EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology 2023, 24(4), 27–34
https://doi.org/10.12912/27197050/162106
ISSN 2719-7050, License CC-BY 4.0

Received: 2023.01.27
Accepted: 2023.03.27
Published: 2023.04.10

A Healthy Environmental Evaluation Study of the Antibacterial Activity of Polyethylene Oxide Against Some Bacteria Isolated from Azo Dyes

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ABSTRACT

The maintenance of the human body, including any actions that may be taken to keep it free from disease and intoxication and to facilitate access to treatment, are all part of good health. Having a wide variety of molecular weights, polyethylene oxide (PEO) is a hydrophilic, uncrosslinked, nonlinear system polymer. It's made from ethylene oxide, which has a lot of advantages for medicine administration and antimicrobial purposes. Polyethylene oxide bactericidal activity at different PEO concentrations value (80, 40, 20 and 10 µg/ml) against five isolates of *Bacillus cereus* isolated and identification from azo dye is investigated in this work (random selection from total isolates). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each isolate were calculated, and PEO's antibacterial activity was evaluated using the disk diffusion test. 85 Bacillus cereus isolates were collected from total azo dyes, PEO has a broad-spectrum antibacterial effect against tested bacteria, with an inverse connection between inhibitory zone diameter and PEO concentration, also even exceeds the activity of some drugs. The MICs of PEO ranged from 10 to 20 µg/ml, with MBCs ranging from 20 to 80 µg/ml. In other trials, PEO was shown to be strongly attached to bacterial cells, which might explain its effect on bacterial inhibitory growth and their invasion. At an appropriate concentration, PEO significantly inhibited bacterial growth. To avoid the development of antibiotic-resistant bacterial strains, it is strongly recommended that PEO be used as a cost-effective antibacterial agent, particularly when mixed with deys used at home or in enterprises.

Keywords: polyethylene oxide, antibacterial activity, *Bacillus cereus*, Azo dyes.

INTRODUCTION

The term azo comes from the French azote, which comes from the Greek a (not) zoe (to live). Its discovery of azo dyes was a watershed moment in the history of the chemical industry. Azo dyes comprise one or more azo linkages and are made up of a diazotized amine linked to an amine or aphenol. Aromatic amines are the most important precursors for azo dyes. Azo dyes are chemical molecules with the functional group R-N=N-R′, with R and R′ generally being aryl. They are azo compounds with the bond C-N=N-C, which are commercially relevant [IUPAC, 2014]. Azo pigments are chemically related to azo dyes and are insoluble in water and other solvents [Fuck WF et al., 2018, Ortega-Gómez et al., 2015]. Although many azo pigments are non-toxic, some are mutagenic and carcinogenic, such as di-nitro aniline orange, ortho-nitroa niline orange, or pigment orange [Harrington et al., 2015, Engel et al., 2007]. Azo dyes are the most common and versatile dyes, accounting for more than half of all dyes made globally. Azo dyes produced from

benzidine are carcinogenic, and exposure to them has been linked to bladder cancer in the past. As a result, in the 1980s, "the most important Western industrialized countries" stopped producing benzidine azo dyes [Puvaneswari, 2007].

At least 3,000 azo dyes were available in the past and were widely used to color textiles, leather, some foods, coloring pens, shoes, printing inks, paints, varnish, lacquer, tattoo inks, cosmetics, hair dyes, waxes, and wood, some of which probably a risk to human health and environmental due to their toxic and harmful properties also contamination by various organisms [Innocenti and Breton, 2020, Anon, 1996].

This problems of azo dyes can moving by existence many microorganisms like bacteria, fungi have been reported to eco-friendly and other may case infection disease [Yu, 2007]. Many of these organisms such bacteria are pathogenic and caused infection to humans in very high, especially these dyes are used daily by humans and children. Only a few aerobic bacteria, such as *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Enterococcus fecalis*, *Escherichia coli*, *Streptomycescereviceae*, and *Candidazeylanoides*, have been discovered in azo dyes under aerobic conditions [Ito et al., 2006, Liu et al., 2007, Shamloo et al., 2015, Wang et al., 2007, Liger et al., 2004, Šlosarčíková et al., 2020].

