*Egypt. J. Chem.* **Vol. 67**, No. 1 pp. 353 - 370 (2024)

# Egyptian Journal of Chemistry

**http://ejchem.journals.ekb.eg/**

## Dcolorization and detoxification of real textile dyes wastewater using free and immobilized indigenous bacterial consortium in semi-pilot scale

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**Abstract**

Most of the liquid and solid effluents from textile industries are treated by physical and chemical methods such as flocculation, adsorption, filtration and oxidation. Accordingly the physical methods, however, simply accumulate and concentrate dyes and create solid wastes, and this problem of disposal still exists. Therefore, the main aim of this study was to evaluate the recently developed a semi-pilot system using combined biological and nanocomposites treatment for decolorizing and degrading dyes from real textile effluent. Moreover, biodegradation of synthetic and real wastewater using bacteria have been used on bench scale to optimizing the biodegradation conditions. After optimizing the condition a semi-pilot plant was operated by three mixed biocarriers, (free and immobilized mixed consortium, and bio-sorption using composite nanoparticles). The obtained results showed that, a good biodegradation and decolorization process where, following all phases and semi-pilot system effluents, all measured parameters significantly decreased. Moreover, the removal percent of chemical oxygen demand (COD), biological oxygen demand (BOD) and color of the effluent of real textile wastewater in semi-pilot system under anaerobic conditions reached to 67, 78.71 and 87.80%, respectively. The removal percent of COD, BOD and color reached 72.35, 67.71 and 90.81% under aerobic condition, respectively. The toxicity results indicated that the treated effluent of real wastewater in the semi-pilot system was nontoxic ($EC_{50} \geq 100$) under both aerobic and anaerobic conditions.

**KeyWords:** textile wastewater, decolorization, detoxification, Bacterial consortium, biological treatment, nano-composites

## 1. Introduction

By preventing light from reaching the deeper layers, the discharge of wastewater containing dye reduces the photosynthesis of aqueous ecosystems. It also impacts natural water bodies' visual appeal and clarity [1]. With a market share of between 60 and 70 percent, azo dyes are the most popular dyes. In comparison to other types, reactive azo dyes are utilized the most. Reactive azo dyes' main drawback is their propensity to hydrolyze, which causes a significant amount of unfixed colors to be washed out during the process. According to Fitzgerald and Bishop in 1995 [2], the dye effluent contains up to 50% of the original dye load. Although various initiatives have been taken to protect and enhance surface and groundwater quality, the amount of wastewater produced by the textile industry is still rising. Developing safe and practical alternative approaches for wastewater management is urgently needed [3].

Physical and chemical procedures are primarily used to treat textile effluent, however, they are not only expensive but also challenging to implement because of the intricate molecular makeup of dyes. The biological treatment of wastewater containing dye has drawn increased attention recently [4]. A promising replacement or addition to the current dye treatment procedures is bioremediation. The variety of microbes provides various genetic resources for environmental cleanup. Encapsulation is a popular biotreatment method because it shows promise for degrading contaminants in an immobilized matrix [5]. Biocarriers for immobilizing microorganisms must have a rough, uneven surface and be harmless. The matrix must be porous and hydrophilic to encourage proliferation and adhesion. The biocarriers are colonized by a high concentration of chemical-degrading microorganisms; their

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Receive Date: 05 June 2023, Revise Date: 11 July 2023, Accept Date: 17 July 2023

DOI: 10.21608/EJCHEM.2023.215695.8094

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attachment is facilitated by the porous structure and surface texture offered by the utilized biocarriers [6]. The single most crucial factor in immobilization is carrier selection. Finding an immobile carrier with consistent performance, good mass transfer, high intensity, extended lifespan, and affordable price is very important. The majority of immobilized materials identified are chemicals, which can only be produced industrially and hence have high costs. Natural biocarriers are gaining interest due to their advantages of being more affordable, simpler to obtain, and not requiring extensive experimental preparation upfront [7]. These characteristics make them suitable for production-scale application. When immobilized, bacteria are often contained by a substance, frequently a gel matrix, which prevents their flow. Increasing bacterial population through immobilization can result in a faster rate of pollution breakdown [8]. Several researches have been published on the biodegradation of reactive dyes using free cells. However, relatively little research on immobilized cells has been conducted. In order to decolorize Congo red, Lade et al. in 2015 [9] looked into the capacity of an immobilized consortium that could grow on wheat bran. The employed microbial community was immobilized using polyurethane foam (PUF). Within 12 hours, employing PUF-immobilized consortium, the entire decolorization process was completed. Suganya and Revathi in 2016 [10] used immobilized *Pseudomonas putida* and *Bacillus licheniformis* cells to test the decolorization effectiveness of reactive dyes (RR 195, RO 72, RY 17, RB 36). Polyacrylamide and sodium alginate (SA) beads were used for immobilization under static and shaking circumstances. Reactive orange 107, a sulfonatedazo dye, was examined by Frindt et al. in 2017 [11] in anaerobic-aerobic two-step batch mode studies. In order to study the correlation between the decolorization of the dyes and glucose levels, Akpor (2018) [12] decolored crystal violet and bromothymol blue in a batch procedure employing *Bacillus subtills* and *Pseudomonas aeruginosa* in both free and immobilized forms. Numerous studies have recently drawn attention to the toxicity (cytotoxic, genotoxic, and mutagenic) of wastewater and textile dye [13]. The treatment of textile wastewater is thus currently the most pressing necessity in terms of environmental safety. The use of biological oxidation was suggested as a pre-treatment or a post-treatment to reduce the need for needless chemical and energy consumption and, as a result, operating costs. Contrarily, the highly biodegradable portion of the wastewater is first biologically eliminated before the recalcitrant contaminants are degraded in AOPs post-treatment [14].

The chemical pre-treatment functions as partial oxidation of the biologically persistent part to produce biodegradable reaction intermediates, however, because many organic compounds released from dye houses are hazardous or resistant to biological treatment, the standard biological methods may not always yield good results, especially for industrial textile effluent. Therefore, the employment of a combination of biodegradation, advanced oxidation processes (AOPs), and nanomaterials widely acknowledged as very effective treatments for refractory wastewater is the only practical alternative for such biologically persistent wastewater [14].

There are enough studies on the application of nanomaterials in water treatment in the literature to justify a review of the developments in nanotechnology in AOPs. Carbohydrate biopolymers including cellulose, alginate, and chitosan have gotten a lot of attention during the last 20 years as substitute effective adsorbents in terms of economic viability and environmental relevance [15]. This is because of their exceptional benefits, including their abundance in nature, environmental friendliness, biodegradability, modification simplicity, and low manufacturing cost [16].

The ability of biopolymer-based adsorbents to bind to different heavy metals, harmful dyes, phenolic compounds, pharmaceutical residues, and oil spills has been the subject of numerous investigations [17]. Chitosan is a common, preferred carbohydrate biopolymer with amazing qualities among these biopolymers [18]. On the other hand, composites have caught the attention of the scientific community as effective adsorbents for the removal of metals, dyes, and other pollutants from wastewater. However, the composites of polyaniline, starch, polypyrrole, chitosan aniline, and chitosan pyrrole with peanut waste biomass have been produced and used for the adsorption of CV dye [19]. Consequently, the key objective of the investigation is to evaluate the recently developed semi-pilot system for decolorizing and degrading dyes from actual textile effluent.

