DUCKWEED-DERIVED ADSORBENT FOR EFFICIENT METHYLENE BLUE REMOVAL FROM SYNTHETIC WASTEWATER

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09-262242-001

DEPARTMENT OF EARTH & ENVIRONMENTAL SCIENCE

BAHRIA UNIVERSITY ISLAMABAD CAMPUS

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A Thesis Submitted in Fulfillment of the Requirements for the Award of the Degree of Master of Science in Environmental Science

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I dedicate this thesis work to myself and to those who helped me, including my professor, friends, and family members. Whose prayers and support have been a beacon for me to climb to the top of a greasy hill.

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ABSTRACT

The main problem is the untreated azo-reactive pollutants and textile dyes, such as methylene blue, which are toxic to both the environment and human health. Chemical and physical treatment methods are not only expensive but also have subordinate environmental weaknesses. Duckweed was used as an economical, sustainable, and cost-effective adsorbent to remove methylene blue from synthetic wastewater. The main objectives of the study include isolation and conversion of DW into an eco-friendly adsorbent, to evaluate the effect of physical parameters, including pH, adsorbent dose, time, and contaminant concentration, and to apply reaction kinetics and reaction isotherm models. The adsorbent was prepared and then examined using various methods, including scanning electron microscopy, X-Ray diffraction spectroscopy, and Fourier transform infrared spectroscopy. Different concentrations of methylene blue were prepared, and batch experiments were performed involving various conditions of pH, adsorbent dose, time, and contaminant concentration to evaluate the optimal removal efficiency of MB by the prepared adsorbent. Citric acid was used as an activator that increases the efficiency of duckweed and reduces the leaching of chlorophyll color. The results were analyzed based on absorbance using a UV spectrophotometer at 580 nm, and the maximum removal efficiency of 93.3 % was achieved at a pH of 10, MB concentration of 50 mg/L, an adsorbent dose of 50mg, and in 30 minutes. Desorption studies were done to verify the removal efficiency, which decreases after two to three uses, so it is suitable for a particular use. Duckweed acts as a favorable substance for regulating methylene blue dye pollution in industrial wastewater.

Keywords: Methylene Blue, Duckweed, Adsorption, Spectrophotometer.

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ANALYTICAL INSTRUMENTS AND EQUIPMENT

Filter Paper	GE Healthcare Whatman 125mm Filter Paper
UV spectrometer	ORI Germany UV 4000 Spectrometer
Oven	Memer + Oven
Analytical Balance	UniBlock Analytical Balance
Shaker incubator	Labo tonics Ashless Shaker Incubator
SEM	JEOL IT100LA Scanning Electron Microscope
XRD	Panalytical X-Ray Diffraction with X'Pert HighScore analytical software
FTIR	ThermoFisher Scientific Fourier Transform Infrared Spectroscopy

LIST OF SYMBOLS

ppm	-	Parts Per Million
mL	-	Milliliter
mm	-	Micrometer
V	-	Volume
UV	-	Ultraviolet
rpm	-	Rotations Per Minutes
g	-	Grams
nm	-	Nanometer
C	-	Concentration
m	-	Mass
t	-	Time
A_o	-	Initial Absorbance
A_t	-	Final Absorbance
C_o	-	Initial Dye Concentration
C_t	-	Residual Dye
C_e	-	Equilibrium Concentration
Q_{max}	-	Monolayer Adsorption Capacity
q_t	-	Absorbance capacity
qe	-	Absorbance at Equilibrium
ln	-	Natural Log
qt	-	Absorbance at Time

TSS	-	Total Suspended Solids
TDS	-	Total Dissolved Solids
BOD	-	Biochemical Oxygen Demand
COD	-	Chemical Oxygen Demand
XRD	-	X-Ray Diffraction
FTIR	-	Fourier Transform Infrared Spectroscopy
SEM	-	Scanning Electron Microscope
x	-	Resolution
wt.%	-	Weight Percentage
μm	-	Micrometer
cm^{-1}	-	Wavelength
π	-	Pie
K	-	Rate Constant
K_1	-	Rate Constant at Pseudo First Order Reaction
K_2	-	Rate Constant at Pseudo-Second Order Reaction
mg/L	-	Milligram per Liter
mg/g	-	Milligram per Gram
NTU	-	Nephelometric Turbidity Unit
μS/cm	-	micro Siemens per centimeter
b	-	Langmuir Constant
R_L	-	Separation Factor
kJ/mol	-	Kilojoules per Mole
g/mol	-	Gram per Mole

