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Food Color Allura Red Induced Aquatic Pollution Analysis with Artificial Intelligence & Approach to Bioremediation with Yeast

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ABSTRACT

Allura red (E129) is a dark-red, water-soluble, azo dye . It is used as food dye for the replacement of amaranth. Allura red is originally derived from petroleum. It is added into soft drinks, children's medications, and cotton candy. This food color is found as natural trace components of the aquatic environment, but their levels have been increased due to industrial wastes, geochemical structure & hotel kitchen run off . The aim of the paper is to monitor the biomarkers that is used in the determinations and diagnosis of Allura red impact and pollution. Besides the chemical methods of water quality determination, this study also incorporates the using of modern technology Artificial Intelligence for detecting the changes of nature of water contaminated with food color for more accuracy. This causes water to be brightly red colored, have an alkaline pH, and have substantially lower levels of dissolved oxygen, all of which have an impact on the surrounding environment. The study collects wastewater from the food color industrial drainage channel at each stage of the process and analyses it to identify its parameters. These parameters include pH (Analytical value is 6.6 to 13.1), BOD (Analytical Value is 432 to 1840mg/l), COD (Analytical Value is 635 to 4459 mg/l), Total Dissolved Solids – TDS (Analytical Value is 6530 to 21989 mg/l), TSS (Analytical Value is 275 to 1189), and Ammonium Nitrogen (Analytical Value is 34.2 to 49.4), Since these are all baseline variables, the aquatic system physico-chemistry is deteriorating. This allows for the deduction of the state authorities' final alleviation standards for the ensuing treatment process. We further have treated the water sample with yeast & reached the insight on bioremedial approaches.

Keywords: Food color industries, Allura red, Physicochemical Parameters, Multispectral spectroscopic sensors, Artificial intelligence , Bioremediation, Yeast.

1. INTRODUCTION

Allura Red AC, also known as FD&C Red 40 or E129, is a red azo dye commonly used in food. It was developed in 1971 by the Allied Chemical Corporation, who gave the substance its name.

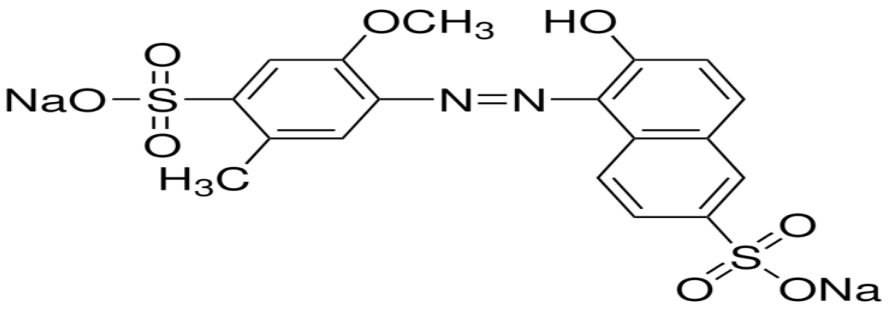

Fig 1 : Chemical Structure of Allura Red

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It is used is ice-creams, desserts, Biriyani , Pulao or sweet yellow rice , mountain dews, string energy drinks, popcorn , chewing gums, fruit cordials ,cotton candy , soft drinks , watermelon juice , rooh-afza , cherry-flavored products, children's medications, and dairy products. It is occasionally used to dye medicinal tablets, fruit fermented alcohol based beverages, cotton candy, puddings, laddu-modaks, jelly, pickles, marmalade, instant soups, custard faluda powder and many more as it brings bloody red and brown colour.

The present research focuses on the effect of Allura red on water parameters in the aquatic medium contaminated by Allura red concentration which are released from food colorant production industries, public hotels and restaurants in a huge amount of left-over stale foods as well as processed by product liquids from kitchens which are discarded into local streamlines on a regular basis. Sample water was taken from the nearby pond of the food color industry in Avanti Bihar, Raipur, India.

The goal of the current study was to evaluate the actual physico-chemical characteristics of industrial wastewater. Various salts, acids, enzymatic ingredients, dyes of various colors, and other chemicals are used during the entire process.All of the leftover materials are eventually flushed into the water supply as industrial effluent. This indicates that the food color industries produce a lot of waste water. The status of the generated effluent should be clarified before discharging into the natural environment. It also helps to improve the effluent quality with chemical or biological treatment if needed. In order to navigate these multifaceted challenges, innovative approaches leveraging edge computing and machine learning technologies have emerged. These technologies offer promising solutions for developing smart optical sensors for water quality monitoring (Whaiduzzaman et al., 2022; Alshami et al., 2024).