## 2. Experimental

### *1- Application of biocarriers for decolorization of real textile wastewater in batch reactor*

#### *1.1. Preparation of seed bacterial consortium*

The mixed culture of five bacterial strains consortia of *Citrobacter freundii* (Accession number

MW485549), *Kelbsiella pnesumonia* (Accession number MW485585), *Enterobacter cloacae* (Accession number MW485599), *Pseudomonas aeruginosa* (Accession number MW485597) and *Aeromonas veronii* (Accession number MW485582) as used for decolonization of real textile wastewater. The initial bacterial counts of the consortium were determined using poured plate method according to APHA (2017) [20].

### *2.1. Biocarriers materials*

Egyptian Loofah (*Luffa cylinderica*), sponge, foam, palm leaf raffia, rice straw and grinded corn qgualh were used as biocarriers for bacterial consortium. The biocarriers were washed three times with distilled sterilized water and dried at 37°C for 4 h. Biocarriers are illustrated in Figure 1. Estimation of total bacterial counts in immobilized biocarriers was carried out according to APHA (2017) [20]. Moreover, Viability of bacterial consortium attached on the biocarriers was determined before and after the adsorption and desorption.

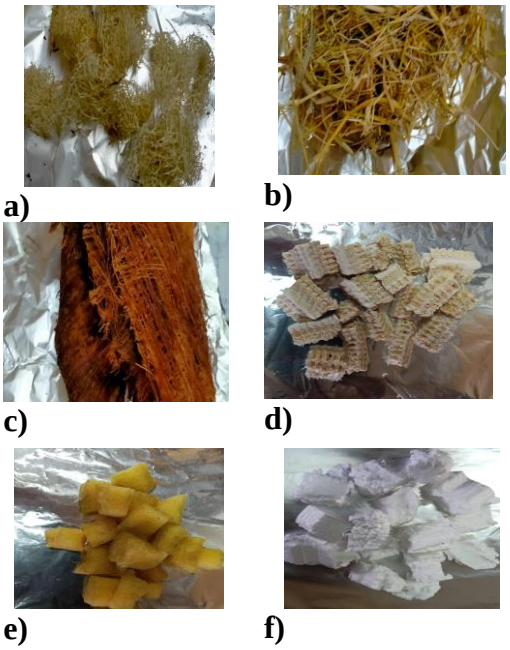



**Figure 1.** The biocarriers used in this study. a) Egyptian Luffa (Luffa cylinderica), b) Rice straw, c) Palm leaf raffia, d) grinded corn qgualh, e) sponge, f) foam

### *1.3. Immobilization and acclimatization of the mixed bacterial cells*

The carrier materials were immersed in culture flasks each contains 150 ml of mineral salts medium (MSM) with aeration for periods extended to 7 days with shaking incubator at 120 rpm/min. The bacterial counts were enumerated to determine the counts in one gram of each biocarriers using pour plate method according to APHA (2017) [20]. The immobilized bacteria either by adhesion and/or entrapment were checked by scanning electron microscopy (SEM). The saturated biocarriers with the mixed bacterial strains were acclimatized with 10 liter of real textile wastewater under aerobic condition for four weeks at lab., temperature (~ 25$^{o}$C ± 5$^{o}$C). The acclimatization was carried out using real textile wastewater samples during 10 days to adapt the bacterial consortium for dye degradation and optimize the reconstituted dying wastewater decolorization.

### *1.4. Scanning electron microscopy (SEM)*

During the treatment processes, the five natural biocarriers before and after biofilm formation and bacterial community structure were visualized through SEM (JSM5910, JEOL, Japan). Each natural biomaterial before biofilm formation was cut into 1 × 1 cm pieces and preserved in phosphate-buffered saline (PBS). The preserved samples were gold-coated prior to SEM, to get clear images. The microscopic images were scanned at 2–200-m resolutions, and 10 kV voltages. However, the biofilm-loaded biocarrers

were fixed with 2.5% glutaraldehyde solution for 4hrs of retention time and washed using low ionic 20 mM potassium phosphate buffer, pH: 7.3 (PBS). Afterward, the electrodes were dehydrated using a 10-100 % gradient alcohol series for 30 min for each stage with very gentle periodic agitation and then dried thoroughly. The desiccated samples were vacuum dried, mounted onto stubs, sputtered with gold, and then captured the images.

### *1.5. Application of biocarriers for decolorization of textile wastewater*

#### *1.5.1. Batch decolorization reactor*

#### *a. On synthetic wastewater*

Laboratory batch decolorization experiments were performed at room temperature (25±5$^{o}$C) in two litre glass flask containing 300 mL of mineral salt media solutions (MSM) supplemented with mixed selected dyes. The aerobic MSM was performed according to Marrot et al (2006) [21]. The MSM solutions were adjusted at pH 7 and autoclaved for 20 min at 121$^{o}$C and then cooled at room temperature. Two grams of each biocarrier that previously saturated by 8.2x10$^{8}$ CFU/mL of mixed bacterial strains were prepared. Some physicochemical

parameters (pH, color, EC, COD and TDS) were determined after 24 h.

**b. On real textile wastewater**

The real textile wastewaters were studied. Laboratory batch decolorization experiments were performed at room temperature (25±5ºC) on two liter glass flask containing 300 mL of real textile wastewater. Some physicochemical parameters (pH, color, EC, COD and TDS) were determined after 24 h under aerobic condition.

**1.6. Adsorption capability of studied nanocomposites**

In this study, the effects of adsorbent dosages (mostly in mg), solution pH (7.5), time taken for shaking (15, 30, 45, 60 min), initial concentration (in mg/L), effect of interfering ions, and temperature on adsorption were examined in most of the adsorption studies.

The adsorption capacity of nanocomposites as represented in Table (1) was performed in 250 mL iodine flasks containing 100 mL water containing mixed dyes namely, Crystal violet (CV), Direct blue (DB), Reactive red (RR), and Reactive yellow (RY) by 50 mg/L for each dye. The samples were mixed under stirring rate of 120 rpm at ambient temperature 25 °C for different contact time (15 min, 30 min, 45 min and 60 min). A hundred mg/L of studied nanocomposites were added in flask. After the mixing time elapsed, the suspension was allowed to settle, and the supernatant was analyzed for the residual dye. After completion of the adsorption process, the nanoparticles were isolated and then the concentration of azo reactive dyes was measured using a UV-visible spectrometer at maximum absorbance wavelength and using the calibration curve presented for each dye. The dye removal efficiency was determined using the following expression:

Dye removal efficiency (%) = $(C_0 - C_f/C_0) \times 100$

Where, $C_0$ and $C_f$ represent the initial and final (after adsorption) dye concentrations, respectively.

***1.7. Application and decolorization of textile wastewater in semi-pilot scale***

***1.7.1. Design and configuration of semi pilot-scaled system***

The semi pilot scale consisted of four tanks. The system automatically transfers the wastewater through pumps and was controlled by electrical control panel. The first tank was used a real textile wastewater container with 100 litres capacity and inoculated with the mixed free bacterial strains (100 mL/L/24h). While, the second tank contained on two columns with 50 ml in length and 7 cm in diameters. These two columns were packed with 200 grams of three biocarriers (loofah, rice straw and palm raffia) saturated with mixed bacterial strains after adaptation. The capacity of the second tank is 50 litres. On the other hand, the prepared nanoparticles were inoculated into the third tanks (with 50 litre capacity). The detention time of real wastewater inside tanks number two and three was 2 h. The fourth tank received 100 litres of the treated wastewater effluents.