CHAPTER 1

INTRODUCTION

1.1 Water Quality and Its Importance

Water is an essential natural resource, pivotal for most biological roles, which makes it essential for all known living organisms. It is found profusely in oceans, rivers, and lakes in different forms, including saltwater, brackish water, and freshwater, and in the environment, it occurs in the form of solid ice, liquid, and gaseous vapors. Chemically, it is a polar compound holding one oxygen and two hydrogen atoms, making it an excellent solvent for biological mechanisms. It shows distinctive physicochemical properties that make it a regulator of Earth's temperature (Atkins, De Paula, & Keeler, 2023). It has a high latent heat of vaporization and specific heat capacity property that helps in regulation and distribution through the hydrological cycle across the surface and subsurface (Mays, 2010). Water Quality is described by the physical, biological, and chemical characteristics of water that have a profound influence on human and animal health. Although it is complicated, it is a measurable factor. Modern predicaments like industrialization, urbanization, and increased population growth have had a huge effect on freshwater quality, leading to an increase in the demand for freshwater. Regulating and monitoring freshwater quality has become a great obstacle in the past ten years, especially in urban areas where people use means such as concealed boreholes and municipalities to fulfill their demands (Bharti, Khandekar, Sengupta, Bhadwal, & Kochhar, 2020). Access to secure food and clean drinking water is a basic human right that must be on the priority list to achieve the highest well-being of the population (Imran, Bukhari, & Gul, 2018).

The chemical parameters that affect water quality are turbidity, nutrients, pH, TSS, TDS, EC, DO, BOD, COD, contaminants, and physical factors, including taste, color, and odor, measured against parameters set by WHO to check suitability for agriculture, drinking, ecosystem health, and industrial use (Poonam, Tanushree, &

Sukalyan, 2013). Total Suspended Solids (TSS) and Total Dissolved Solids (TDS) are two of the key quality parameters of water, which measure undissolved suspended elements like bacteria, organic matter, salt, etc., and dissolved particles like minerals and inorganic salts that indicate water quality and purity. pH is the hydrogen ion concentration that measures the alkalinity and acidic nature of water, which has a vital influence on metal toxicity, nutrient availability, chemical reactions, and aquatic life. EC is the measure of the electrical conductivity of water that indicates dissolved ions like minerals, salt, and chemicals. BOD is biological oxygen demand, COD is chemical oxygen demand, and DO is dissolved oxygen, which measures the organic pollutant content, treatment efficiency, and pollutant load. According to the WHO report, TDS should be less than 500, TSS should be less than 10 mg/l, pH between 6.5 and 8.5, EC should be less than 800 μS/cm, and Turbidity should be less than 5 NTU for drinking water; it should be free from any color, microbes, and odor. (Organization, 2022).

Bioaccumulation and the toxic nature of heavy metals released from pharmaceuticals, industries, pesticides, production houses, and other sectors pose a long-term risk even in minute quantities that can lead to serious health effects like cancer, kidney failure, and neurological disorders. Water quality depends on the factors of anthropogenic activities, the geological composition of the earth, and other activities that use water as a source of production (Organization, 2022). Many insoluble and soluble substances are added to water, moving towards the groundwater table, which influences the properties of water, causing effects on the availability of safe drinking water (Burge, Hoffman, Hartman, & Venedam, 2005). Natural lakes and streams are becoming contaminated, leading to the problem of eutrophication after many nutrients are added due to running water that washes nutrients like phosphorus, potassium, and nitrogen through agricultural waste, human excreta, and other sources. The effluents discharged from anthropogenic activities have a significant influence on the environment, especially in degrading the quality of water bodies, as they contain a high number of chemicals, pathogens, nutrients, and toxic materials, which result in harmful effects for both humans and other species by degrading their natural habitats. Additionally, these anthropogenic activities, like urbanization, deforestation, and dam construction, have affected the natural recharge system and water quality (Vörösmarty et al., 2010).

Water quality is continuously depleting, and it has been deteriorating in the past decade due to an increase in anthropogenic activities, improper water use, and a lack of

treatment that led to an increase in demand for clean drinking water and has caused adverse health effects. The World may face a global water shortage challenge in the year 2030 if daily water use remains the same. There is a need to monitor, assess, and identify efficient water usage technologies that can be advantageous for water conservation and long-term use.