Bioremediation approaches using ecofriendly yeast *Saccharomyces cerevisae* was implemented in laboratory condition and it showed the striking capability of degrading Allura red present in the sample.

2. MATERIALS AND METHOD

2.1.Reagents

Reagents associated with water parameters analysis

1.Phenolphthalein indicator
2.N/44 NaOH
3.$MnSO_4$
4.Alkaline KI
5.Concentrated H_2SO_4
6.1% starch
6.0.025 N $Na_2S_2O_4$
7.$KCrO_4$
8.0.02 N AgNO3
9.Methyl Orange
10.0.1N HCl
11.0.1N NaOH
12.NH4Cl Buffer
13.Erioshrome Black T indicator
14.0.01M Standard EDTA

2.2. Artificial Intelligence Tool used

An efficient ingenious spectroscopic sensor system unified with artificial intelligence (AI), designed for the detection and analysis of presence of any dye in water sample. The system architecture incorporates various components, each engineered for optimized data collection and processing, as visualized in **Fig. 2**. The principal component of the system is a circuit board containing the AS7341 sensor, which is serially connected to NODEMCU ESP32 board via an I2C interface cable. configuration is crucial for capturing detailed spectral data from water samples. The data collected by the spectral sensor is then transmitted to the NODEMCU ESP32 and then to a

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personal computer via the ESP32's Wi-Fi module. This data transmission process is a critical step for the subsequent storage and which is fundamental to ensuring accuracy in water quality analysis.

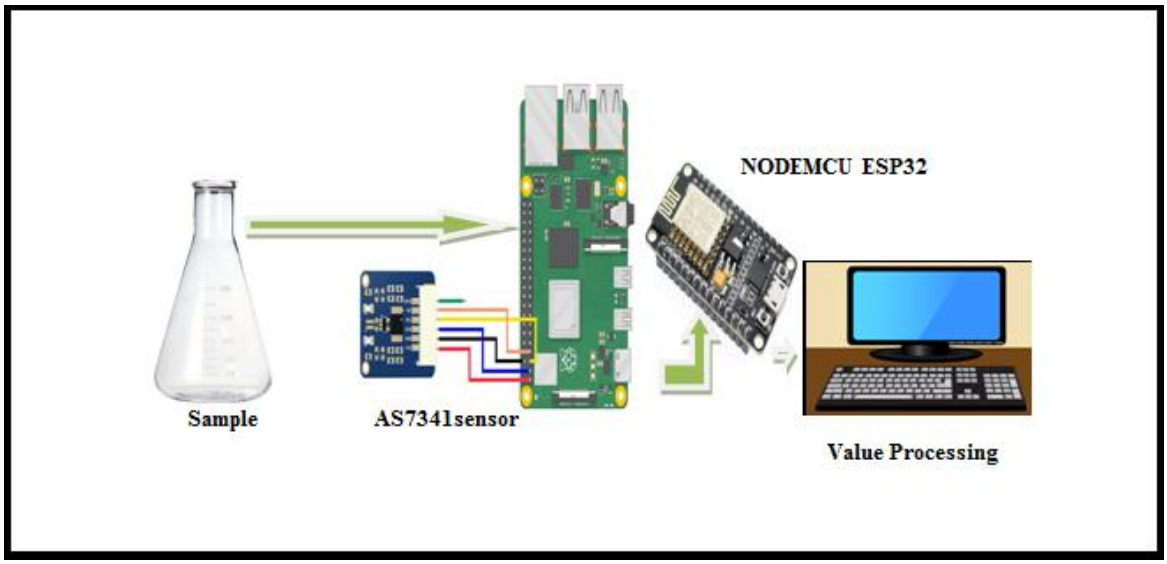

Fig.2: Artificial intelligence enabled sensor system

2.3. Methodology used for studying water parameters

2.3.1. Study area of water sample collection

Avani Bihar, Raipur, Chattisgarh, India, which is 6.1 km from railway station. It has five manufacturing factories of food colors mainly Allura red directly channeled with two local ponds. Water samples were collected from both pipeline drainage of the factories and the ponds. Collection was for 4 consecutive days done at the mid-day and the evening time when effluents concentration is very high and very less respectively as assumed according to the timetable of factories.