**Table 1.**The abbreviations of studied nanocomposites

| Matrix I | |
|---|---|
| Cs-Cell | Chitosan cellulose |
| 1 Mg-Cf | 0.2mg Co Fe2O4 (Mg CF) |
| 2 Mg-Cf | 0.4mg Co Fe2O4 (Mg CF) |
| 3 Mg-Cf | 0.6mg Co Fe2O4 (Mg CF) |
| Matrix II | |
| Cs-PVA | Chitosan polyvinyl Alchohol |
| $Fe_2O_3$ | Pure $Fe_2O_3$ |
| 1-Cs-PVA-Cu | 0.3 mg Cu- Chitosan polyvinyl Alchohol ferrite |
| 2-Cs-PVA-Cu | 0.5 mg Cu- Chitosan polyvinyl Alchohol ferrite |
| 3-Cs-PVA-Cu | 0.7 mg Cu- Chitosan polyvinyl Alchohol ferrite |

***2. Working of the bioreactor***

Real textile wastewater was tested for decolorization and dyes removal. To begin with in tank 1, the collected wastewater (70 L) was injected with mixed bacterial strains (100 mL/L) and incubated at room temperature for 24 hr before the operation of system. Afterwards, system was operated with flow rate 10L/min, and 50L of wastewater were successfully transferred from tank 1 into tank 2 within 5 min, which had the natural biocarriers saturated with mixed bacterial strains as a biological treatment process. For increasing the bacterial activities, the tank 2 was facilitated with an aerator to supply $O_2$ for 60 min and promote the bacterial evolution. Under agitation (250 rpm) for 60 min, the effective dose of used nanocomposites/nanosorbent (100 gm/L) was inoculated in Tank 3 for degrading the residual dyes in tested wastewater received from tank 2 after biological treatment (Figure 2). The experimental trial was repeated in triplicate by operating the system 3 runs along 3 different intervals.

Physicochemical parameters including; pH, total suspended solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total kjeldahl nitrogen (TKN), total phosphorous (TP), Oil and grease, settleable solids, color, turbidity, nitrite, and nitrate, in addition to total bacterial counts and toxicity assay, were determined in the untreated and treated textile wastewater samples.

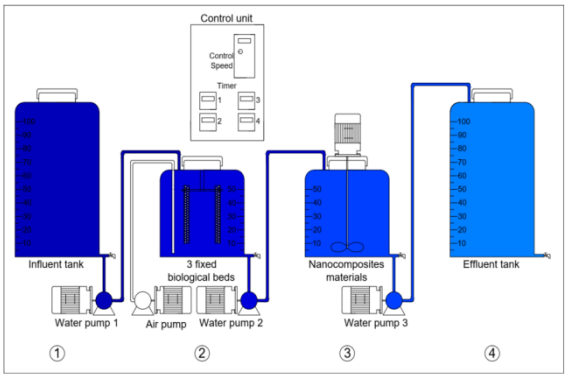



**Figure 2.** A schematic diagram of designed semi pilot-scaled system

Tosun et al. (1988) [22], Hemdan et al. (2016) [23] demonstrated that, the Reynolds number was calculated as a function of multiple ducts design, using the hydraulic equivalent diameter ($D_h$), defined as:

$D_h$ = 4 × Flow area/wetted perimeter

**For Semi-pilot model with single duct; (1)**

Flow area = $\pi/4\times d^2$

Where d, is the semi-circular duct diameter

Wetted perimeter = $\pi\times d/2 + d$

The Reynolds no. depends on hydraulic diameter is:

$Re = ( D_h\times u\times \rho)/ \pi$

Where;

- u: Flow velocity (m/s)
- ρ: Fluid density ($kg/m^3$)
- π: Fluid viscosity (kg/m.s)

**For multiple ducts with n ducts; (2)**

$D_h$ = 4 × Flow area/wetted perimeter

for n multiple ducts,

Flow area = $\pi/4n\times d^2$

Wetted perimeter = $\pi\times d/2 + d$

**1. Pumps specification and energy required for 70 L**

Mixer motor energy= 370 W/hr.
Mixer speed = 1:1390 RPM variable speed
Energy of water Pump 1, 2, 3 = 330 W/hr
Energy of Air pump 4 = 250W/hr
Air flow rate 84 L/min
Total air in one hour = 5040 L

***2.1. Phyisco-chemical characterization and total bacterial counts of the tested real wastewater***

***a. Analytical parameters and techniques***

The collected samples from each step were subjected to laboratory analyses, namely; pH, total suspended solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total kjeldahl nitrogen (TKN), total phosphorous (TP), oil and grease, settleable solids, color, turbidity, nitrite, and nitrate. All the analyses, unless specified, were carried out according to the Standard Methods for the Examination of Water and Wastewater APHA (2017) [20].

***b. Total bacterial counts***

TBC were carried out according to APHA (2017) [20] using plate count agar medium. One ml from each water sample or itis dilution was transferred into a sterile Petri dish; cooled melted agar was poured into Petri dish. Two plates were incubated at 37±0.5°C for 24 h and one un-inoculated plate served as a control. After the incubation period, all plates were counted with the use of colony counter.

***c. Toxicity Test***

Toxicity test was carried out using Microtox analyzer 500 instruments for all samples types. 10 µL

from tested water samples or from its dilution were added to 0.5 mL of Microtox diluent. The Microtox analyzer Model 500 is fully automated and temperature controlled and needs no daily adjustment or calibration. A marine luminescent bacterium *Vibrio fischeri* (earlier referred as *Photobacterium phosphoreum*) has been widely used for acute toxicity estimation with several commercial tests. The toxicity bioassay is based on the detection of light output changes through the light production (which directly relative to the metabolic activity of the bacterial population) and any inhibition of enzymatic activity causes a corresponding decrease bioluminescence. $EC_{50}$ and Toxicity levels were calculated according to Niemirycz et al. (2007) [24] and Mansour et al. (2022) [25].

## 3. Results and discussion

The preservation of the natural world has long since grown in importance on the political and economic fronts. The prudent management of water resources stands out as one of the priorities. Particularly in industrialized areas, the application is urgently needed. Agriculture accounts for over 70% of all water withdrawals worldwide. Domestic and industrial applications consume the final 30%. Manufacturers are urged to lessen the impact of their operations on the environment in light of the tightened waste restrictions worldwide and the mounting demand for the water supply. A maximum reduction in wastewater discharges is, therefore, the trend. In order to meet demand and safeguard resources simultaneously, local or regional wastewater recycling or reuse solutions can be put into place [26]. The utilization of secondary sources, such as wastewater, is, on the other hand, a need due to the global shortage of water supplies. In this context, local or regional recycling and/or reuse of treated wastewater, particularly in heavily water-intensive industrial sectors provide an intriguing method to meet demand and safeguard priceless resources [27].

### *3.1. The mixed bacterial strains that used*

Textile wastewater effluents are not stable and it varies often in a wide range depending upon the process practiced. Adaptation of microorganisms to varying pH and temperatures conditions makes them more suitable for the degradation of industrial effluents. The mixed bacterial culture of *Citrobacter freundii* (Accession number MW485549), *Kelbsiella pnesumoniae* (Accession number MW485585), *Enterobacter cloacae* (Accession number MW485599), *Pseudomonas aeruginosa* (Accession number MW485597) and *Aeromona sveronii* (Accession number MW485582) are able to decolorize the textile dyes at broad pH and temperature range. The optimum pH and temperature for dye decolorization were 7.5 and 35ºC, respectively .All the used bacterial strains are Gram negative bacteria and facultative anaerobic.