1.2 Types of Wastewaters

Wastewater is one of the oldest subjects of concern, as it is increasingly becoming the cause of cancerous diseases and ecosystem collapse due to the overburden of chemicals and nutrients, which not only impacts water bodies but also impacts soil and vegetation. It can provoke food chain contamination, cancer, neurological damage, hepatitis, and waterborne diseases as water bodies become enriched with contaminants like pathogens, heavy metals, herbicides, pesticides, and other pollutants, depending upon the type of contamination. Wastewater has increased the effects on land and ecosystems, as well as raising public health concerns, especially in underdeveloped nations and rural regions, so it also requires expensive treatment. Wastewater primarily consists of four major types: agricultural wastewater, domestic wastewater, Stormwater, and industrial wastewater (Joshua, 2021). Agricultural wastewater involves water originating from agricultural runoff and municipal sources for irrigation; thus, it carries sediments, salts, nutrients like phosphorus and nitrogen, biological, and chemical contaminants containing many pesticides, herbicides, and heavy metals that lead to an increase in food demand and water scarcity (Obijianya et al., 2025).

A substantial amount of agricultural wastewater holding nutrients can be recycled. However, it contributes to chemical stress and high salinity levels, thus requiring proper treatment. The other category is domestic wastewater that includes water released because of household activities like laundry, good housekeeping, bathing, dishwashing, etc. It mainly contains detergents, pathogens, organic matter, and other impurities (Yadav et al., 2024). This type of wastewater contains a high number of TDS, TSS, and organic load, which makes it unfit for drinking (FAO, 2021). Domestic wastewater is usually classified into two sub-categories, including blackwater with high pathogenic and organic contents and greywater comprising low organic and pathogen levels, which differ in rural and

urban areas (Cheng et al., 2021). Noticeably, domestic wastewater is a reason behind water source contamination, eutrophication, groundwater contamination, and vector breeding. To treat the impurities, methods include primary treatment involving sedimentation and screening, secondary biological treatment involving filters and activated sludges, and tertiary treatment.

The third type is stormwater, involving wastewater born out of natural events such as snow and rainfall. As a result of these natural activities, water merges with pollutants, contaminants, nutrients, and sediments, entering directly into water streams and groundwater (Subramaniam, Zambrano-Monserrate, Permatasari, & Guangling, 2026). In this water, there is low organic content but a variety of oils and chemicals responsible for soil erosion and floods during the process. Meanwhile, climate change, urbanization, and land use patterns also influence the impacts and severity of stormwater (Kumar et al., 2024). Stormwater is a non-traditional type of wastewater, which combines with sewerage systems and severely affects the infrastructure. Like other types of wastewater, it can also be collected and reused as it holds nutrients. However, it needs to be treated under strict supervision to ensure safety protocols since it severely increases water quality degradation, urban flooding, and sewerage overflow that directly or indirectly become the reason for health problems.

Industrial wastewater is contaminated water released from industries because of commercial and manufacturing processes, which is enriched with impurities like oils, pollutants, heavy metals, toxicants, and other solids. Industrial wastewater can be reactive, toxic, ignitable, and carcinogenic; therefore, it requires proper treatment owing to its potentially hazardous nature and instigator of adverse health effects (Ahmed, Thakur, & Goyal, 2021). Industrial wastewater has the potential to cause irreversible health effects and damage to aquatic life and other animals, but it is casually disposed of in irrigated lands or water bodies, which causes serious effects to the food web. Due to its interaction with the food ecosystem, cancer, cholera, diarrhea, jaundice, and hepatitis are increasing day by day due to the consumption of contaminated water. Industrial wastewater includes a variety of heavy metals, including copper, iron, nickel, lead, arsenic, zinc, and mercury, mainly released from pharmaceutical, fine paint, textile, chemical, and dye manufacturing, where phenolic compounds and phenols act as major pollutants (Rajkumar & Palanivelu, 2004). Many organic and biodegradable pollutants are also present. Their biological treatment becomes challenging as it is a slow process

and still holds the probability of conversion into secondary pollutants post-treatment. Consequently, these secondary pollutants cause organ damage, nervous system damage, reduced growth, and oxidative stress (Bilal et al., 2022). The composition of industrial wastewater is complex, as it holds many dyes, non-biodegradable materials, biocides, lignin compounds, urea, phosphorus, and nitrogenous waste released through textile, printing, pharmaceutical, and dyeing industries (Wang, Hipel, Wang, & He, 2011).

The major categories of industrial wastewater include textile and pharmaceutical industries, where cadmium, phenolic compounds, nickel dyes in pharmaceuticals, NaOH, iron, chlorinated compounds, salts, urea, chromium, and surfactants in textile industries are major water pollutants that increase BOD and COD of water (Ahmed et al., 2021). The water that is enriched with pharmaceutical active ingredients released from hospitals, municipal sewage, manufacturing plants, improper disposal, antibiotics, and waste from patients' excretion is termed pharmaceutical wastewater. It is biologically active and toxic, thus requires proper and advanced treatment, as it is the source of pollutants that repeatedly cause undesirable effects to the environment, even in minute amounts (Zampelli & Verna, 2025).