2.3.2. Chemical Method of Water Parameters analysis

A. Free CO_2

Free CO_2 will be determined in mg/L by Titrimetric method using phenolphthalein as an indicator at pH 8.3, as described in APHA-AWWA-WPCF (1980). [Bhattacharjee et al., 2018]

B. Dissolved O_2

The Winkler method will be done as per standard protocol. [Bhattacharjee et al., 2018]

C. Salinity

The salinity of water sample will be determined as described in APHA-AWWA-WPCF (1980). [Bhattacharjee et al., 2018]

D. Alkalinity

The alkalinity of water sample will be determined as prescribed in APHA-AWWA-WPCF (1980). [Bhattacharjee et al., 2018]

E. Acidity

The acidity of water sample will be determined as prescribed in APHA-AWWA-WPCF(1980).[Bhattacharjee et al., 2018]

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F. Total Hardness

Total hardness will be determined by EDTA titrimetric method, following APHA-AWWA-WPCF (1980). [Bhattacharjee et al., 2018]

G. pH

It was done using pH meter.

H. BOD

The BOD test and its calculation are standardized by organizations like the American Public Health Association (APHA) [Bhattacharjee et al., 2018]

I. COD

The COD test and its calculation are standardized by organizations like the American Public Health Association (APHA) [Bhattacharjee et al., 2018]

J. Total Dissolved Solid

The TDS of water sample will be determined as prescribed in APHA-AWWA-WPCF (1980). [Bhattacharjee et al., 2018]

K. Ammonical Nitrogen

AN will be determined by EDTA titrimetric method, following APHA-AWWA-WPCF (1980). [Bhattacharjee et al., 2018]

2.3.3. Multispectral sensor Detection of dyes

2.3.3.1. Sample Preparation

A. **Normal Control Water Sample (Sample 0) :** A sterile container holding clean water sample for spectral measurement. This clean water state provides a reference point for the system's capability to detect the presence of dye.

B. **Mid day Sample (Sample 1) :** Another sterile container holding sample water containing Allura red collected between 12-2pm (hence mid-day sample).

C. **Evening Sample (Sample 2) :** Another sterile container holding sample water containing Allura red collected between 6-8 pm (hence evening sample).

D. **Additional UV disinfection :** For complete eradication of microbes present in the two samples to obtain the clear spectrometric data the UV disinfection was done. The wave length of UV lamp was 254 nm and intensity was 40 µW/cm2. UV-treated water samples were subjected to UV disinfection using a 14-watt low-pressure mercury lamp emitting UV light at 254 nm for a specific duration to ensure effective disinfection for sample 1 and sample 2.

Three samples were taken into the sample tube and measured. In the first stage, sample 1 weighing about 100 ml was taken in a silver bowl, placed on top of the water filled bowl with the distance between the sensor system and the water 1 in. to obtain better observations. Then the relevant spectral readings for sample 0 are observed. Then followed by water sample 1 and water sample 2 , spectral measurements were made. Then, the sample 1 and 2 were placed in the environment where the closed vessel type UV reactor was located. The microorganisms in the dirty water were disinfected with UV light. The disinfected water samples were again prepared for spectral measurements.

2.3.3.2. Multispectral Detection

In each case, the circuit board containing the AS7341sensor (Durgun, 2024) detects light reflected from the water samples and transmits this data to the NODEMCU ESP32. This data is then sent to the personal computer via the Wi-Fi module for processing and analysis. In this system, the computer fulfills the tasks of data storage as well as

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data processing and water quality analysis through AI software. Each of these three scenarios represents specially designed situations to evaluate various aspects of water quality and provides valuable data on how the system design can detect and analyze different states of water.

The sensor system employed in this study consists of an AS7341 sensor serially connected to a NODEMCU ESP32 board via an I2C interface cable. The AS7341 sensor is pivotal for capturing detailed spectral data from water samples. The collected data is transmitted serially to the NODEMCU ESP32, and then wirelessly to a personal computer via the ESP32's Wi-Fi module. This process is crucial for ensuring accuracy in water quality analysis. Fig. 1 illustrates the system components: Clean Water Containers (Sample 0), mid day sample (Sample 1), and evening sample (Sample 2). The AS7341 sensor detects light reflected from the water samples and transmits this data to the NODEMCU ESP8266, which subsequently sends it to a personal computer for storage and analysis. This setup allows for comprehensive data collection and real-time monitoring of water quality.