#### *3.1.1.Estimation of total bacterial counts in immobilized biocarriers in synthetic textile wastewater (Preliminary experiment)*

Before preceding any biodegradation and decolorization experiments it was important to determine the bacterial consortium counts that will be immobilized on each biocarriers. The initial count of free bacterial cells was $8.2x10^8$ CFU/mL. The counts of bacterial consortium that immobilized on sponge, foam, loofah, rice straw palm raffia and grinded corn qgualh were $5.3x10^7$, $1.2x10^7$, $4.6x10^7$, $3.2x10^7$, $2.1x10^7$ and $1.0x10^7$ CFU/gm, respectively after seven days.

### *3.2. Acclimatization of the immobilized mixed bacterial strains on biocarriers*

To study the stability of mixed bacterial strains on the three selected biocarriers in real textile wastewater, the bacterial counts were estimated. The initial count of free bacterial strains was $5.3x10^{10}$ CFU/mL. The bacterial cells that released after 24 h from one gram of loofah, rice straw and palm raffia were $9.6x10^9$, $4.8x10^9$ and $8.0x10^9$ CFU/gm, respectively (Table 2). The bacterial counts were increased after 7 days with the increase of contact time. It means that the bacterial strains were able to adapt with the real textile wastewater under aerobic conduction to accelerate biofilm development and to provide information on azo dye degradation [28].

**Table 2:** Acclimatization of the immobilized mixed bacterial strains on biocarriers

| Time | CFU/gm | | | |
|---|---|---|---|---|
| | **First basin (Loofah)** | **Second basin (Rice straw)** | **Third basin (Palm raffia)** | **grinded corn on flask** |
| **After 24 h** | $9.6x10^9$ | $4.8x10^9$ | $8.0x10^9$ | $1.5x10^9$ |
| **After 7 days** | $1.9x10^{12}$ | $8.5x10^{12}$ | $3.6x10^{11}$ | $6.5x10^{12}$ |

### *3.3. Viability test*

In this work, the survivability of the immobilized bacterial cells on various naturally

derived biocarriers was evaluated on actual textile wastewater on a bench scale to guarantee the mixed bacterial strains immobilized remained viable without losing their variety [29]. After becoming accustomed to actual wastewater for seven days in an aerobic batch setting at room temperature (25ºC), the adapted bacterial community was trained to remain alive in the biocarriers for 96 hours. After 24 and 48 hours, the decolorization rates were around 48 and 37.8 percent, respectively. The result was one of the benefits of immobilization since, over the four days, the examined loofah's bacterial counts ranged from 1.2 $x10^6$ to 1.7 $x10^{10}$ CFU/mL. While the examined rice straw had counts that vary from $5.0x10^7$ to $5.0x10^8$ CFU/mL. The studied palm riffia's bacterial consortium counts, however, ranged from $1.0x10^8$ to $1.9x10^9$ CFU/mL. Between $1.3x10^9$ and $2.9x10^9$ CFU/mL were found to be attached to the biocarriers mix (3 gm of loofah, 3 gm of rice straw, and 3 gm of palm riffia) (Table 3). After 24 and 48 hours, the mixed biocarriers had the maximum color removal (60 and 52%) (Table 3). The benefit of maintaining viability is that it may be used again for sub-sequencing procedures. The stable and enhanced enzyme activity in the immobilized state may be the origin of these phenomena [30]. Because the foam and sponge employed in this study were not naturally occurring, we opted to use the naturally occurring biocarriers (loofah, palm raffia, and rice straw) at a semi-pilot scale. According to Khouni et al. (2020) [30], the decolorization rate was very high, 91-100%, at dye mass loading rates that ranged between 1.25 and 7.5 mg/g/d following the acclamation stage of biomass to the biocarriers (around 4 days).

**Table 3.** Viability of mixed bacterial consortium immobilized on the biocarriers and inoculated to real dyes wastewater at bench scale

| **Biocarriers** | **Color(Co/Pt)** | | **The initial bacterial counts $5.3x10^{10}$CFU/mL** | | | |
|---|---|---|---|---|---|---|
| | **After 24 h** | **After 48 h** | **The first day** | **The second day** | **The third day** | **The fourth day** |
| **Control** | **425** | **425** | | | | |
| **3 gm of Loofah** | **251** | **254** | $6.2x10^8$ | $8.0x10^8$ | $7.5x10^8$ | $8.2x10^8$ |
| **6 gm of Loofah** | **307** | **256** | $7.1x10^9$ | $6.2x10^9$ | $5.1x10^9$ | $5.3x10^9$ |
| **12 gm of Loofah** | **302** | **306** | $8.6x10^9$ | $1.0x10^{10}$ | $1.2x10^6$ | $1.7x10^{10}$ |
| **3 gm of rice straw** | **355** | **251** | $5.0x10^7$ | $2.3x10^8$ | $2.0x10^8$ | $2.9x10^8$ |
| **6 gm of rice straw** | **278** | **259** | $1.1x10^8$ | $3.6x10^8$ | $3.2x10^8$ | $3.6x10^8$ |
| **12 gm of rice straw** | **304** | **259** | $4.3x10^8$ | $5.0x10^8$ | $4.1x10^8$ | $4.5x10^8$ |
| **3 gm of palm raffia** | **246** | **242** | $1.0x10^8$ | $2.5x10^8$ | $2.1x10^8$ | $3.1x10^8$ |
| **6 gm of plam raffia** | **291** | **270** | $5.2x10^8$ | $9.2x10^8$ | $7.5x10^8$ | $8.4x10^8$ |
| **12 gm of plam raffia** | **285** | **258** | $1.9x10^9$ | $9.6x10^8$ | $8.4x10^8$ | $9.1x10^8$ |
| **Mix of biocarriers** | **170** | **204** | $1.3x10^9$ | $2.9x10^9$ | $1.7x10^9$ | $2.1x10^9$ |

**Control :** Real textile wastewater
**Mix of biocarriers:** 3 gm of each biocarriers (Loofah+ rice straw+ palm raffia)

### 3.4. The used Biocarriers

In the annals of history, the twentieth century is remembered as a time of extraordinarily rapid advancements in culture and technology. Environmental issues were caused by industrialization, conflict, and the usage of synthetic xenobiotics and heavy metals on a broad scale [31]. Numerous microbes are capable of degrading a variety of contaminants [32]. The physiological status of the microorganisms, susceptible to various environmental influences, determines the biodegradation rate. According to Wasilkowski et al. (2014) [33], immobilization is necessary to increase microorganisms' resilience to adverse environmental effects. Many agricultural wastes in Egypt can serve as biocarriers [34]. Examples include used sponge [31], palm leaf raffia, maize qgualh, and Egyptian loofah (Luffa cylindrica) [35]. These carrier materials are economical [36] and safe for the environment (nontoxic, nonpolluting materials). Large surface area and powerful adsorption capabilities are provided by biocarriers. They also increase the activity of dehydrogenase and promote oxygen transport [35]. A crucial component of bioremediation involves

preserving substantial biomass of microbial populations on biocarriers [36].