Pharmaceutical wastewater includes a wide range of drugs like paracetamol, Ibuprofen, Indomethacin, Diclofenac from the NSAID pharmacological Class, Sulfadiazine, Atenolol, Ciprofloxacin, Ofloxacin, Carbamazepine, etc. from the antibiotic class. pharmacological class and Propranolol, Metoprolol, and Ranitidine from the Antihypertensive pharmaceutical class, and cocaine from the CNS Stimulant Pharmaceutical class (Gros, Petrović, Ginebreda, & Barceló, 2010). These drugs are removed from water by implementing the methods of Electrocoagulation, Coagulation, Ozonation, AOP+ Oxone, and active carbon adsorption, which removes them with an efficiency of 64 to 96 percent, depending on the type (Mortazavi et al., 2019). Pharmaceutical wastewater is becoming the main reason for ecotoxicity in aquatic organisms, as untreated continuous discharge can lead to chronic toxicity of water in lakes, groundwater, rivers, and other water bodies, which inhibit the growth of fish by causing reproductive organ failures, endocrine disruption, population imbalance, and interference with hormonal regulation. (Susilawati, Levita, Susilawati, & Sumiwi, 2023). It also causes antimicrobial resistance and a human health risk through consumption or exposure to contaminated water.

Among the world's greatest and most sophisticated industrial sectors are the textile industries that involve cultivation, fiber production, processing of raw elements, dyeing, garment construction, finishing, but releasing a large amount of wastewater and solid waste, amalgamated with nonbiodegradable pollutants, salts, dyes, heavy metals, and other chemical pollutants (Zhang et al., 2025). These pollutants cause water pollution, microplastic release, soil degradation, acute to chronic human effects, and aquatic habitat destruction (Bartram, 2002; Zhang et al., 2025). It compounds the load on fossil fuel use and energy demands that require proper treatment. An example of textile industry wastewater is dye-based wastewater. It is a type of wastewater that holds diverse types of dyes in different proportions, usually discharged from textile industries as an effluent. It acts as one of the harmful types of wastewater, as this kind has the capacity to increase COD and BOD, block sunlight that can hinder photosynthesis, and cause organ failure, especially in fish.

Dyes are well known as coloring agents, and they are used in the coloring or dyeing of substances like paper, clothes, and other materials. They are strong since their color cannot be altered after being affected by factors like heating, washing, light, or other factors (Lellis, Fávaro-Polonio, Pamphile, & Polonio, 2019). They also affect plants and soils as it alters soil chemistry, causing a visible reduction in the fertility of crops by inhibiting microbial activity. Few dyes are biodegradable, but most of them are non-biodegradable and have negative effects even in minute amounts (add reference here). The solutions for the removal of these dyes from water include adsorption, resource recovery, advanced oxidation processes, and bioremediation. According to research, there are almost ten thousand dye products that annually produce 7 lakh tons of dyestuff, and almost 10 percent of the total is released into water bodies. This ratio is increasing day by day due to an increase in demand, and once they alter the water's natural mechanism, it is difficult to remove them completely (Eshaq & ElMetwally, 2019).

One of the types of dye-based wastewater toxicity is methylene blue wastewater, which encumbers photosynthesis and blocks sunlight, affecting processes like photocatalysis and adsorption in aquatic life. In contrast, it also causes neuromuscular and cardiovascular issues in humans. For example, trace concentrations of synthetic dyes like MB can bioaccumulate, leading to severe health complications such as impaired neurological development, while simultaneously degrading the visual and aesthetic quality of water systems. It can also become a major source of an increase in health risks,

leading to cancer, anemia, and nausea, due to its ecological persistence (Eshaq & ElMetwally, 2019).

1.3 Methylene Blue and Its Health Impacts

Methylene blue is a highly concentrated, dark blue dye widely utilized in industries and laboratory settings. Historically, it was first synthesized in 1876 by the German chemist Heinrich Caro via the oxidation of aniline derivatives, following the broader mid-19th-century boom in synthetic dye development initiated by William Henry Perkin. Due to its intense chromophore properties, methylene blue possesses a strong affinity for biological substrates and effectively stains human tissues (Howland, 2022). In its solid state, it exists as a dark green crystalline powder that dissolves readily in water at room temperature, exhibiting a maximum light absorbance at 665 nm. It has recently been used as a fluorophore agent in surgical treatment, microbial staining, and in numerous clinical methods with minimal negative effects on patients, including the detection of enterovesical, intestinal, breast cancer, tumors, and bronchopleural sinuses. In contrast, it is helpful in the treatment of eczema and herpes labialis. It is toxic for microalgae as it suppresses cellular growth and reduces photosynthetic pigments, causing a great phytotoxic effect, and it also interferes with hemoglobin oxygen, causing skin irritation, gastric distress, and severe health effects (Cwalinski et al., 2022; Howland, 2022).

