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ASSESSMENT OF BACTERIAL PHOTODYNAMIC INACTIVATION MEDIATED BY METHYLENE BLUE IN THE PRESENCE OF POTASSIUM IODIDE

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Abstract:
Background: The increasing environmental problems associated with traditional methods of water disinfection, such as chlorination, have prompted the hunt for sustainable substitutes. Photodynamic treatment (PDT) with photosensitizers has emerged as a viable approach to water purification due to its advantages over conventional approaches. The objective of this research is to evaluate the effects of immobilized photosensitizers, specifically potassium iodide (KI) and methylene blue (MB), on improved long-term water disinfection and bacterial inactivation.
Methods: When examining how photosensitizers affect bacterial inactivation, a methodical sequence of steps is utilized. Potassium iodide and methylene blue are co-extrusioned into a polymer matrix to create an efficient and safe immobilization procedure. The final beads undergo a thorough testing procedure that includes measurements of absorbance using UV-visible spectrophotometry, evaluation of photosensitizer leakage, and tests for antibacterial activity against both Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria.
Findings: Examining photosensitizer in polymers reveals three different concentration gradients (C1, C2, and C3) with different amounts of methylene blue and potassium iodide. Following the deployment of the beads, data from the UV-visible spectrophotometer show an inverse relationship between absorbance and photosensitizer inclusion levels, with C1 showing the least inclusion and C3 the highest. Days 1 and 2 of the polymer matrix test show rapid leaking of photosensitizer, which is followed by a decrease in activity on the subsequent days. According to antibacterial activity tests, C1 is the concentration that works best for both types of bacteria, with C2 and C3 displaying different levels of potential for breakdown.
Conclusion: The study comes to the conclusion that immobilised photosensitizers are very effective at disinfecting water and inactivating germs, especially when potassium iodide is present. For photosensitizer immobilisation, the suggested co-extrusion approach works well and is both economical and ecologically benign. Reactive iodine species are produced in conjunction with potassium iodide, which is thought to have a synergistic impact that improves bacterial death during methylene blue photodynamic therapy. This creative method, which highlights the use of potassium

iodide and immobilised photosensitizers in environmental stewardship, has the potential to advance sustainable water disinfection techniques.

Keywords: Photosensitizer, Methylene Blue, Photocatalysis, Spectrophotometry

Introduction:

The environmental dangers associated with traditional methods of water disinfection, such as chlorination and ozonization, have led to a great deal of attention being paid to the search for alternatives. The undesirable consequences that these methods usually produce are what drive research into sustainable alternatives. Porphyrins, phthalocyanines, and related chemical dyes have emerged as key players in photodynamic treatment (PDT), an alternative to water filtration that shows promise.

PDT exposes cells to light through photosensitizers, which causes the creation of reactive oxygen species (ROS), especially singlet oxygen, which is lethal to cells. This method not only works to disinfect water, but it also works to treat infections, microbiological diseases, and chemical contaminants. By making use of these materials' versatility, photosensitization provides a useful means of generating microorganism-free water, enhancing techniques for water purification.

Wastewater that has been cleaned and disinfected might be a helpful irrigation alternative in areas with limited water supplies. According to Thandu et al. (2015), photosensitizers can be used to photodynamic treatment instead of more conventional methods like UV radiation or the use of chemicals based on chlorine.

Photosensitizers can cause Type I and Type II reactions when they are exposed to visible light. Energy transfer to molecular oxygen is a feature of Type II, which produces a variety of ROS, including singlet oxygen. Type I collagen generates radical ions and active free radicals. Bacteria can be rendered inactive by singlet oxygen due to its resistance to microbial defense-related enzymes. According to Garcia-Fresnadillo and associates in 2018.

Applying photosensitizers (PSs), which can be liposomally encapsulated, free-floating, or placed on solid supports, eliminates microorganisms. Studies have shown that free porphyrin supplements (PSs), such as meso-substituted cationic porphyrin, rose bengal (RB), and methylene blue (MB), are effective in treating wastewater (Jemli et al., 2002). Benefits of PSs immobilized on a solid phase include improved resistance to bleaching, reusability, and ongoing processing.