In order to immobilize the dye-degrading bacterial cells for decolorizing mixed dyes in synthetic solution and natural real textile wastewater samples, four natural bio-materials (loofah, rice straw, palm raffia, and ground corn) were chosen. These materials were selected for their affordability, accessibility, and reproducibility. The establishment of distinct complex bacterial consortia on all examined biocarriers was discovered by SEM examination of the samples from each biocarrier (Figure 3). In the stationary phase of cell development, bacteria have a negative surface charge, which interacts with the majority of positively charged surfaces of natural biomaterials to form a biofilm [37]. The literature can also guide the analysis using SEM procedures [38]. Similar findings were made by Balapure et al. (2015) [39] over the surface of a pumice stone used as a support material. In this investigation, SEM showed that the bacteria were connected to the four tested biocarriers in the form of a short rod. The SEM demonstrated that bacterial metabolism and the release of their EPS chemicals were the primary sources of energy for the surface of biocarriers. In addition, the SEM revealed an excellent porosity structure that offered enough protection to prevent bacteria from being readily washed away [40].

### *3.5. Application of nanocomposites for textile dyes decolorization*

Dye degradation was possible under anaerobic, aerobic, and anaerobic-aerobic conditions, as well as with the assistance of bacteria. Hydrogenation, which yields hydrazobenzene, and oxidation by hydrogen peroxide and peracids, both of which promote the molecule, are azo group addition reactions [41]. AD removal from textile effluent in minutes is also made possible by technological advancements in nanoparticle microbe enzyme conjugates [42].

To treat actual dye pollutant samples, it is necessary to design appropriate adsorbents [43]. We employed two nanoparticle matrixes to adsorb the textile colors in this investigation. All adsorption studies were conducted in a batch system utilizing various dye and nanoparticle solution concentrations and contact times at a regulated temperature of 25 ºC, pH 7.5, and 120 rpm. For nanoparticle matrix I (Cs-Cell, 1 Mg-Cf, 2 Mg-Cf, and 3 Mg-Cf) and matrix II (Cs-PVA, Fe2O3, 1-Cs-PVA-Cu, 2-Cs-PVA-Cu, and 3-Cs-PVA-Cu), the influence of contact or stirring time on the efficacy of adsorbing dyes was separately studied. All test solutions' starting dye concentrations ranged from 10 to 50 mg/L.

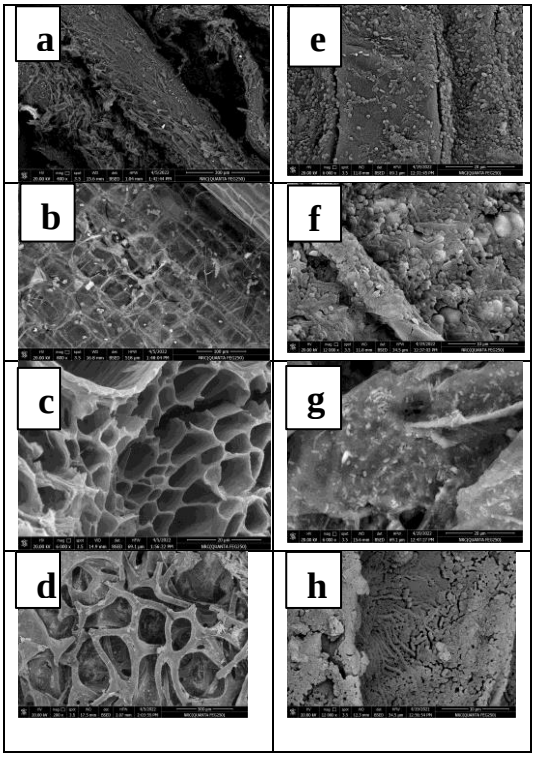



**Figure 3.** Scanning electron micrographs (SEM) of selected biomaterial before biofilm formation (a-d): a) Loofah, b) Rice straw, c) Palm raffia, and d) grinded corn and after biofilm formation as a biocarriersloaded immobilized bacterial cells (e-h) e) Loofah, f) Rice straw, g) Palm raffia, and h) grinded corn

#### *3.5.1. Evaluation of matrix I of magnesium nanocomposite.*

When combined with $CoFe_2O_4$, the chitosan cross-linking can enhance the mechanical and chemical characteristics, resulting in a magnetic composite that is more stable in acid solutions, has a high capacity for adsorption, and is simple to remove from the liquid once the adsorption operation is complete [44]. According to the literature, investigations have yet to be done on synthesizing chitosan/$CoFe_2O_4$ materials [45, 46]. Without any obvious application, all of these investigations merely addressed the production and characterization of the substance.

#### *e. Mixture of dyes*

To our knowledge there is no available information regarding the removal of synthesized

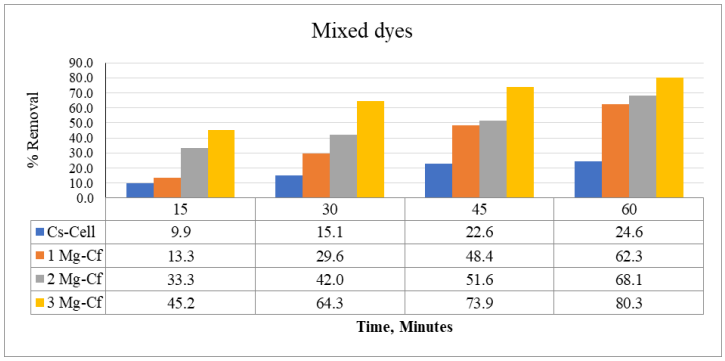
mixed dyes from aqueous solutions. The majority of the works used single dye as a group model. In the present work, a mixture of the four dyes (CV, DB, RR, and RY) was prepared with a concentration of 50 mg/L for each dye. The mixture solution was then scanned for the determination of its wavelength. The color removal was calculated based on the difference between the absorbance before and after contact with nanoparticle. After 60 min contact time; about 80% removals was achieved using 3Mg-Cf (Figure 4). Also, 2Mg-Cf showed better performance on mixed dyes removal than 1Mg-Cf NPs where the removal was 68% for 2Mg-Cf after 60 min reaction time while it reached only 62% for 1Mg-Cf.

### *3.5.2. Evaluation of matrix II of Copper nanocomposite*

According to Cheung et al [47], chitosan, a biodegradable polymer, is being extensively researched as a potential adsorbent for the removal of dyes. Chitosan's amino and hydroxyl groups are its active sites, and they aid in the adsorption of organic dyes. In extreme environments, it is less stable. A cross-linker, polyvinyl alcohol, can make chitosan more stable. It adds more active sites for dye adsorption and strengthens the chitosan by creating a -H bond with the amino group of the chitosan on chitosan together with other binding substances [50]. Additionally, magnetic materials tend to interact with pollutants through a variety of processes, including as electrostatic attraction and surface complexation. They would therefore contribute to the overall adsorption process. Due to the ease with which magnetic nanocomposites are drawn to magnetic fields, any reactor system may readily have then removed. At the same time, silver and other metal nanoparticles are said not to have as good molecule [48]. Although difficult to separate following the adsorption process, chitosan may be solubilized in dilute acid solutions. These two factors to determine disadvantage of chitosan's industrial use [7]. Therefore, several researches documented the use of magnetic iron oxides covered with polymers, such as chitosan, as magnetic composites with adsorbent characteristics, which may be separated from the aqueous medium by a straightforward application of an external magnetic field [49].