This AS7341 module integrates three distinct optical sensors: i) AS72651,ii) AS72652, and iii) AS72653. Each sensor is equipped with a set of six on board optical filters, which together provide comprehensive spectral coverage across the ultraviolet (UV), visible (Vis), and near-infrared (NIR) ranges, from 410 nm to 940 nm. This configuration allows for the precise and sensitive capture of spectral information, which is crucial for the detection and quantification of various water contaminants. As illustrated in the accompanying **Fig. 3**, the 18 channel spectral response of the AS7341sensors demonstrates the normalized responsivity of each channel across the monitored wavelength spectrum. Each peak represents the responsivity of a particular channel corresponding to a specific wavelength, with peaks labeled from A to U, denoting wavelengths from 410 nm to 940 nm. The varying heights of these peaks reflect the relative sensitivity of each sensor channel at different wavelengths. This range allows for precise detection and quantification of various contaminants. The spectral data collected is then processed and analyzed to determine water quality.

The different spectral wavelengths, which is essential for identifying and analyzing a wide range of substances present in water samples. For instance, the peaks in the blue region (410 nm–485 nm) are critical for detecting substances that absorb or scatter light in the UV spectrum this is crucial for detecting the dye and its concentration, while the red and NIR peaks (610 nm–940 nm) are indicative of the sensors' ability to detect organic and inorganic matter.

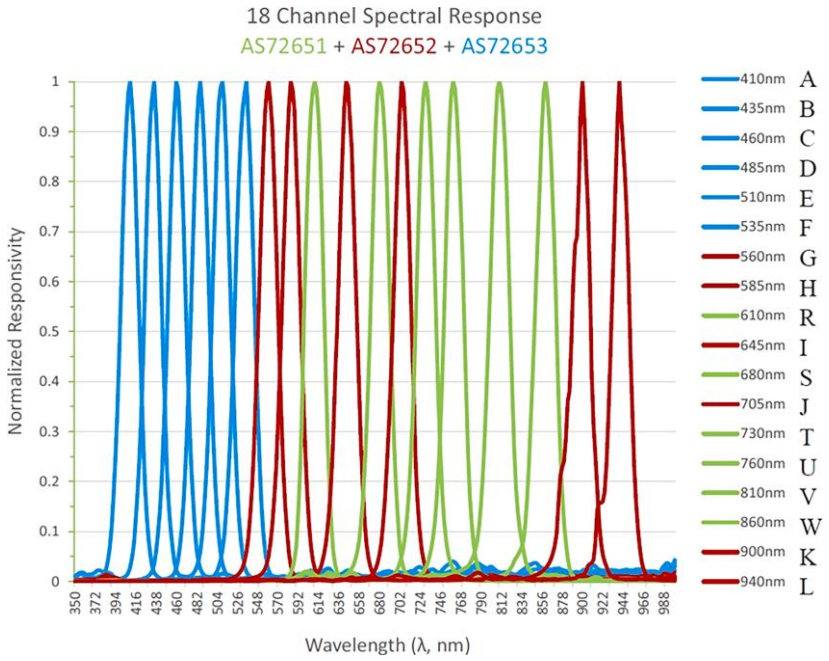

Fig 3 : 18 channels spectral response of AS7341 sensors (Alshami et. al 2024)

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2.3.3.3. Data collection and processing

The spectral data was collected using the AS7341 sensor module with readings taken every 150 ms. for the three samples each. The data acquisition process was automated using the Arduino IDE software, and the data was transmitted wirelessly to a personal computer for storage and analysis.

2.4. Microbial degradation of sample water containing food color

Saccharomyces cerevisae was cultured in laboratory and was introduced to the medium containing sample water with Allura Red in a dose & time depended pattern. Yeasts are not widely used in dye decolorization and have not been as extensively studied as bacteria and filamentous fungi. However, yeasts hold potential in this field especially because they present a biotechnological advantage as they are fast-growers and can also thrive in harsh condition. The experiment was carried out in a 100 ml flask containing the mid-day collected effluent sample with inoculating loop transferred yeast and glucose. The pH was fixed with 7±0.5 using soduium hydroxide & weak hydrochloric acid solution. Autoclave of flask was done at 121° for 30 mins. These were then inoculated with 10 ml of yeast inoculums of three isolates of strains of yeast. After mechanical thorough shaking, these were kept for 10 days at room temperature . samples were drawn at 24 hrs of interval and were subject to centrifugal run with 10 ml solution at 5000 rpm for 15 mins. Decolorisation of growth medium was assessed by the absorbance of the supernatant with mass spectrophotometer(MALDI TOF).

3. RESULTS & DISCUSSION

3.1. Characterization of wastewater samples

A. Free CO_2

The normal water free co2 is 0.460 mg/L , which showed variation from 0.434 to 0.418 mg/L during mid-day & 0.453 to 0.418 mg/L at evening. The declination of free co2 indicates the algal dysphoria and indicative eutrophication in water.

