradation. Therefore many previous studies have shown that the rate of decolorization decreased gradually by increasing the dye concentration, this can either be because high concentrations of dyes lead to toxicity in living cells, or that the high concentrations of dyes require the use of higher biomass as an inoculum for growth medium [27,28]. Sciences were studied [29] the effect of dye concentration on the rate of decolorization using isolate *Escherichia coli* NG188, the results showed that the rate of decolorization of the studied dye decreased with increasing concentration and that the highest decolorization was 59%, while the lowest decolorization was 35% when using concentrations 50 and 1000 ppm respectively [29]. In a previous study, the bacterial isolate *Acinetobacter baumannii* YNWH 226 was used to decolorize and degrade the Congo red dye using a liquid growth medium containing three different concentrations of Congo red dye (100, 200, and 500 ppm), where the bacterial isolate showed a good ability to decolorize the two concentrations (100 and 200 ppm) at a high decolorization rate reached to 98.62% and 96.31% respectively after two days of incubation, while the third concentration (500 ppm) was decolorized with 80.73% only after the same period [30].

Identification of best isolates:

According to results obtained from macroscopic characteristics, biochemical tests and microscopic characteristics, for the isolates WR7, WR18, and WR30, the three Isolates with the highest efficiency were identified as *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Bacillus cereus* respectively.

Conclusions:

The results of the current study indicate that wastewater samples are a good source in obtaining local bacterial isolates capable of decolorizing the Congo red dye. These isolates showed a discrepancy in their ability to consume the Congo red dye. This may be due to the toxic effect of this dye.

References:

- [1] A. K. Pandey, DV. Sarada, and A. Kumar, "Microbial decolorization and degradation of reactive red 198" *Proceedings of the National Academy of Sciences, India*, pp. 805–15, 2016.
- [2] MA. Hassaan, and AE. Nemr, "Health and Environmental Impacts of Dyes: Mini-Review". *American J Environ Sci Eng*. Vol. 1, pp. 64–76, 2017.
- [3] G. Nanis, and Allam, "Bioremediation efficiency of heavy metals and Azo dyes by the individual or consortium bacterial species either as free or immobilized cells: A comparative study". *Egypt. J. Bot.*, vol. 57, pp. 555 – 564, 2017.
- [4] L. Zhao, M. Lu, S.C. Liang, L.Y. Zhao, D.B. Li, and B.B. Zhang, "Production and synthetic dyes decolorization capacity of a recombinant laccase from *Pichia pastoris*" *J. Appl. Microbiol.*, vol. 107, pp. 1149-1156, 2009.
- [5] R.G. Saratale, G.D. Saratale, D.C. Kalyan,, J.S. Chang, and S.P. Govindwar, "Enhanced decolorization and biodegradation of textile azo dye Scarlet R by using developed microbial consortium-GR". *Bioresour. Technol.*, vol. 100, pp, 2493–2500, 2009.
- [6] N. Loncar, N. Gligorijevic, N. Bozic, and Z. Vujcic, "Congo red degrading laccases from *Bacillus amyloliquefaciens* strains isolated from salt spring in Serbia". *Int. Biodeterior. Biodegrad.*, vol. 91, pp. 18–23, 2014.
- [7] [M. E. M. Mabrouk](#), and [H. H. Yusef](#), "Decolorization of fast red by *Bacillus subtilis* HM. " *J. Appl. Scien. Res.*, vol. 4, pp. 262-269, 2008.
- [8] M. Gou, Y. Qu, J. Zhou, F. Ma, and L. Tan, "Azo dye decolorization by a new fungal isolate, *Penicillium* sp. QQ and fungal-bacterial cocultures". *J. Hazard. Mater.*, vol. 170, pp. 314-319, 2009.
- [9] M. Leelakriangsak and [S. B. Songklanakarin](#), "Characterization of the decolorizing activity of azo dyes by *Bacillus subtilis* azoreductase AzoR1". *J. Sci. Technol.* vol. 34, no. 5, pp. 509-516, 2012.
- [10] [10] K.C. Chen, J.Y. Wu, D.J. Liou, and S.C.J Hwang, "Decolorization of the textile dyes by newly isolated bacterial strains". *J. Biotechnol.* Vol. 101, pp. 57 -68, 2003.
- [11] A.C. Jalandoni-Buan, A. Decena-Soliven, E.P., Cao, V.L. Barraquio, and [W. L. Barraquio](#), "Characterization and Identification of Congo red decolorizing bacteria from monocultures and consortia. *Philipp. J. Sci.*, vol. 139 , no. 1, pp. 71-78, 2010.
- [12] K. Perumal, R.B. Malleswari, A. Catherin, and T.A.S. Moorthy, "Decolourization of Congo Red dye by bacterial consortium isolated from dye contaminated soil" *J. Microbiol. Biotech. Res.*, vol. 2, no. 3, pp. 475-488, 2012.
- [13] [S. S. Jaiswal](#), and [A. V. Gomashe](#), "Bioremediation of textile azo dyes by newly isolated *Bacillus* sp. from dye contaminated soil" *Intern. J. Biotech. Biochem.* vol. 13, no. 2, pp. 147-153, 2017.
- [14] [Y. N. Shukur](#), "Determination of Optimal Conditions for the Production of Laccase Enzyme by Local Isolate of *Bacillus* sp. " *Iraqi Journal of Science*, vol 56, no.1A, pp. 132-139, 2015.
- [15] [A. K N. Khudair](#), and [S. S. Mahmood](#) "Detection of the Antiseptic Resistance Gene among *Pseudomonas aeruginosa* isolates" *Iraqi Journal of Science*, vol. 62, no. 1, pp. 75-82, 2021.
- [16] [R. A. Hashem](#), R. Samir, [T. M. Essam](#), [A. E. Ali](#), and [M. A. Amin](#), "Optimization and enhancement of textile reactive Remazol black B decolorization and detoxification by environmentally isolated pH tolerant *Pseudomonas aeruginosa* KY284155" *AMB Expr.*, vol. 8, no. 83, pp. 1-12, 2018.
- [17] [M. A. M. Abo-State](#), [Y. E. Saleh](#), and [H. A. Hazaa](#), "Decolorization of Congo red dye by bacterial isolates" *J. Eco. Heal. Env.*, vol. 5 no. 2, pp. 41-48, 2017.
- [18] S. Kumari, and P. Rajoriya, "Textile Industrial effluent treatment by Azo dye decolorizing bacterial consortium" *Int. J. Curr. Microbiol. App. Sci.*, vol. 8, no. 11, pp. 884-907, 2019.
- [19] J.G. Holt N.R. Krig, P.H.A. Sneath, , J.T. Staley and S.T. Williams, "Bergey's manual of determinative bacteriology (9th edn) " *Baltimore, Maryland: Williams and Wilkins*. 1994.
- [20] M.B. Kurade, T.R. Waghmode, R.V. Khandare, B.H. Jeon, and S.P. Govindwar, "Biodegradation and detoxification of textile dye Disperse Red 54 by *Brevibacillus laterosporus* and determination of its metabolic fate" *J. Biosci. Bioeng.*, vol. 121, pp. 442-449, 2016.
- [21] C.G. Whiteley, "Bioremediation of textile dyes" *Ind. Bioproc.*, vol. 29, pp. 7, 2007.
- [22] A. Pandey, P. Singh, and L. Iyengar, "Bacterial decolorization and degradation of azo dyes. *Int. Biodeterior. Biodegrad.*, vol 59, pp. 73–84, 2007.