Increased resistance to bleaching, reusability, and continuous processing are just a few advantages that immobilized PSs offer for disinfecting water (Wagner et al., 1998). A variety of immobilization techniques, including covalent bonding, ionic bonding, adsorption, and integration into polymers, are available to enhance photosensitization performance (Sabbahi et al., 2010). According to Jenkins et al. (2006), PSs covalently bonded to polymers demonstrated noteworthy singlet oxygen quantum yields, with values as high as 0.91. Photosensitization is necessary for the formation of reactive oxygen species (Chong et al., 2010). Photosensitizers are materials that absorb light energy and transfer it through a process known as photosensitization, which is a crucial component of photodynamic therapy, according to Hamblin et al. (2018). The many uses of various photosensitizers are highlighted by Vatansever et al. (2013), Kasimova et al. (2014), and Fotinos et al. (2008). Heme-derived porphyrin-based photosensitizers are commonly employed in PDT, but red-spectrum-effective chlorin-based ones. Bacteriochlorin-based and ruthenium-based photosensitizers explore near-infrared regions, and organic dyes like methylene blue and rose Bengal, along with quantum dots, find broad applications.

Wanger et al. (1998) and Sabbahi et al. (2010) emphasize factors influencing phototreatment, including the type and concentration of photosensitizer, pH levels, water quality, and incident light parameters. Nisnevitch et al. (2014), involve Type I and Type II mechanisms, with Type II often involving singlet oxygen production. Visible light and UV-based photodisinfection, described by Braun et al. (2011) and Nisnevitch et al. (2014), showcase different mechanisms, utilizing organic dyes and semiconductor materials, respectively.

The effectiveness of photodisinfection against various microorganisms, inducing changes in cell membranes and DNA, is noted by Manjon et al. (2009) and Luz et al. (2011). Environmental applications extend to water disinfection, fisheries, aquaculture, and corrosion control, promising positive outcomes in these domains.

Vecchio et al. (2015) describe the role of KI along with methylene blue (MB) in a photodynamic system, acting as a co-reactant to enhance electron transfer. This electron relay or electron catalysis process generates ROS, including singlet oxygen, amplifying overall photodynamic activity.

Important contributions to the field of spectroscopy by Chong et al., Hamblin et al., Vatansever et al., Kasimova et al., Fotinos et al., Wanger et al., Sabbahi et al., Braun et al., Nisnevitch et al., and Vecchio et al. will be found in their works. This law is used in spectrophotometry for quantitative analysis and describes the relationship between solution concentration, light path length, and light absorption. It may vary depending on certain conditions, but it allows for the determination of solute concentrations based on absorbance measurements.

Background of the Study:
Concerns about the impacts of conventional methods like chlorination have prompted study into the vital topic of ecologically friendly water disinfection. Porphyrins, phthalocyanines, and organic dyes—three important participants in photodynamic therapy—emerge as a cutting-edge method of water filtration. The study attempts to clarify the intricate connections between various photosensitizers and how these interactions affect the photo-treatment environment by closely examining the intricate process of photosensitization.

In regions where water is scarce, the study proposes using treated wastewater for irrigation and incorporating photosensitizers into photodynamic treatment methods. Photosensitizer activation in the presence of visible light not only ensures microorganism-free water but also demonstrates the adaptability of these compounds in a variety of applications.

The study emphasizes the usefulness of photosensitizers in the treatment of wastewater, whether they are free, encapsulated, or immobilized. The immobilization strategy onto solid phases is emphasized because it provides continuous processing, reusability, and enhanced bleaching resistance, all of which boost the feasibility of sustainable water disinfection.

With a focus on practical applications, the research aims to move beyond scholarly debate and offer helpful insights into improving sustainable methods for water disinfection. It seeks to significantly advance the ongoing discussion on environmental stewardship and water resource management by doing this.

Statement of the Research Problem:
The research looks on green alternatives to conventional methods such as chlorination to address the issue of water disinfection. Because photodynamic therapy (PDT) poses environmental risks, one unique alternative that has garnered attention is the use of photosensitizers in PDT. Reactive oxygen species (ROS), especially singlet oxygen, are produced during photosensitization, which advances water filtering methods and offers a practical path to water free of microorganisms.