Chemically, methylene blue is a 3,7-bis (diethylamino)sulfur and nitrogen-containing heterocyclic organic chloride salt. It also acts as an antidepressant and antioxidant compound that is decomposable but carcinogenic, typically released from food processing, tanning, paper production, and cosmetics industries. Its common names include methylthionine chloride, CL basic Blue 9 methanediyl as a radical, methylidene as an IUPAC name, or CH_2 in solid form, and Methylene Dichloride, Methylene Chloride, and Dichloromethane or DCM as a solvent having a molar mass of 14.0266 g/mol, molar entropy of 193.93 J/(K.mol), and 386.39 kJ/mol enthalpy of formation. It is related to compounds such as Silylene (SiH_2), carbide, and methyl (CH_3). It also causes aesthetic pollution as it is highly pigmented, which gives visible colors to streams and other water bodies (Khan et al., 2022)

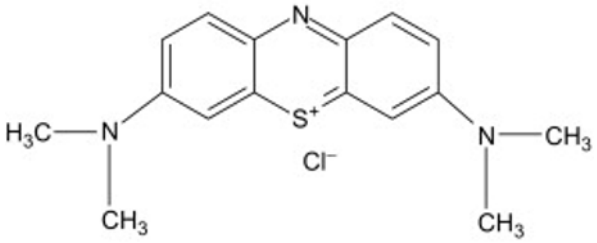


Figure 1.1: Chemical Structure of MB Dye (Tang Shu Hui & M. Abbas, 2015)

A low dose of methylene blue is favorable, but if the dose increases, it can cause toxicity that can lead to bluish skin or urine. It has a half-life of 5 to 6.5 hours when excreted in urine for examination after 4 to 24 hours. Historically utilized as a cotton-dyeing agent, methylene blue was later transitioned into medicine as a treatment for malaria and a standard therapeutic intervention for methemoglobinemia. Beyond its diverse clinical history, its physicochemical properties make it a prominent subject in environmental remediation. As a strongly cationic, pH-dependent dye, its removal efficiency from aqueous solutions is significantly optimized at alkaline pH levels. This optimization is governed by the strong electrostatic forces of attraction that develop between the cationic dye and negatively charged adsorbent surfaces (Hamad & Idrus, 2022)

Beyond its use in visualizing enlarged glands and treating urinary tract infections, methylene blue serves as a primary intervention for methemoglobinemia. Methemoglobinemia is a rare hematological disorder characterized by an abnormal accumulation of methemoglobin, which causes red blood cells to exhibit an impaired capacity to bind and release oxygen, resulting in systemic tissue hypoxia. The etiology of methemoglobinemia includes genetic predispositions as well as exposure to oxidizing agents such as nitrites, benzocaine, and dapsone. Clinically, the condition manifests through symptoms such as cyanosis, dizziness, dyspnea, and fatigue. While diagnosis is confirmed via pulse oximetry and co-oximetry blood tests, treatment strategies vary by severity: mild cases are typically managed with supportive care and intravenous hydration to facilitate clearance, whereas high-risk cases require the administration of specific antidotes, most notably methylene blue itself. Paradoxically, while valuable in clinical settings, methylene blue poses severe ecological challenges when discharged into the environment. As a highly stable heterocyclic aromatic compound, it exhibits significant resistance to conventional wastewater treatment methods. Consequently, the dye persists

for extended periods in soil and aquatic media, necessitating advanced oxidation or remediation strategies (Hamad & Idrus, 2022).

In emergency medicine, methylene blue is indicated for the treatment of cyanide poisoning, a condition characterized by acute symptoms such as seizures, cardiac arrest, cyanosis, and unconsciousness. It similarly functions as a vital redox agent to improve the blood's oxygen-carrying capacity during methemoglobinemia crises, though its use requires caution due to the risk of triggering serotonin syndrome. At the cellular level, the compound provides notable antioxidant benefits, boosting mitochondrial respiration, cellular energy production, and cognitive memory retention. It further serves as a therapeutic agent for Vasoplegia by blocking the guanylate cyclase pathway to stabilize blood pressure. While certain jurisdictions have restricted or banned its broader commercial applications, its high efficacy and low cost sustain its targeted use in micro-dosages within specialized medical frameworks (Pandey, Daverey, Dutta, Yata, & Arunachalam, 2022).