Dye bacteria from textile azo dyes have been isolated and described in a number of investigations [Domagk et al., 1935, Ellis et al., 2011, Miller et al., 1957, Yang et al., 2019, Polman EMN et al., 2021, Leena et al., 2008]. Many investigations isolated bacteria (*Bacterium firmus* and *Halomonas* sp., respectively) and other dye bacteria (*Pseudomonasaeruginosa*) [Feng et al., 2012] and *Comamonas* sp. UVS [Gueugnon et al., 2013]. However, due to the alkaline environment, only a few studies have been done to extract and describe bacteria that have contaminated the azo dye [Xue Zet al., 2015, Quémener Det al., 2005, Bates et al., 2015] This environment necessitates the existence of alkaliphilic and halophilic bacteria that can adapt and operate physiologically under such extreme circumstances. Humans are exposed to xenobiotics such as azo dyes by food, skin contact, or inhalation, and some pathogenic microorganisms found in azo dyes can cause illness [Neugebauer, 2007].

Electrical composites with cosmetology (emulsions, personal lubricants, skin creams), gene therapy, pharmaceutical products and carbon black, are just a few of the possibilities for polyethylene oxide (PEO), a non-toxic, biocompatible, and water-soluble polymer [Feng et al., 2012, Zhou et al., 2014, Liao et al., 2014, Liu et al., 2012, Johnson et al., 2010, Radder et al., 1996, Gasteier et al., 2007]. It has been investigated how to enhance and adjust the capabilities of PEO-based graft copolymers as well as a variety of possible properties [Johnson et al., 2010, Radder et al., 1996]. These materials have been used for biomedical implants, lithium batteries, elastomer production, drug delivery systems [CLSI, 2020], and nanotechnology [Radder et al., 1996, Gasteier et al., 2007].

The objective of this study is to isolate and identify microorganisms that have contaminated various azo dyes can used daily by human like lether clothes dyes, shoes dyes, childrens coloring pens may be cause toxicity and many human infection, and using PEO as antibacterial to proofing their capacity to lessen azo dyes bacteria to attempt to mixing any type of polymer with azo dyes in their industry to reduce the human infection by bacteria.

MATERIALS & METHODS

Samples collection

The 100various samples, specimens were gathered of coloring dyes, dyes shoes and leather clothes dyes). Azo dye samples are collected, and the samples size included total azo dye samples (N=100) these were from shoos dye (N=35), from lether dyes sample (N=44), and from children coloring pens (N=21). Azo dyes collected from different locations in many house in Al-Hilla city. Various culture medium, including blood agar and Macconkey agar plates, were utilized for 24–48 hours at 37 °C as part of standard microbiological protocols must isolate and purify these samples in order to identify the species of bacteria present. All of the isolates were validated by the VITEK-2 compact system (Biomérieux).

Media and solution

We bought Mueller-Hinton media and Mueller-Hinton agar from Hi-Media (Mumbai, India). Different concentrations of PEO (80, 40, 20 and 10 g/ml) were provided by (Zhengzhou Dongyao Co., Ltd., China). Different antibiotic disks

Nitrofurantion (F-300), Cefotaxime (CTX-30), Aztronam (ATM-30), Chloramphenicol (C-30), Amoxicilin (AMC-30), and Mrthicillin (ME-5) were purchased from (Bioanalyse, Turkey).

Overview of the study design and data collection

An observational cross-sectional research was conducted. A number of azo dye samples were collected from homes as part of the study to identify the species of pathogenic bacteria that may cause illness in humans, particularly youngsters. To collect and deliver them to the DNA research facility, sterile bags were employed. A sterile cotton swab dampened with sterile normal saline was used for sampling. Using different swabs, numerous samples of each color were obtained, and they were then promptly inoculated in culture media (Blood agar, MacConkey, and Chocolate agar). were injected onto common culture medium utilizing various bacterium media. Swabs were inoculated in bacterial medium, and plates were then incubated for 24–48 hours at 37 °C. Gram staining from swabs and culture plates was done for differentiation. These samples were then thoroughly defined and identified by VITEK-2 system compact in AL-Hilla teaching hospital compact. Before collecting the samples, put on gloves to prevent contamination. All tests may be completed quickly, safely, and easily.