The study proposes a feasible irrigation substitute in water-scarce places by recommending the addition of photosensitizers to the photodynamic treatment of treated wastewater. The ability of photosensitizers to be free, encapsulated, or immobilized is demonstrated by their effectiveness in wastewater treatment. Immobilization onto solid phases is a tactical technique that enhances resistance to bleaching, reusability, and continuous processing.

The study's main focus is on the need for ecologically friendly ways to disinfect water, with a particular emphasis on the potential applications of PDT's photosensitizers in water filtration. Owing to the intricacy of photosensitization and the adaptability of these substances in various settings, a comprehensive study is required to furnish valuable insights for the creation of ecologically sustainable methods of treating water.
The study aims to address this problem and offer solutions that promote sustainable water resource management and environmental stewardship by looking at the intricate interactions between different photosensitizers and how they affect photo-treatment efficiency.

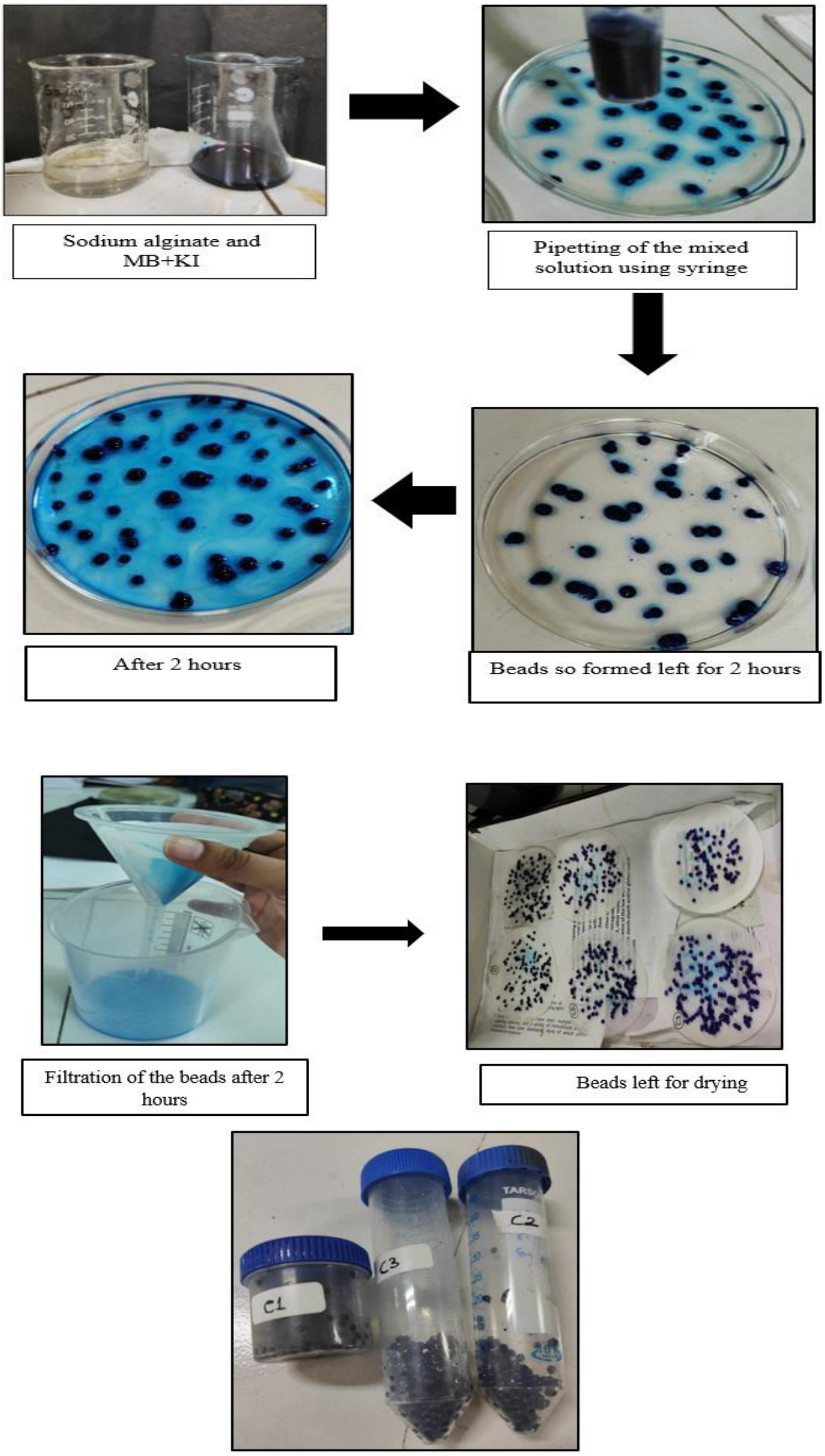
Aim/Objectives:

1) Using a polymer (Sodium alginate), potassium iodide, and photosensitizer (Methylene blue), beads with varying concentrations of potassium iodide were formed.
2) Use a random selection technique to ascertain the weight and average weight of the beads.
3) Assessment of the MB's incorporation in the beads.
4) Observing the leakage of the photosensitizer from the beads to perform its activity in sunlight.
5) The serial dilution method of the spread plate approach was used to carry out the examination of bacterial degradation.

Material and Methodology:

The study approach consisted of a methodical set of steps to examine how photosensitizers affect bacterial inactivation and evaluate their potential for long-term water disinfection. The careful preparation of methylene blue (MB) stock and working solutions marked the beginning of the experimental procedure. The stock solution was made by carefully combining phenol, methylene blue chloride, distilled water, and ethyl alcohol. Then, at a consistent 1:10 ratio, feasible solutions were produced employing this mixture. A crucial component of the study was creating gel beads, which required dissolving sodium alginate in distilled water to create an alginate solution. Different volumes of potassium iodide (KI) were added to a fixed volume of methylene blue working solution in parallel fashion. After this combination was gradually added to a 0.3 M calcium chloride solution over a predetermined amount of time, crosslinked gel beads began to form. The beads were extensively washed in 50 mM Tris-HCl buffer after gelation to remove any remaining calcium chloride and non-encapsulated cells. The concentration gradients of KI and methylene blue were carefully controlled over three tiers (C1, C2, and C3), each with different amounts of KI and methylene blue working solution. The evaluation of photosensitizer inclusion in polymers necessitated the measurement of absorbance in the $CaCl_2$ solution following bead deployment. Subsequently, an examination of photosensitizer leakage from polymeric matrices involved the immersion of photosensitizer beads in tap water. Regular monitoring of washings via spectrophotometry at 665 nm enabled the quantification of leaked photosensitizers. The bacterial growth experiments were differentially conducted for Gram-negative (E. coli) and Gram-positive (S. aureus) bacteria. The formulation and sterilization of LB broth, inoculation with starter cultures, and controlled incubation at optimal growth temperatures exemplified the rigor in experimental design. The antibacterial activity assay, a pivotal segment of the research, incorporated a serial dilution technique for both E. coli and S. aureus broths. The subsequent exposure of samples to sunlight, coupled with their application onto nutrient agar media plates, aimed at scrutinizing the degradation of bacterial colonies. The preparation of plates and control plates, alongside the sunlight irradiation process, collectively constituted a comprehensive approach to gauging the efficacy of photosensitizers in sustainable water disinfection.

Result:
Formation of Beads:

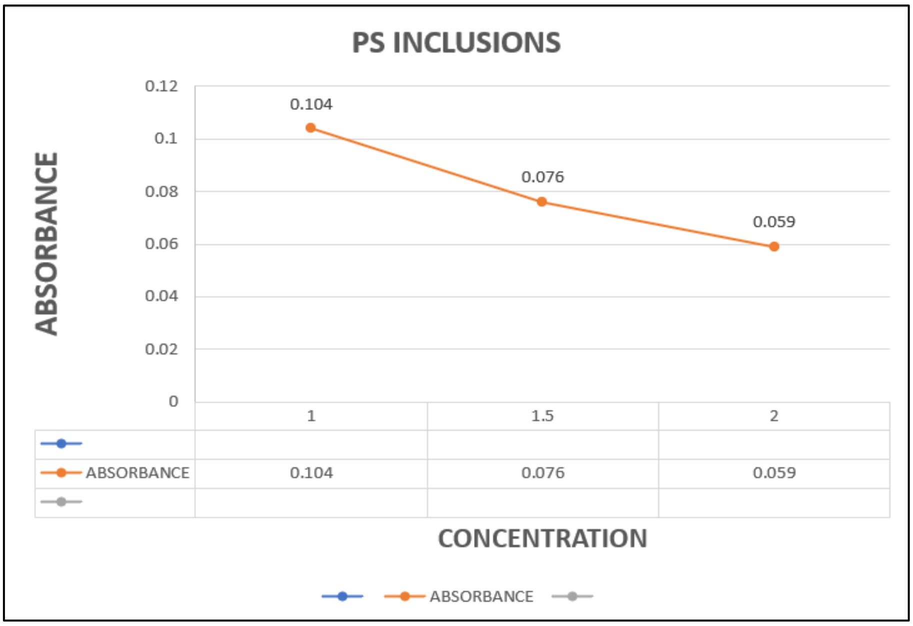


Bead Formed

Evaluation of Photosensitisers Inclusion in Polymers:
Absorbance was evaluated for all the 3 samples and result was interpreted from the graph obtained. The OD so obtained is mentioned in the table below:

TYPE	**CONCENTRATION(g) OF KI IN 2ml OF WORKING SOLUTION OF MB**	**OD (665nm)**
Blank	0	0.001
C1	2	0.059
C2	1.5	0.076
C3	1	0.104

Absorbance of the washings after 2 hours of formation of beads



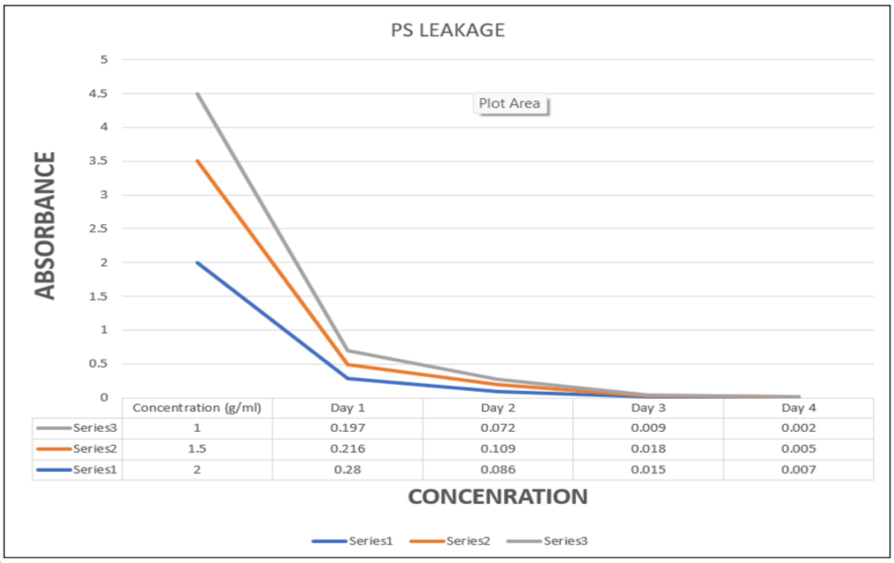
The assessment of UV-visible spectrophotometer readings operates on the principles of the Beer-Lambert law, establishing a direct proportionality between the concentration of a substance and its absorbance. In the context of our study, this relationship was applied to evaluate the inclusion of MB+KI in the solution. According to the law, higher absorbance values indicate a greater concentration of MB+KI in the sample, while lower absorbance values correspond to lower concentrations. Therefore, in our experimental results, the absorbance values followed the order: C1 < C2 < C3.

Interpreting these results in terms of inclusion of MB+KI, the inverse relationship holds true. Greater absorbance (as demonstrated in C1) implies a lower concentration of MB+KI in the solution, whereas lower absorbance (as demonstrated in C3) suggests a higher concentration of MB+KI in the solution. In summary, the levels of MB+KI inclusion in the solution are inversely correlated with the absorption values. The least inclusion is displayed by C1, and the most inclusion is displayed by C3.