Methylene blue serves as an essential diagnostic aid across various surgical specialties. In thoracoscopic interventions, it is applied to localize pulmonary nodules and malignant tissues, while in pelvic and abdominal surgeries, it facilitates the intraoperative visualization of the ureters to reduce negative surgical complications. Methylene blue, even in small quantities, can cause coloration, reducing sunlight that suppresses photosynthesis, causing effects in the aquatic food web. It also causes reproduction problems, oxidative stress, cellular damage, and environmental effects (Khan et al., 2022; Howland, 2022; Ahmed et al., 2021). The colored dye is unsuitable for drinking, domestic, agricultural, and industrial use as it depletes natural resources (Vörösmarty et al., 2010).

Following administration, mild side effects may include transient injection-site pain, which can be managed via saline irrigation. Other documented dermatological reactions include rashes, abscesses, burning sensations, and superficial ulcers. Systemically, the compound typically causes a distinct but benign green or bluish discoloration of the urine, which may be accompanied by a brief period of transient hemolysis that rapidly normalizes. However, high doses of methylene blue pose significant toxicological risks. Elevated serum concentrations can trigger dyspnea, mental confusion, severe headache, nausea, abdominal pain, and vertigo. At critical thresholds,

acute systemic toxicity may result in life-threatening complications, including cardiac arrest, renal failure, and severely decreased peripheral blood flow rates (Peter, Hongwan, Küpfer, & Lauterburg, 2000). It is also used in the diagnosis of ureters during operations, which reduces negative effects on patient health, and it works on the principle of the light ureter catheter. Alternatively, it has the potential to cause an allergic reaction when used. It causes an impact on photosynthesis and leads to eutrophication due to its high molecular stability (Khan et al., 2022). At the industrial level, primary, secondary, and tertiary treatments are used as removal treatments at the commercial level. Using biological, physical, and chemical processes.

1.3.1 Removal Analysis of Methylene Blue from Wastewater

Conventional treatments, including both physical and chemical methods, are available for treatment, but they are not only costly but also have many secondary environmental disadvantages, so there is a need to find an environmentally friendly, safe possibility for the treatment of methylene blue. A substantial number of Technologies have been used for the removal of dyes, such as photochemical, adsorption, Fenton reaction, and ozonation, but they possess some negative effects, like secondary pollutants (Crini et al., 2019). Many strategies are also studied for effective removal of methylene blue, involving bioremediation, membrane filtration, enzymatic procedures, electrochemical extractions, chemical coagulation, and physical adsorption. One of the methylene blue removal methods is photodegradation, which is a complex oxidation process having advantages like mineralization, as it uses catalysis under UV-vis light. But it has some limitations and side effects that include the formation of secondary pollutants and byproducts from catalysts, changes in pH, and residual dye toxicity. Sometimes it reacts with catalysts and causes catalytic fouling by clogging the surface of the catalysts, which directly causes a reduction in the efficiency of the reaction. Photodegradation is also affected by factors like pH, retention time, type of catalyst, and concentration(Khan et al., 2022)

Methylene blue has numerous applications due to its negative and favorable qualities and antagonistic nature. Another treatment method is through Chemical and Physical processes that involve flocculation, coagulation, electrochemical, and membrane

filtration methods. The coagulation-flocculation method is universally used as an efficient technique for methylene blue removal. In this process, a suitable coagulant is added that eliminates coloring agents from the water (Castillo-Suárez, Sierra-Sánchez, Linares-Hernández, Martínez-Miranda, & Teutli-Sequeira, 2023). This is an effective technique that is divided into three stages, including coagulation, flocculation, and decantation. At the initial stage, a chemical coagulant, either ferric chloride, aluminum sulfate, or another, is added that destabilizes suspended particles by neutralizing their charges. In the second step, a synthetic cationic or anionic flocculant is added that helps in settling destabilized particles. This step is slow, and in the ultimate step, these sediments are separated. In the case of methylene blue, natural bentonite is used as a coagulant due to its greater availability and inexpensiveness (Oussalah & Boukerroui, 2020)A large amount of clay minerals is also used for removing diverse types of dyes (Adesina, Elvis, Mohallem, & Olusegun, 2021).