Susceptibility test for antibiotics

Utilizing Kirby-Bauer disk diffusion methods, the clinical laboratory standard Institute (CLSI) 2018 guidelines were applied to assess antibiotic susceptibility. The six antibiotics utilized to assess the drug resistance of five Bacillus cereus isolates included Nitrofurantion, Cefotaxime, Aztronam, Cephalosporins, Amoxicilin, and Mrthicillin (chosen randomly bacteria from all total isolate). The percentage of resistant isolates among all discovered bacterial isolates served as an expression of the findings. MDR is characterized as having three or more antimicrobial classes of resistance. MDR is represented by the bacterial strains.

Antibacterial properties of PEO

PEO's antibacterial abilities were tested against a number of human pathogens that were grown on nutritive agar slants with azo dyes. While evaluating antibiotic activity, the guidelines provided by the Clinical and Laboratory Standards Institute were adhered to [CLSI, 2012]. Using a disk diffusion experiment, triplicates are employed in dilutions of PEO concentrations [80, 40, 20 and 10 g/ml] in solvent to assess antibiotic sensitivity and PEO against the study microorganisms. The first step, the isolates were incubated for 15 min at room temperature, then incubated at 37 °C overnight when treated bacterial isolates with PEO against bacterial isolates under study. A digital Vernier caliper used to measure the inhibitory zone's breadth after an incubation period during which the inhibition zone could be visible around the well [CLSI, 2016]

The determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) [CLSI, 2006]

Before being used to create 0.5 McFarland, the bacterial isolates were grown overnight at 37 °C. After making a total of 10ml tube nutritional broth medium, each sample was infected aseptically with 1ml of the applicable bacterial suspension (approximately $1.5 \cdot 10^8$ CFU/mL). In addition to four PEO dilutions (80, 40, 20 and 10 µg/ml) prepared in solvent, The absence of PEO was employed as a negative control. Each isolate underwent three examinations. At 37 °C, the infected sections were incubated over night. The visual turbidity in all tube was assessed following the incubation time. The lowest broth concentration without turbidity is what the MIC for the tested isolates. Nutrient agar plates were used to cultivate turbidity-free tubes at 37 °C overnight. The MBC reflects the concentration that exhibited no growth when bacterial colonies were tested.

RESULTS AND DISCUSSION

A total 85 isolates of *B. cereus* were isolated then identified by stander microbiological laboratory procedure (biochemical tests), then confirmed by VITEK-2. The rate of *B.cereus* from lather clothes dyes was 24 (28.23%), The rate of *B.cereus* from shoes dyes was 30 (35.29%),and The rate of *B.cereus* from children coloring dyes was 31 (36.47%), by Vitek-2 compact system (Biomérieux) verification of all isolates was performed, Table 1.

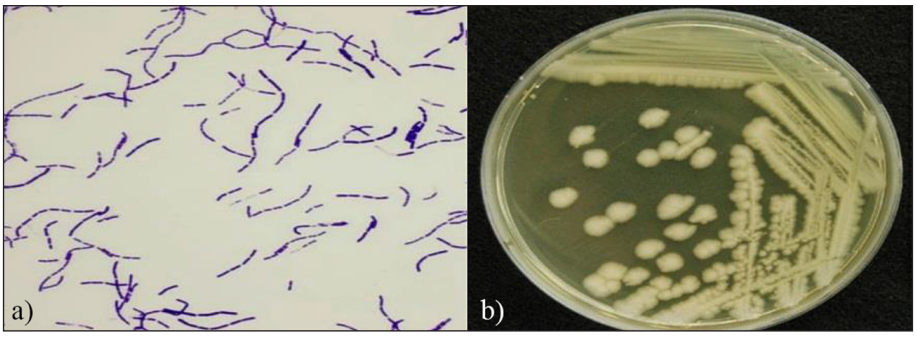

Figure 1. (a) Bacillus cereusis a Gram positive, rod shaped (b) colony on nutrient agar