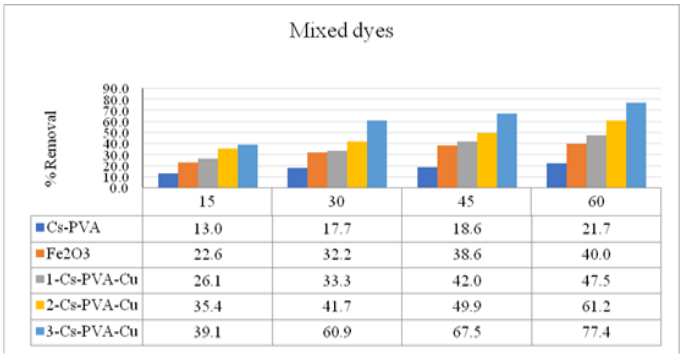
## e. Mixture of dyes

Figure (5) showed that the maximum removal efficiency was achieved by 3-Cs-PVA-Cu with value of 77%. Chitosan is a remarkable adsorbent with a remarkable chelation activity that enables it to bond firmly with various organic and inorganic substances, including polluting ones. However, pristine chitosan has some evident disadvantages, including limited mechanical and chemical stability and difficulties retrieving the adsorbents from the water after adsorption [43]. The prevention of oxidation, increased strength, and easier separation of the adsorbent from the reaction mixture are all benefits of loading magnetic elements adsorption [51]. So, using the Sol-Gel technique, two distinct nanocomposites matrix were created. The more effective magnetic nanocomposites for removing dyes were employed and tested for adsorption. Therefore, in this work, these magnetic nanocomposites can function as better nano adsorbents in the controlled environment of a semi-pilot-sized system for the treatment of synthetic dyes and real textile effluent.




| | 15 | 30 | 45 | 60 |
|---|---|---|---|---|
| Cs-Cell | 9.9 | 15.1 | 22.6 | 24.6 |
| 1 Mg-Cf | 13.3 | 29.6 | 48.4 | 62.3 |
| 2 Mg-Cf | 33.3 | 42.0 | 51.6 | 68.1 |
| 3 Mg-Cf | 45.2 | 64.3 | 73.9 | 80.3 |

**Figure 4.** Mixture dyes removal efficiency as a function of time using different nano-particles adsorbent




| | 15 | 30 | 45 | 60 |
|---|---|---|---|---|
| Cs-PVA | 13.0 | 17.7 | 18.6 | 21.7 |
| Fe2O3 | 22.6 | 32.2 | 38.6 | 40.0 |
| 1-Cs-PVA-Cu | 26.1 | 33.3 | 42.0 | 47.5 |
| 2-Cs-PVA-Cu | 35.4 | 41.7 | 49.9 | 61.2 |
| 3-Cs-PVA-Cu | 39.1 | 60.9 | 67.5 | 77.4 |

**Figure 5.** Mixture dyes removal efficiency as a function of time using different nano-particles adsorbent

### *3.6. Application of the mixed biocarriers for decolorization in batch reactor*

#### *3.6.1. In synthetic wastewater*

In this stage, we used mixture dyes of nine dyes including; reactive red (R.R) , reactive yellow (RY), reactive blue (RB), reactive orange (RO), direct red (DR), direct blue (DB), crystal Violet (CV), Congo red (CR) and methylene blue (MB). The highest color and COD removal were observed with foam and sponge and followed by loofah, grinded corn, rice straw and palm raffia (Table 3).

From the results in table (3) it can be concluded that the removal percentage of color and COD in control sample (free cells) was lower than the removal percentage in immobilized sample. The slightly low decolorization rate of free cells compared to the immobilized cells might be attributed to the mass transfer restriction arising from cell entrapment. The results are disagreeing with those reported by Saratale et al. (2011) [52] where free *P. vulgaris* required about 5 h for complete decolorization of reactive blue "172" while PVA-immobilized beads achieved only 8% decolorization at the same period. However, the results in this study were in agreement with the findings outlined by Cheng et al. in 2005 [8] where 90% decolorization was achieved using immobilized cells of *Burkholderia vietnamiensis* compared to 85% color removal by using free cells.

#### *3.6.2. In real wastewater*

The total bacterial counts at 37 and 22°C were $2.1x10^3$ and $6.3x10^3$ CFU/mL, respectively (Table 4).

**Table 3.** Comparison between different biocarriers for removal of mixed dyes from synthetic wastewater

| Mixed dyes | | | | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **TBC** | **pH** | **Color** | **COD** | **TDS** | **EC** |
| **Unit** | **CFU/mL** | **---** | **Co/Pt** | **mg/L** | **mg/L** | **µs/cm** |
| **Blank** | --- | 7.46 | 1990 | 360 | 7350 | 12250 |
| **Control** | $8.2x10^8$ | 7.42 | 1700 | 255 | 7290 | 12150 |
| **R%** | | | 14.57% | 29.16% | 0.81% | 0.81% |
| **Loofah** | $6.2x10^8$ | 7.44 | 1640 | 248 | 7300 | 12178 |
| **R%** | | | 17.5% | 31.1% | -0.68% | -0.68% |
| **foam** | $9.2x10^8$ | 7.49 | 870 | 178 | 7230 | 12050 |
| **R%** | | --- | 56.2% | 50.5% | 0.95% | 0.96% |
| **sponge** | $9.5x10^8$ | 7.41 | 1100 | 220 | 7220 | 12033 |
| **R%** | | --- | 44.7% | 38.8% | 1.09% | 1.10% |
| **Grinded corn** | $2.3x10^8$ | 7.43 | 1670 | 270 | 7240 | 12067 |
| **R%** | | | 16.0% | 25% | 0.82% | 0.82% |
| **palm raffia** | $1.0x10^8$ | 7.44 | 1789 | 240 | 7210 | 12017 |
| **R%** | | --- | 10.1% | 33.3% | 1.23% | 1.90% |
| **Rice straw** | $5.0x10^7$ | 7.42 | 1720 | 249 | 7250 | 12083 |
| **R%** | | --- | 13.5% | 30.8% | 1.36% | 1.3% |

**Blank :** without mixed bacterial strains and biocarriers

**Control :** with mixed bacterial strains (Free cells) and without biocarriers

This stage involved testing several biocarriers to see which ones were effective at removing colors from actual textile effluent (Table 5). Grinded corn (136 Co/Pt) showed the greatest color reduction. While utilizing foam and then ground corn produced the highest COD elimination rates. Given that even up to 50% of the dyes used are not fixed to the textile fibres but instead persist as pollutants in the liquid phase [5,8,28]. It is evident from the results in table (5) that the biodegradation rate for real textile wastewater was marginally higher than in synthetic textile wastewater (Table 3).

A diverse combination of different substances, including salts, metals, and organic molecules, is also present in the batch final effluent. Unexpectedly, under all experimental circumstances, decolorization failed to occur in the leftover dye batch effluent. This shows that treating the entire textile wastewater is preferable to treating only the effluent from the dyeing process. This might be as a result of the ultimate wastewater coming from all the industrial processes that consume water being significantly more diluted than the batch effluent from leftover dyes [30].

It is important to note that dyes are persistent and challenging to degrade because of their complex aromatic structure and synthetic origin. Furthermore, depending on the specific textile process, such as scouring, desizing, mercerizing, bleaching, dyeing, printing, and finishing, detergents, surfactants, dispersants, levelling agents, toxic organics (phenols), chlorinated compounds (AOX), sulphide, and formaldehyde, as well as inhibitory compounds, grease and oil, and many other compounds, are present in the effluents of the textile industry [14].

Our research shows that the native bacteria that evolved in the bioreactors efficiently decomposed both colors and metabolites in real textile effluent. Prior study employing synthetic dye solutions suggested an excellent potential for the regulated treatment of azo dyes [51]. The current work significantly builds on previous findings by building and analyzing a biodegradation process utilizing actual textile effluent that also contained process chemicals.