Another treatment process, called the electrochemical oxidation process, is used for the degradation of blue methylene with greater efficiency. On comparison with electrocoagulation and electro-Fenton processes, electrochemical oxidation is more advanced as it operates easily, is environmentally friendly, and operates easily (Berruti, Oller, & Polo-López, 2021). In this process, a graphite rod acts as the cathode, an iron rod acts as the anode, current is then applied through electrodes, and pH is maintained between 3 and 11. It performs a destabilization reaction of colloidal fragments using a resolvable anode substance. Mostly metallic aluminum and iron are used as anodes. On the other hand, EC has some negative effects, as it consumes a large amount of energy and electrode consumption (Song et al., 2018). It is a green and reliable method that has two steps of occurrence: the direct oxidation step, where pollutants lose electrons after touching the anode, and the indirect oxidation step, where water gets oxidized, forming an effective radical that further attacks pollutants like methylene blue and degrades it.

Many biological treatments are also used, including enzymes, microbial degradation, and the bio adsorption process. In the case of enzymes, mostly yeast is used, which releases proteases and lipases that degrade pollutants. In microbial degradation, yeast or fungi are used, and in bio adsorption, microbes are grown on biochar that performs the removal process (Song et al., 2018). One of the safest means of methylene blue treatment is bioremediation. In which adsorption acts as a main phenomenon, which stands out as one of the most rational, cost-effective, and effective techniques among

respective methods like ion exchange, reverse osmosis, and photocatalysis. Adsorption is a straightforward physicochemical treatment process for high and efficient removal of methylene blue, and additionally, it prevents secondary contaminants from forming due to the reaction. Carbon-based adsorbents are mostly used with a nano-catalyst application for the efficient removal of methylene blue. Recently, biowaste-based adsorbents and green adsorbents have been a great topic of discussion due to their performance and friendliness (Ghosh, Singh, & Ratan).

1.4 Bioremediation Treatment Analysis

Bioremediation is an ecologically sustainable remediation process that uses living organisms like plants, microbes, or their specific enzymes, and animals to immobilize, detoxify, remove, or convert harmful pollutants from a toxic to a less toxic state in the environment and, as a result, reduce the negative effects and change in production of secondary pollutants from treatment. In comparison with conventional treatment methods, bioremediation is cheaper and easier, as it relies on natural processes that make it more result-oriented and cost-effective. Bioremediation is an advanced, efficient, and effective treatment process, especially for pollutants like pesticides, polycyclic aromatic hydrocarbons, petroleum, dyes, heavy metals, and inorganic pollutants. Phytoremediation, bioaugmentation, bio stimulation, composting, bioventing, mycoremediation, and microbial remediation are some types of bioremediations that are characterized in ex situ and in situ treatment types that use anaerobic and aerobic mechanisms through direct and co-metabolism processes. This is a natural process that usually takes place on its own or with little effort, and the organisms that perform bioremediation processes are called bioremediators (Tomassoni, Santos, Santos, Carpinski, & Silveira, 2014). The knowledge related to bioremediation was limited till early twenties, when contaminated soil was usually dug, evacuated, and treated at a different site (ex situ treatment). This treatment was not as effective for contaminated soil as it poses a significant risk during handling, digging, and evacuating; it gained attention, and an on-site remediation process was proposed. Bioremediation has two major types: in situ and ex situ bioremediation. In Situ means in place; it is a type of remediation process in which remediation or treatment takes place at the contaminated site. In this

case, a condition, microbes, special nutrients, reagents, or a mixture of these is provided to the contaminated site that activates bioremediation. It is preferable when the contamination proportions are greater, and excavation becomes more challenging or has negative environmental effects. It is more workable, cost-effective, and sustainable, but challenging due to complexity and low speed. Another type is ex situ, which means out of place; it is a type of remediation process that involves the extraction of contamination or material to treat it outside its location, either in a laboratory or another suitable place that has controlled conditions like pH, temperature, Oxygen level, humidity, etc. It has more predictable results but requires proper technology. It is less environmentally friendly and costly than in situ but has a high monitoring and controlled environment that hastens its speed (Gomez-Eyles et al., 2013).

In the case of aerobic mechanisms, microbes use oxygen as a source of energy and degrade pollutants; examples include the degradation of aromatic compounds and hydrocarbon compounds. Alternatively, anaerobic bioremediation takes place in the absence of oxygen, and sources of energy are iron, nitrates, carbon dioxide, or sulfur that ease the process, for example, dichlorination, and petroleum hydrocarbons. Here, two processes take place: a direct metabolism where contaminants are directly utilized as a source of energy or carbon by microbes, and co-metabolism, where specific enzymes are produced first through a breakdown process that only occurs in the presence of a substrate; then these enzymes work on the main food and chemically destroy it.