Testing PS Leakage from the Polymer:

The goal of the test protocol was to determine how long it would take for the dye that was extracted from the sodium alginate to show signs of activity, in order to assess photosensitizer (PS) leakage from the polymer. To conduct this test, 0.2g of beads from each of the three concentrations were weighed first. After that, these beads were immersed in 250 cc of water at room temperature. Over the course of many days, the absorbance of the solution was tested at predetermined intervals using a UV-Visible spectrophotometer. The time-dependent release of the ingested dye could be monitored thanks to this methodical assessment, which also shed light on the kinetics of PS activity and its possible uses.

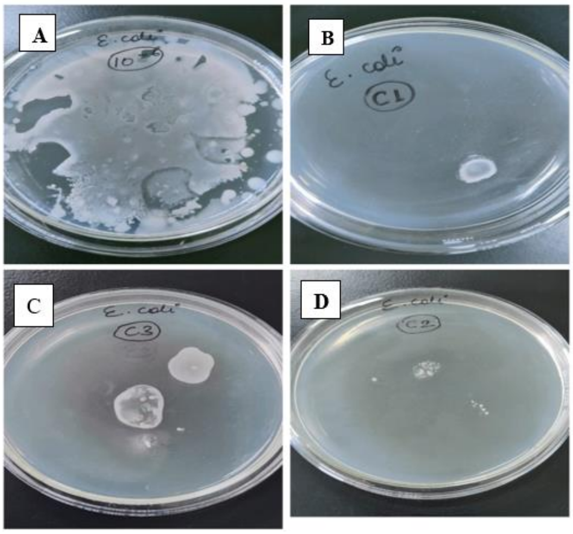
DAY	TIME		CONCENTRATION	ABSORBANCE OD (665nm)
	IN	OUT		
DAY 1	9;00 AM	6:15PM	Blank	0.001
			C1	0.280
			C2	0.216
			C3	0.197
DAY 2	6:20PM	10:00AM	Blank	0.001
			C1	0.086
			C2	0.109
			C3	0.072
DAY 3	10:10AM	6:00PM	Blank	0.001
			C1	0.015
			C2	0.018
			C3	0.009
DAY 4	6:10PM	11:20AM	Blank	0.001
			C1	0.007
			C2	0.005
			C3	0.003



Examining the graph obtained from the OD measurements, it is clear that Day 1 and Day 2 had the dye's maximum concentration seeping into the solution. After that, there was a discernible drop in the quantity of MB+KI on Days 3 and 4. The data were collected in ambient temperature settings. Noteworthy, if sunlight were to fall on the experimental setup, the leaching process might manifest faster kinetics.

Anti-bacterial activity Assay:
This evaluation was carried out for two different kinds of bacterial colonies: S. aureus (Gram-positive bacteria) and E. coli (Gram-negative bacteria). The assay produced visible and discernible findings, offering insightful information on how the investigated conditions affected each variety of bacterium.

For *E. coli-*



A- Plate with 10^-6 diluted *E. coli* growth.

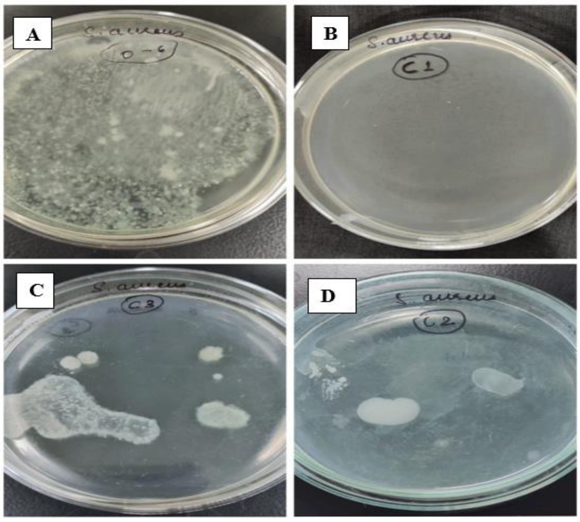
B- Effect of C1 after irradiation for 5 hours.