**3.7. Decolorization and degradation of real textile wastewater using semi-pilot system**

As a result of our analysis, the only practical alternative for the industrial application of textile wastewater treatment was to utilize chemical oxidation techniques as a pre-treatment and biological processes as a post-treatment, or vice versa. The majority of the investigations mentioned thus far were bench-scale, laboratory experiments. Only a few papers on pilot plants and even fewer about commercial applications of integrated biological processes and AOPs are included in the scaling up literature study. The main issues for scaling up microbial mediated dye degradation are the creation of sustainable, financially feasible, and time-efficient. The best alternative strategy for improving the effectiveness of dye removal from wastewater was to use bioreactors, which could effectively regulate the process parameter conditions. In general, bioreactors have been used for dye degradation studies in two ways: free cell systems, in which microorganisms are directly inoculated into the bioreactor, and immobilized systems, in which microorganisms are either encapsulated in biocarrier support or grown on the surface of the bio-carrier. The immobilized technology involves benefits, including higher productivity, a larger surface area for microbial growth, and improved process parameter monitoring. Due to better microbial endurance in harsh settings, such as pH and temperature, and specially designed process conditions to treat at greater concentrations, immobilized microbial carriers in the bioreactor are the most appropriate choice for dye degradation. In this work, a semi-pilot plant was operated by three mixed biocarriers, three free and immobilized mixed consortiums, and bio-sorption using composite nanoparticles.

**Table 4.** Some physicochemical characteristics and total bacterial counts for the tested real textile wastewater collected from Factory 3

| Parameters | Unit | Results |
|---|---|---|
| **pH** | -- | 8.36 |
| **TSS** | mg/l | 103.325 |
| **TDS** | mg/l | 7822.75 |
| **Color** | Co/Pt | 278.5 |
| **Turbidity** | NTU | 81.275 |
| **COD** | mg/l | 319.6667 |

| | | |
|---|---|---|
| **BOD** | mg/l | 140.75 |
| **TKN** | mg/l | 12.87 |
| **Ammonia** | mg/l | 3.386667 |
| **Nitrite** | mg/l | 0 |
| **Nitrate** | mg/l | 136.6 |
| **TP** | mg/l | 9.806667 |
| **Oil and grease** | mg/l | 14.575 |
| **Total bacterial counts at 37°C** | CFU/mL | $2.1x10^3$ |
| **Total bacterial counts at 22°C** | CFU/mL | $6.3x10^3$ |

**3.7.1. Decolorization rate of real wastewater**

Real textile wastewater was tested for decolorization and dyes removal. Before treatment real textile wastewater was placed in dark container made of PVC for 24 h to settle any insoluble particulate materials and used as a raw influent for the semi pilot (Table 6). To begin within tank 1, the collected wastewater after settling (70 L) was injected with mixed bacterial strains (100 ml/L) and incubated at room temperature for 24 h before the operation of system. Afterwards, system was operated with flow rate 10L/min, and 50L of wastewater was successfully transferred from tank 1 into tank 2 after 5 min, which have the natural biocarriers saturated with mixed bacterial strains as a biological treatment process. For increasing the bacterial activities, the tank 2 was facilitated with aerator to supply $O_2$ for 60 min to promote the bacterial evolution. Under agitation (250 rpm) for 60 min, the effective dose of used nanocomposites / nanosorbent (100 gm/L) were inoculated in Tank 3 for degrading the residual dyes in tested wastewater, which received from tank 2 after biological treatment. The study was conducted under low level of COD (126 - 336 mg/L) which is the actual load of factory at this time. All stages of the experiment were operated under lab temperature (~25 °C±5). The experimental trial was repeated in triplicate by operating the system 3 runs with 3 different day's interval. The averages counts of mixture bacteria were $5.2x10^9$, $4.7x10^{10}$, $3.4x10^8$ and $8.5x10^3$ CFU/mL in tanks 1, 2, 3 and 4, respectively.

**Table 5.** Comparison between different biocarriers for removal of dyes from real textile wastewater

| **Real textile wastewater** | | | | | |
|---|---|---|---|---|---|
| | **pH** | **Color** | **COD** | **TDS** | **EC** |
| **Unit** | **---** | **Co/Pt** | **mg/L** | **mg/L** | **µs/cm** |
| **Blank** | 7.55 | 328 | 180 | 4500 | 7500 |
| **Control** | 7.52 | 259 | 148 | 3470 | 7283 |
| **R%** | 7.52 | 21.04 | 17.7% | 2.28% | 2.97% |
| **Loofah** | 7.53 | 203 | 148 | 4470 | 7500 |
| **R%** | --- | 38.1% | 17.8% | 0.66% | 0.66% |
| **foam** | 7.5 | 189 | 110 | 4370 | 7310 |
| **R%** | --- | 42.37 | 38.8 | 2.88% | 2.53% |
| **sponge** | 7.51 | 249 | 156 | 4420 | 7367 |
| **R%** | --- | 24.08% | 13.3% | 1.7% | 1.7% |
| **grinded corn** | | 136 | 130 | 4450 | 7417 |
| **R%** | --- | 58.53 | 27.7% | 1.11% | 1.1% |
| **palm raffia** | 7.5 | 230 | 153 | 4496 | 7493 |
| **R%** | --- | 29.8% | 15% | 0.08% | 0.09% |
| **Rice straw** | 7.5 | 240 | 160 | 4490 | 7420 |
| **R%** | --- | 26.8% | 11.1% | 0.22% | 1.06% |

**a. Decolorization rate of real textile wastewater without aeration**

As shown in Table (7), the biodegradation performance of textile wastewater in designed semi-

pilot scaled system was reported by measuring some the physicochemical characteristics. The efficiency of the semi-pilot system (Fig. 2) was calculated by comparing between intake and treated effluent of each step. Raw and treated effluent after each process including biological and nanosorption. From the obtained results, it could be reported that there is an excellent biodegradation and decolorization activity in which the all measured parameters were dramatically decreased during different steps of semi-pilot system. The backing biocarriers were replaced and changed after each run of operation.

**Table 6.** Pollutants reduction after 24 hours of plain settling

| parameters | Unit | Initial | After sedimentation | R % |
|---|---|---|---|---|
| **pH** | -- | 8.08 | 8.1 | --- |
| **TSS** | mg/L | 48 | 27 | 43.7 |
| **TDS** | mg/L | 2421 | 2211 | 8.6 |
| **EC** | mg/L | 3764.8 | 3504 | 6.9 |
| **COD** | mg/L | 305 | 195.8 | 35.8 |
| **BOD** | mg/L | 140.8 | 81 | 42.4 |
| **Color** | Co/pt | 258.5 | 227.3 | 12 |

**b. Decolorization rate of real wastewater with aeration**

Three batches of biodegradation performance in designed system supported with aeration to explore the effect of oxygen on biodegradation rate. Oxygen was pumped into tank 1 after free dye-degrading bacteria were injected into the textile wastewater to provide the oxygen requirements for biological processes and to mix the free bacterial cells with the real wastewater. As shown in Table (8), the biodegradation performance of textile wastewater in designed semi-pilot scaled system facilitated with aeration was reported by measuring some the physicochemical characteristics raw and treated effluent after each process including biological and nanosorption. From the obtained results, it could be depicted that there is an excellent biodegradation and decolorization activity in which the all measured parameters were dramatically decreased after all steps and effluents of semi-pilot system.