Table 1. Distribution of *B. cereus* from many clinical sample

Isolates source	Specimens No.	Percentages %
Lather clothes dyes	24	28.23%
Shoes dyes	30	35.29%
Children coloring pen	31	36.47%
Total	85	100%

B. cereus isolates tested negative for growth in 5% and 7% NaCl, urea hydrolysis, catalase, lecithenase and indole. Conversely, all isolates tested positive for Gram stain, spore forming, motility, citrate utilized, oxidase, gelatin hydrolysis, nitrate reduction, voges proskauer, anaerobic growth, growth at 42 °C, protease and starch hydrolysis [May et al., 2011] as shown in Table 2.

Table 2. Biochemical characteristics obtained from *Bacillus cereu*s isolates

Test	*Bacillus cereus*
Gram stain	+
Spore forming	+
Cell shape	Rod
Motilty	+
Citrate utilized	+
Oxidase	+
Urea hydrolysis	-
Gelatin hydrolysis	+
Catalase	-
Lecithenase	-
Nitrate reduction	+
Voges proskauer	+
Starch hydrolysis	+
Indole	-
Growth in 5% NaCL	-
Growth in 7% NaCL	-
Anaerobic growth	+
Growth at 42 °C	+
Protease	+

Table 3. The ability of bacteria (*Bacillus cereus*) to ferment different sugars

Sugar	*Bacillus cereus*
Glocose	+
Arabinose	-
Lactose	+
Mannitol	-
Galactose	+
Maltose	+
Salcin	-
Xylose	+
Fructose	+
Sucrose	=

The data provided in Table 3 illustrate how well various isolates ferment different sugars; the positive (+ve) results suggest that the bacterium was able to ferment sugars under study, because they have a certain enzyme that is necessary for the fermentation of sugars and the formation of acid and/or gas [43].

Antibacterial activity of PEO

The modified Kirby-Bauer disc diffusion technique was used to determine the antibiotic sensitivity of each kind of bacterium. Selective antibiotics are frequently used to treat infections caused by *B. cereus* in order to show their impact on different populations as indicated in the Figure 2-6 [Hamzah et al., 2020].

Strong, broad-spectrum antibacterial effectiveness against multidrug-resistant bacteria is evaluated, as demonstrated by PEO. There was comparison between the outcomes of several antibiotics on bacterial isolates. Figures 2 to 6's outcome demonstrated that not all identified bacteria under research were successfully combatted by the chosen medications. PEO showed a notable decrease in inhibitory zone width as PEO