B. Dissolved O_2

The normal range is 11.19 mg/L, but it varies from 9.24-8.26 mg/L &10.24-10.26 mg/L during mid-day & evening respectively. The declination of DO indicates the reduced water flow and increased organic matter also eutrophication in water.

C. Salinity

The normal range is 1621 ppm, but it varies from 1619-1601 ppm & 1620-1615 ppm during mid-day & evening respectively. The declination of salinity indicates the essential demineralization in water.

D. Alkalinity

The normal range is 499 ppm, but it varies from 431-428 ppm & 450-455 ppm during mid-day & evening respectively. The declination of alkalinity suggests the acidification of water.

E. Acidity

The normal range is 9.23ppm, but it varies from 5.2-5.1 ppm & 9.2-9.1 ppm during mid-day & evening respectively. The declination of acidity suggests the acidification of water.

F. Total Hardness

The normal range is 401 mg/L but it varies from 402-403.3 mg/L & 401.1- 402.2 mg/L during mid-day & evening respectively. No such significant changes observed.

G. pH

The normal range is 8.23, but it varies from 5.2-5.1 & 8.2-9.1 during mid-day & evening respectively. The declination of acidity suggests the acidification of water.

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H. BOD

The normal range is 582mg/L , but it varies from 599-610 & 589-600 mg/L during mid-day & evening respectively. This is a result of the variety of chemicals employed in the production processes of Allura red dusts. Further it could be due to resulting anaerobic digestion by water microbes.

I. COD

The normal range is 1280 mg/L , but it varies from 1310-1409 mg/L & 1284-1300 mg/L during mid-day & evening respectively. This is a result of the concentration of higher oxidizable inorganic matter in water.

J. Total Dissolved Solid

The normal range is 9648 mg/L but it varies from 9645-9641 mg/L & 9655-9641 mg/L during mid-day & evening respectively. This slight declination might be due to the use of highly salty chemical materials with the food dyes, in stage of processing . And so these kinds of wastewater should not suggest for drinking and agricultural purposes.

K. Ammonical Nitogen

The normal range is 42.1 mg/L but it varies from 44.2-49.4 mg/L & 44.2-49.7 mg/L during mid-day & evening respectively. It can be decreased chemically or biologically through the denitrification process by bacterial utilization of food dye.

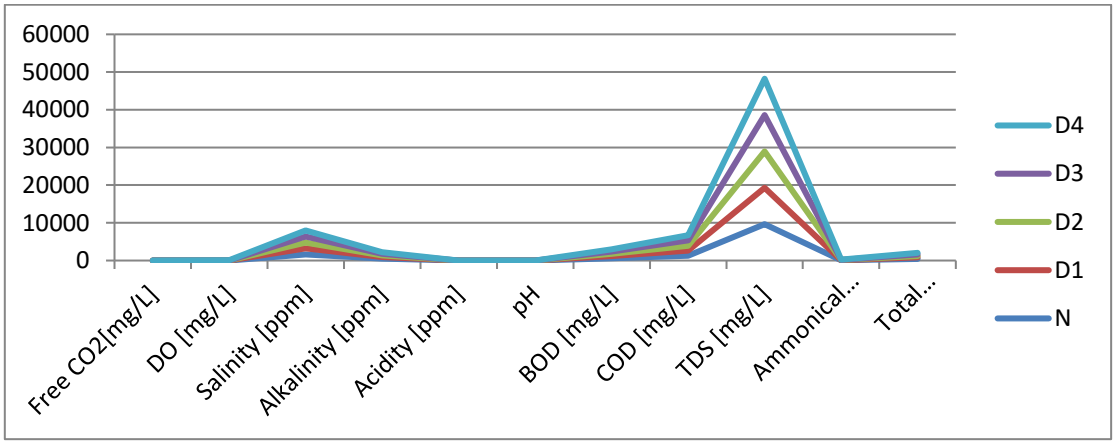

Figure 4 : Showing the several water parameters of collected sample during the mid-day time