C- Effect of C2 after irradiation for 5 hours.

D- Effect of C3 after irradiation for 5 hours.

Bacterial degradation for gram negative bacteria

For S.aureus



A-Plate with 10^-6 diluted *Aureus* growth.

B-Effect of C1 after irradiation for 5 hours.

C- Effect of C2 after irradiation for 5 hours.

E- Effect of C3 after irradiation for 5 hours.

Bacterial degradation for gram positive bacteria

Discussion:

The experimental findings affirm the efficacy of the combined photosensitizer system comprising methylene blue and potassium iodide (KI) in the bacterial inactivation process, addressing both Gram-negative (E. coli) and Gram-positive (S. aureus) bacterial strains. The three distinct concentrations of the photosensitizer yielded differential inclusion levels, with C1 exhibiting the highest, followed sequentially by C2 and C3.

In assessing the oozing-out dynamics, notable dye release occurred within the initial 24 to 48 hours, indicative of a prompt initiation of activity. Methylene blue was added to the water, and days later, there was a steady but diminishing discharge of the chemical, demonstrating how rapidly it became active.

When it comes to bacterial breakdown in water, the experiment's findings showed that C1 was the most effective concentration against both Gram-positive and Gram-negative bacteria. Although C3 demonstrated the ability to break down both kinds of bacteria, C2 shown significant efficacy, particularly against Gram-positive bacteria, over a longer period of radiation.

All concentration ranges exhibited bacterial degradation activity, with C1 demonstrating the highest efficiency. Together, potassium iodide (KI) and methylene blue enhanced the photosensitizer's activity and maximized its ability to filter water. By using photodynamic inactivation, KI can boost the effectiveness of methylene blue in wastewater treatment, as demonstrated by the combined results.

Conclusion:

This work concludes by assessing bacterial photodynamic inactivation mediated by methylene blue (MB) in the presence of potassium iodide (KI) in order to identify sustainable alternatives for water disinfection applications. As part of the study's approach, MB and KI were co-extruded into a polymer matrix, which immobilized them jointly and produced beads with varying quantities of KI. Strong results against Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria were obtained from the systematic evaluation of photosensitizer inclusion, leakage dynamics, and antibacterial activity.

The UV-visible spectrophotometer data demonstrated an inverse relationship between absorbance and photosensitizer inclusion levels, with the highest concentration (C1) displaying the least inclusion and the lowest concentration (C3) displaying the largest. This complex inclusion pattern shows that the recommended co-extrusion method effectively regulates the amounts of photosensitizer in the polymer matrix.

According to an assessment of photosensitizer leakage from the polymer matrix, release happened rapidly over the first 24 to 48 hours and subsequently slowed down over the next few days. The time-dependent release dynamics provided important information on the kinetics of photosensitizer activity, emphasizing how quickly it starts when introduced into the water.

The effectiveness of the immobilized photosensitizers at disinfecting water and deactivating microorganisms, particularly when KI was present, was shown by the results of the antibacterial activity assays. The most effective concentration for both Gram-positive and Gram-negative bacteria turned out to be C1. C2 and C3 demonstrated varying degrees of bacterial degradation capacity, with C2 being particularly efficient against Gram-positive bacteria.

It is notable that KI and MB have been shown to work synergistically to enhance bacterial death during photodynamic therapy. Increased bacterial inactivation is thought to be a result of this cooperative action, which produces short-lived reactive iodine species. The co-extrusion technology proved beneficial in immobilizing photosensitizers, providing an economical and eco-friendly solution without requiring extra chemicals.

When used in conjunction with potassium iodide, the co-extrusion-immobilized photosensitizer system offers a potential approach to environmentally friendly water disinfection. By providing insightful information on the complex dynamics of photosensitizer immobilization, leakage kinetics, and antibacterial activity, the study highlights the potential of this novel strategy to further ecologically friendly water treatment methods. The results open up new avenues for investigation and refinement of this technique for wider uses in environmental stewardship and water purification.

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