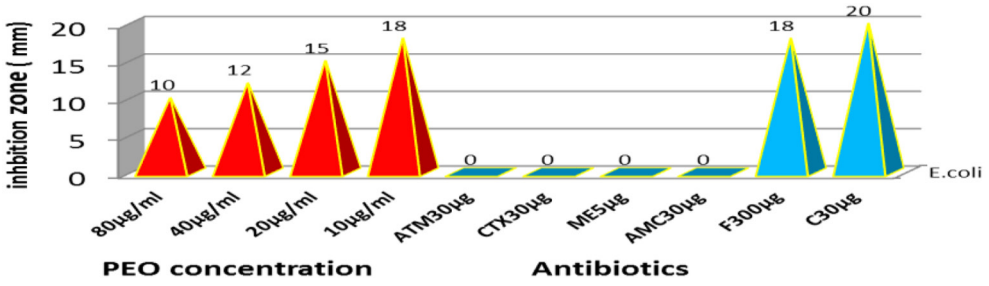

Figure 2. Antibacterial action of on *B.cereus* 1

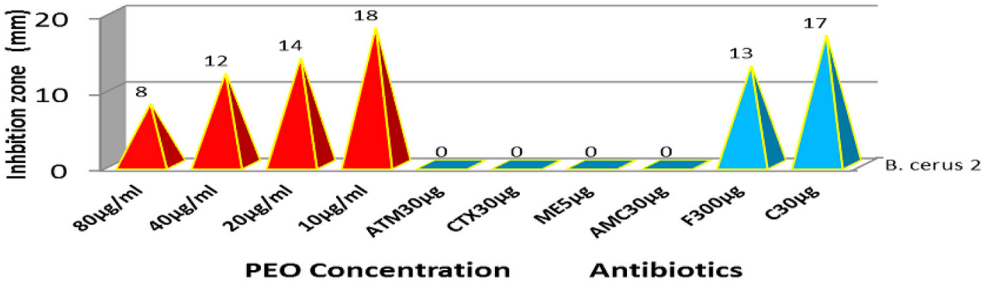

Figure 3. Antibacterial action of on *B.cereus* 2

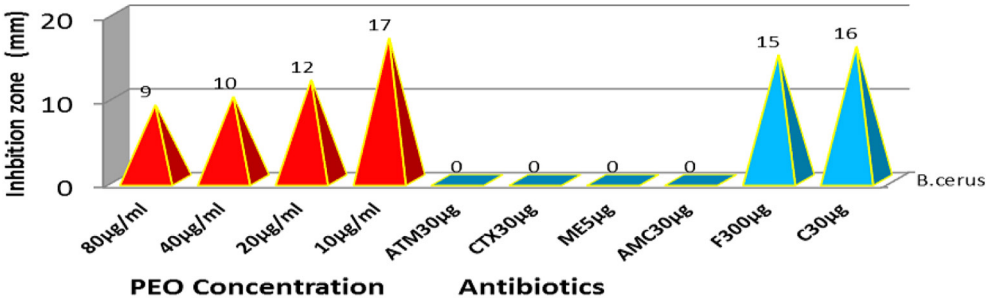

Figure 4. Antibacterial action of on *B.cereus* 3

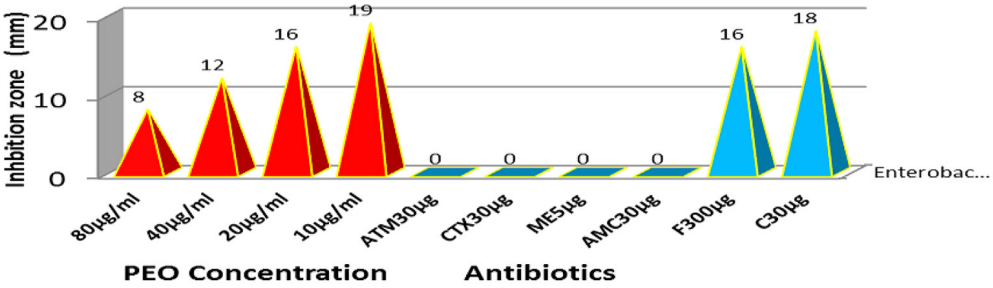

Figure 5. Antibacterial action of on *B.cereus* 4

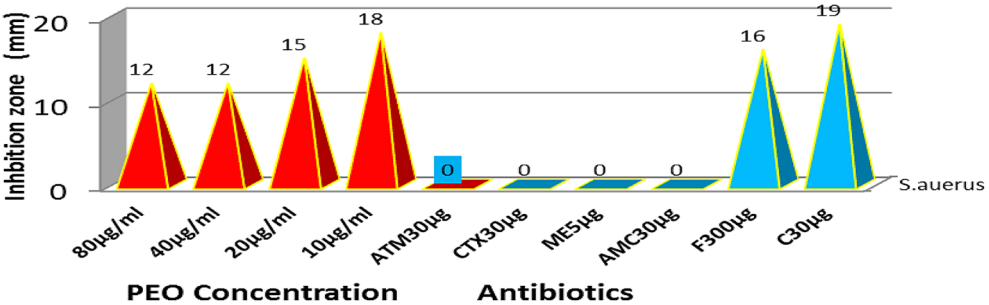

Figure 6. Antibacterial action of on *B.cereus* 5

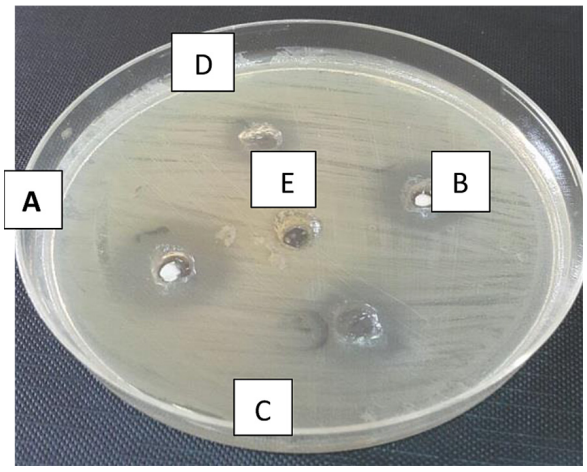

Figure 7. Antibacterial activity of PEO against *B.cereus* (A – 80 µg/ml, B – 40 µg/ml, C – 20 µg/ml, D – 10 µg/ml)

concentration fell, even outperforming the effects of several antibiotics. Maximum zone of inhibition of 19 mm against *B. cereus* 4 Figure 5 and the least sensitive isolate in comparison to the chosen antibiotics appeared, followed by *B. cereus* 2 and 3 Figure 3 and 4, at a dosage that produced the highest zone of inhibition against the test organisms. *B. cereus* is the second PEO-sensitive isolate. 1 and 5 Figure 2 and 6.

In addition to producing reactive oxygen species (ROS), such as superoxide species, which aid in the oxidation of biomolecules, PEO also rapidly reduces the integrity of bacterial cell membranes [Kim and Lee, 2005]. A minimum of three antibacterial antibiotics or categories must be obtained before a condition is considered to have minimal residual illness. The PEO might inhibit the multidrug-resistant (MDR) bacteria, according to a conclusion that has been supported by Zhang and Chen's research [Zhang et al., 2009].

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

B.cereus 4 demonstrated the greatest sensitivity, followed by other bacteria, in Table 4, which reveals that the MIC of polyethylen [PEO] varied from [10 to 20 µg/ml] and the MBC value ranged from [40 to 80 µg/ml].