- [23] [S. S. Jaiswa](#), and [A. V. Gomashe](#), "Bioremediation of textile azo dyes by newly isolated *Bacillus* sp. from dye contaminated soil" *Int. J. Biotech n. Biochem.*, vol. 13, no. 2, pp. 147-153, 2017.
- [24] S. Thangaraj, B. Jooju, and [S. K. Sadasivam](#), "Decolourization of textile Azo dyes by a newly isolated bacterial strain" *Appl. Ecol. Environ. Sci.*, vol. 9, no. 2, pp. 203-208, 2021.
- [25] [K. K. Aqilah Hanis](#), [AR. M. Nasri](#), [WK. W. Farahiyah](#), and MY. M. Rabani, "Bacterial degradation of Azo dye Congo red by *Bacillus* sp." *J. Phys.: Conference Series*: pp. 1-8, 2020.
- [26] M. Aydın, A. Ağaoğlu, and Ö. Barış, "Azo dye decolorization by using four different psychro-tolerant *Bacillus* species" *NEsciences*, vol. 6, no. 1, pp. 19-29, 2021.
- [27] EZ. Gomaa, "Biodegradation and detoxification of Azo dyes by some bacterial strains" *Microbiology J.*, vol. 6, no. 1, pp. 15–24, 2016.
- [28] S. Chakraborty, B. Basak, S. Dutta, B. Bhunia, and A. Dey, "Decolorization and biodegradation of Congo red dye by a novel white rot fungus *Alternaria alternate* CMERI F6" *Bioresour. Technol.*, vol. 147, pp. 662–666, 2013.
- [29] D. E. El-Badan, N. G. Naga, M. E. Mabrouk, and [K. M. Ghanem](#), "Decolorization of Remazol Brilliant Blue dye by *Escherichia coli* NG188 under optimized conditions" *Egypt. J. Bot.*, vol. 60, no. 2, pp. 461-475, 2020.
- [30] [R. Li](#), [X. Ning](#), J. Sun, [Y. Wang](#), J. Liang, M. Lin, and Y. Zhang, "Decolorization and biodegradation of the Congo red by *Acinetobacter baumannii* YNWH 226 and its polymer production's flocculation and dewatering potential" *Bioresour. Technol.*, vol. 194, pp. 233-239, 2015.