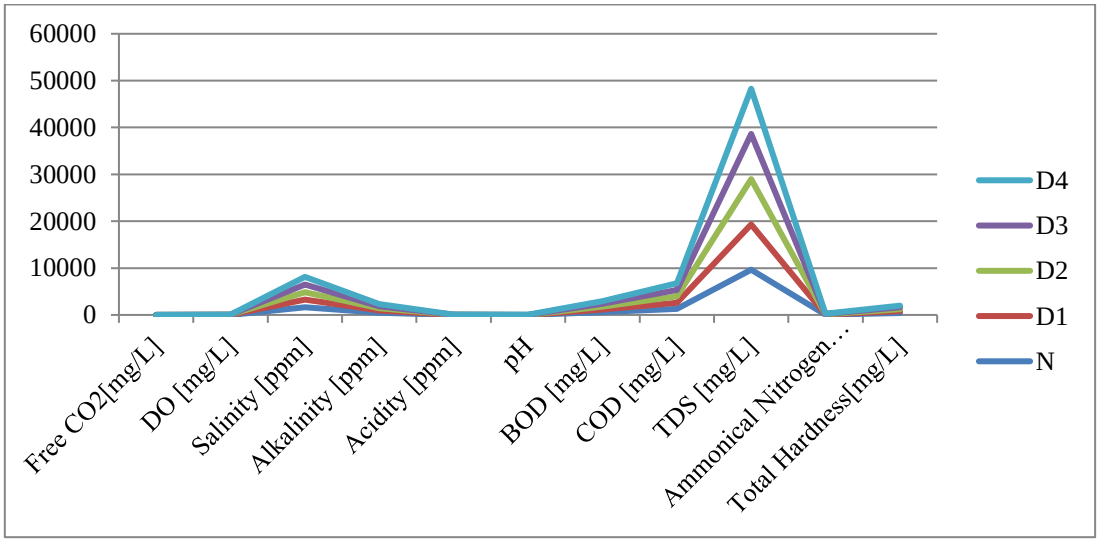

Figure 5 : Showing the several water parameters of collected sample during the evening time

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3.2. Spectral Analysis of water samples

This study presents a spectral analysis of three different water samples. The findings highlight significant indicators of water quality and safety, reflected through changes in optical properties of water.

For the clean water (Sample 0), the spectrum typically displayed low intensity values across all wavelengths. This is consistent with the transparency and minimal contamination of the water, showing no significant absorption or reflection characteristics. In contrast, the mid day water sample (Sample 1) displayed a notable increase in intensity values at certain wavelengths. This increase can be interpreted as the maximum amount of neighboring industrial effluents contamination with Allura red reflecting more light at these wavelengths, indicating a clear alteration in the optical properties of the water due to contamination. Alongside, in case of evening water sample (Sample 2) displayed a declined intensity value at certain wavelengths compared to sample 1. This result serves as a strong testament to the efficacy of Allura red contamination in a periodic manner as in **Fig. 6.**

The use of such analyses for evaluating contamination in water samples and the efficacy of disinfection processes can make significant contributions to the management and preservation of water resources especially those wetlands neighboring to dye industries.

The water samples at specific wavelengths, notably at 610 nm and 860 nm deployed the optical variations corresponding to different states of water quality in three individual samples. The findings are illustrated in **Fig.** 7, which presents the normalized intensity values at these wavelengths for each case.

Analysis at 610 nm Wavelength : The graph depicting normalized intensity at 610 nm demonstrates a significant difference between Sample 1 and Sample 2, clearly pointing out the rate of Allura red contamination at the different time of a day.

Analysis at 860 nm Wavelength : At 860 nm, the disparity in intensity between sample 1 and sample 2 is more evident , suggesting that the rate of contamination exerts a noticeable effect at this wavelength as well. The results at 860 nm further reinforce the findings at 610 nm, providing a comprehensive understanding of the impact of Allura red contamination and its impact on water's optical characteristics.