The findings of this study demonstrated that PEO have a significant inhibitory effect on bacterial strains due to their ability to interact with bacterial cell walls and rupture them. This disruption of bacterial metabolism through bacterial DNA effects and interactions with mitochondria and other bacterial organelles causes disruption of bacterial metabolism [Abdulazeem et al., 2019, Vargas et al., 2012].

Table 4. PEO's MIC and MBC for a several pathogenic microorganisms

Bacterial isolates	MIC	MBC
Bacillus cereus1	20 µg/ml	40 µg/ml
Bacillus cereus2	10 µg/ml	40 µg/ml
Bacillus cereus3	20 µg/ml	40 µg/ml
Bacillus cereus4	20 µg/ml	80 µg/ml
Bacillus cereus5	20 µg/ml	40 µg/ml

Note: MIC – minimal inhibitory concentration, MBC – minimal bactericidal.

CONCLUSION

According to the results of this experiment, PEO has a substantial inhibitory and antibacterial effect on certain pathogenic bacterial isolates from azo dyes with aninverse connection between inhibitory zone diameter and PEO concentration. The MICs of PEO ranged from 10 to 20 µg/ml, and MBCs ranging from 20 to 80 µg/ml. Due to PEO's potent capacity to prevent bacterial development, it is strongly advised that it be used as a more affordable alternative to conventional anti-bacterial agents, particularly with ingredients used to produce coloring colors, dye shoes, and dye leather clothing.

Acknowledgments

We would like to express our gratitude to all members of the research team, particularly the Dean of Al-Mustaqbal University College, represented by Assist. Prof. Dr. Hassan Shakir Majdy, for the financial assistance offered to finish this study.

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