In an immobilized aerated reactor, STE containing a combination of the dyes RB 221, RY 145, and RR 195 was treated. The azo dye decolorization capacity of three different natural bacterial consortia was tracked and assessed. Khouni et al. (2012) [30] also ran unique bacterial consortia in an aerobic sequencing batch reactor with noteworthy results for decolorization. Enzymatic and chemical reductions are involved in the degradation of azo dyes in any environment [52]. Mono- and di-oxygenases of microbial origin involved in dissolving aromatic ring structures in dyes are well recognized for incorporating $O_2$ under aerobic conditions [30]. Additionally, bacterial consortium immobilization offered several benefits over suspended cells [53], and bacterial consortium treatment for textile dyes has received extensive research [54]. The results demonstrated that 71.9% of the dye was decolored after 24 hours in the immobilized sludge reactor. Although the decolorization rate increased with time, no substantial decolorization was seen after 192 hours [55].

The reactor for immobilized sludge has the highest decolorization percentage (91.9%). When bacterial consortia from sludge were immobilized in reactors, a similarly high dye removal rate was observed [56]. The characteristics of the microbial consortia and the reactor's operating circumstances affect the decolorization percentage. As various studies have validated the sequential anaerobic-aerobic treatment of azo dyes, the cause for the reactor's insufficient decolorization may be related to operating circumstances [57]. During the subsequent anaerobic-aerobic treatment, complete mineralization of azo dyes was accomplished [58]. In 24 hours, the immobilized alkalophilic reactor decolored to 53%. The greatest decolorization percentage was achieved in 192 hours and was 85.3%. However, the decolorization efficiency rose progressively. Lalnunhlimi and Krishnaswamy (2016) [59] achieved comparable results using an alkalophilic consortium for the degradation of color. According to consensus [60], high pH significantly negatively impacts the movement of dye molecules across cell membranes. This limits the effectiveness of full decolorization. Therefore, bacteria that are used to higher pH levels will be absolutely important for total decolorization. When the pH was raised from 6 to 11, Eskandari et al. (2019) [61] found that a bacterial consortium called PsGo was seven times more effective in decolorizing materials (85%). Although the bacterial community in this work exhibits good removal rates for BOD and COD in textile wastewater, it could not reduce EC, TSS, and TDS significantly. The result is

that these bacteria are unable to digest highly salinized wastewater.

### 3.8. Toxicity Results

Regarding the toxicity test of textile wastewater samples with aeration that displayed in Table (9) extremely toxic level were observed for raw textile wastewater samples with $EC_{50}$ about 5.2%, light toxic level was observed with sample withdrawn from tank 1 with $EC_{50}$ about 15%. On the other hand, the samples were withdrawn from tanks 2, 3 and 4 were nontoxic (Table 9). The toxicity level of samples which withdrawn from tanks 2, 3 and 4 without aeration was nontoxic. It was clear that extremely toxic level were observed for raw textile wastewater samples with $EC_{50}$ about 5.2%, very toxic level was observed with sample withdrawn from tank 1 with $EC_{50}$ 7.2%. While, light toxicity level was observed with sample that withdrawn from tank 2 with $EC_{50}$ 16.2%. The samples were withdrawn from tanks 3 and 4 were nontoxic (Table 10).

**Table 7.** The average values of some important physicochemical parameters of real textile wastewater in influents and effluents of semi-pilot system

| Items | Raw | Tank 1 | % removal | Tank 2 | % removal | Tank 3 | %removal |
|---|---|---|---|---|---|---|---|
| **pH** | 8.04 | 7.65 | -- | 7.52 | -- | 7.06 | -- |
| **TSS** | 26.6 | 22.25 | 16.3534 | 6.3 | 76.3158 | 7.272 | 72.66165 |
| **COD** | 336 | 174.5 | 48.0655 | 100.5 | 70.0893 | 77.64 | 76.89286 |
| **BOD** | 137 | 75.5 | 44.8905 | 32.85 | 76.0219 | 29.16 | 78.71533 |
| **Color** | 244 | 59.75 | 75.5123 | 37.5 | 84.6311 | 29.76 | 87.80328 |

**Table 8.** The average values of some important physicochemical parameters of real textile wastewater in influents and effluents of semi-pilot system with aeration

| Items | Raw | Tank 1 | % removal | Tank 2 | % removal | Tank 3 | %removal |
|---|---|---|---|---|---|---|---|
| **pH** | 8.17 | 7.67 | --- | 7.60 | --- | 7.03 | --- |
| **TSS** | 29.16 | 10.12 | 64.99 | 7.63 | 73.91 | 4.41 | 84.81 |
| **COD** | 153.66 | 72.17 | 51.13 | 52.73 | 64.58 | 41.86 | 72.35 |
| **BOD** | 62.33 | 26.77 | 54.27 | 22.37 | 62.64 | 19.63 | 67.73 |
| **Color** | 249 | 71.44 | 70.71 | 30.50 | 87.99 | 23.33 | 90.81 |

Bacterial decolorization of dyes is frequently started understatic/anaerobic/microaerophilic conditions by an enzymatic transformation process in which reductive breakage of the azo bond results in the creation of colorless aromatic amines. Under solely aerobic circumstances, these poisonous amines can be further oxidized to produce simpler, non-toxic versions [62]. Most of the research that has been done thus far has focused on the initial anoxic phase of therapy, which only decolorizes colored azo dyes. Secondary amines, the resulting intermediate metabolites that are more poisonous than the original dye, pose a significant health risk to the ecosystem [63]. *Pseudokirchneriella subcapitata*, *Sinorhizobium meliloti*, and *Vibrio fisheri* all saw a reduction in acute microbial toxicity as a result of the biological therapy carried out under aerobic circumstances [64, 65, 66]. Alternating anaerobic and aerobic conditions must be used for azo dye removal and detoxification. Azo dyes are anaerobically discolored, producing aromatic amines that might harm living things. Further therapy is frequently required since these amines cannot be eliminated without oxygen. In the aerobic stage, the aromatic amines that were created due to the azo bond cleavage in the anaerobic step are further broken down [67].

**Table 9.**Toxicity test of textile wastewater in semi pilot scale with aeration

| no. | samples | $EC_{50}$ | Toxicity Level |
|---|---|---|---|
| **1** | Raw Textile wastewater | 5.2 | Extremely toxic |
| **2** | Tank 1 | 15 | Light toxic |
| **3** | Tank 2 | ≥100 | Non toxic |
| **4** | Tank 3 | ≥100 | Non toxic |
| **5** | Tank 4 | ≥100 | Non toxic |

**Table 10.**Toxicity test of textile wastewater in semi pilot scale **without** aeration

| no. | samples | $EC_{50}$ | Toxicity Level |
|---|---|---|---|
| **1** | Raw Textile wastewater | 5.2 | Extremely toxic |
| **2** | Tank 1 | 7.2 | very toxic |
| **3** | Tank 2 | 16.2 | Light toxic |
| **4** | Tank 3 | ≥100 | Non toxic |
| **5** | Tank 4 | ≥100 | Non toxic |

## Conflict of interest statement

The authors declare that they have no conflict of interest.

***Acknowledgment***
The authors would like to thank Science, Technology & Innovation Funding Authority (STDF) for financial support project number 41562. Also, the authors would like to thank Eng. Hasan Mohamed Mostafa, semi-pilot scale Department, National Research Centre (NRC) for drawing the semi-pilot system.

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