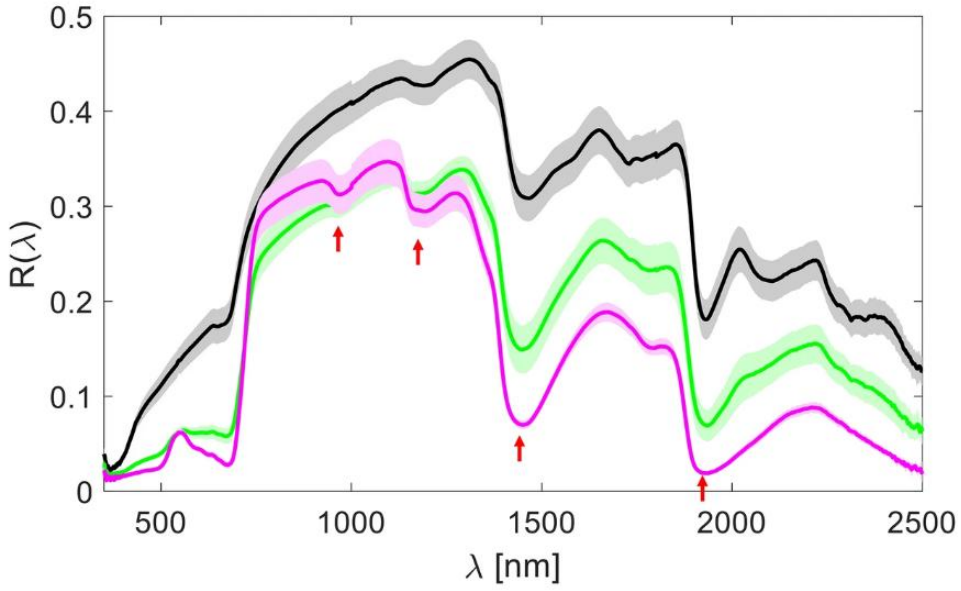

Fig. 6 : Spectral Analysis of three water samples. Curve in pink indicates the sample 0 , black for sample 1 and green for sample 2. The red arrows of the curve for sample 0 indicate the specific major absorptions and the shading in each curve shows the standard deviation from the baseline.

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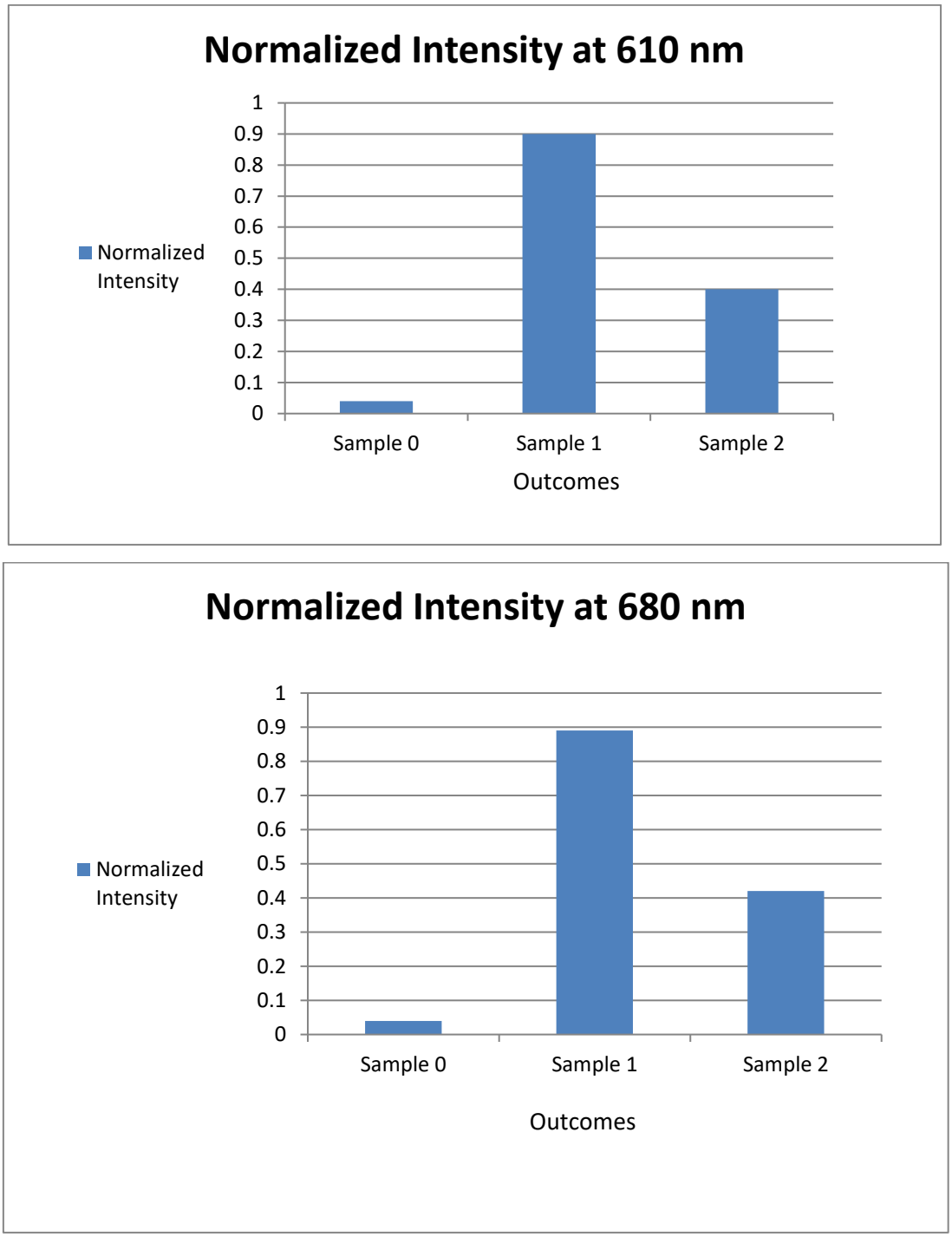

Fig. 7 : Normalized intensity of three water samples at 610 nm (upper)and 680 nm(lower).

3.3. Decolorization assay

It was measured in the form of percentage decororization using UV spectrophotometer as

$$\text{Percentage of decolorization} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100$$

Result shows the Saccharomyces uvarum to be the best efficient degrading bioagent for Allura red.

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Table 1 : Allura Red degradation potential of three yeast strains

Strain Used	Degradation time (days)	Decolorization efficiency (%)
Saccharomyces cerevisae	10	96.0
Saccharomyces uvarum	06	98.0
Saccharomyces lipolytica	08	91.0

Figure 8 : Slurry of three strains of yeast employed

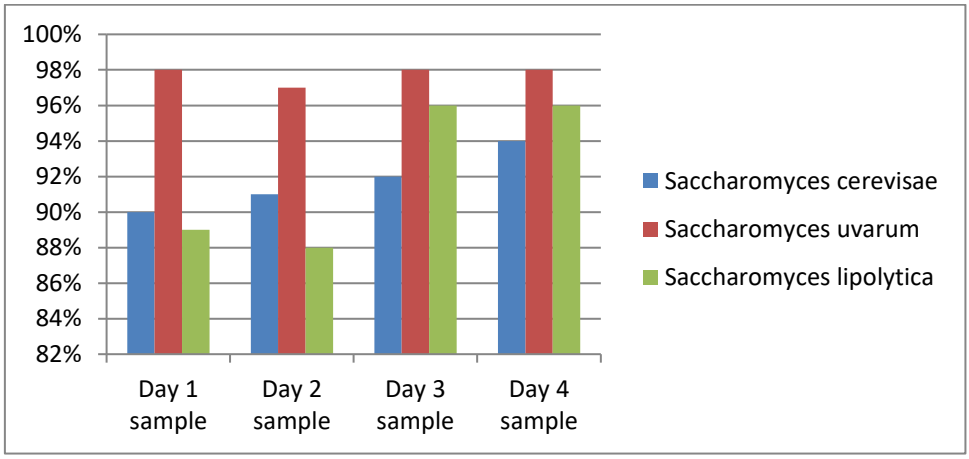

Figure 9 : Bar graph showing the decolorization efficiency of three strains of yeast employed

4. CONCLUSION

Azo dyes have become important in different industries, especially because color plays a huge role in consumer choices. However, with the increased usage of azo dyes, several health and environmental problems have emerged, which are caused by some of these azo dyes and its metabolites. As modern societies are striving toward greener solutions, bioremediation should be taken advantage of microorganisms have shown versatile performance not only in the biomedical field but also in the realms of environmental application. .The ability of these microorganisms to accept a broad spectrum of xenobiotics must also be also looked upon. Modern technology has displayed tremendous progress over the past decades.. This research marks a significant stride in water quality assessment, showcasing the efficacy of an AI-enhanced multispectral sensor system in detecting and analyzing

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water pollution. The combination of these developments and the area of bioremediation, especially in the field of dye degradation, is still an exciting venture to research. Ultimately, these microorganisms can pave the way for a hazard-free conversion of azo dyes and other xenobiotics. Many different approaches i.e. oxidation, photocatalyst, bioadsorption and nanotechnology are used to eliminate these dyes from wastewater effluents to prevent living organisms. These methods are costly and are not suitable to use at low azo dye concentration. Microbial use for dye polluted wastewater treatment is often considered as an eco-friendly method instead of conventional methods. Bacterial, yeast, and fungal capabilities to reduce these dyes are being investigated. Mixtures of aerobic and anaerobic bacteria are considered more efficient to degrade azo dyes.

The outcomes of the study highlight the current water quality of food color industrial effluents discharged into local surface waters and their potential effects on human health and aquatic life. In order to reduce water pollution and promote ecological and economic development, it is necessary to enforce water quality regulations & sustainable aquatic biodiversity.

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Conflict of interest

The authors declare that no conflict of interest among the contributing authors of this paper is there.

Authorship contribution statement

The entire research was conducted by the corresponding author Supriyo Acharya, along with manuscript writing , with the supervision of Dr. Paramesh Chaudhari and Dr. M.S. Gaikwad.

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The research object chemical Allura red was procured from the local market, Kermy synthetic food sellers & all the required reagents were provided by the Department of Zoology, GMRD college Samastipur, Bihar.