1.4.1 Adsorption as a Bioremediation Strategy

Adsorption is a surface-driven phenomenon restricted entirely to the interfacial boundary of a material, rather than extending throughout its volume. In this process, the substance providing the operational surface is designated as the **adsorbent**, whereas the molecular or ionic species accumulating at the interface is defined as the **adsorbate**. Adsorbents typically comprise solid or liquid matrices characterized by high surface areas that facilitate the localized immobilization of adsorbate molecules. Some common examples of adsorbents include activated carbon, zeolites, graphene material, and polymer-based porous material.

It acts as one of the best mechanisms for catalysis, separation, and purification that can be done by two types, either physical or chemical means (weak and strong bonding). In the case of physisorption (physical adsorption), Van der Waals forces are involved, and in comparison, with chemisorption (chemical Adsorption), physisorption usually occurs at low temperatures, does not change the chemical nature of adsorption, and is reversible. Chemisorption is stronger than physisorption as it involves chemical bonding, so it also requires high energy for activation. In adsorption, there is a high concentration of adsorbents on the surface rather than the whole, and factors like pore surface, interaction time, pH, surface area, and adsorbent dose impact adsorption. It is one of the best and cost-effective processes due to its greater efficiency, controllability, simplicity, and removal efficiency for both inorganic and organic impurities from aqueous medium (Ruthven, 1984). Functional groups like the carboxyl group, amine groups, hydroxyl group, and carbonyl group influence adsorption through hydrogen bonding, surface complexation, and electrostatic attraction, specifically for ionic compounds and aromatic contaminants (Ruthven, 1984). Due to the accessibility of numerous binding sites, heterogeneous surfaces mainly possess high adsorption capacities (Foo & Hameed, 2012).

Two types of models are used to describe the reaction rate of adsorption: Adsorption Kinetics and Adsorption Isotherm. Adsorption kinetics are applied to illustrate the rate of reaction of the solution in which adsorbate molecules are attached to the adsorbent surface from the solution. Here, pseudo-first order and second-order kinetic models are used to explain the reaction rate. Alternatively, isothermal models are used to describe the adsorption equilibrium that illustrates the adsorbate molecule dissemination between solid and liquid phases at constant temperature. Here, the Freundlich isotherm model and the Langmuir isotherm model are used to describe the adsorption equilibrium.

1.5 Use of Duckweed as a Sustainable Adsorbent Material

Duckweed is a small, floating, freshwater aquatic plant that belongs to the Lemnaceae family. Having a size of 1 to 15 mm, they are commonly found in slow-moving water bodies. This family includes 38 member species and 5 major aquatic genera that are all phytoremediator species. These plants act as a food source for fish and purify

water, offering various health benefits due to their high nutrient content. They are flowering aquatic plants that are also called Bay root. They consist of a definable leaf and root-like stem, or rootless, depending on the type. They also consist of air pockets that help them float on water. The plant consists of a hydrophobic coating that covers the surface, made up of waxes and cutin, which protects the plant against environmental stress by acting as an interference layer.

Duckweed wax is composed of campesterol, sitosterol, and stigmasterol, which are components of phytosterols. There is a noteworthy difference between the bottom layer, directed towards water, having numerous stomata, and the upper layer, which is directed towards air. The lower layer is enclosed with wax crystals made up of ketones, alkanes, aldehydes, alcohols, and diketones that form a well-organized structure. In contrast, the cuticle's diffusion process acts as a shield against water loss and stress from abiotic and biotic factors. There are ester bonds and cross-links made up of fatty acids that form a flexible polyester structure (Hahn et al., 2024).

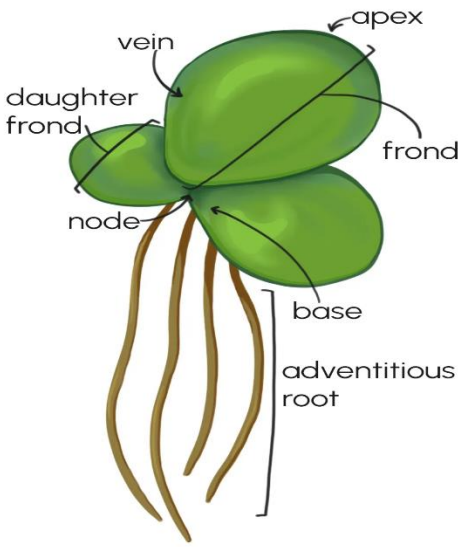


Fig 1.2: Structure of Lemna minor duckweed (iGEM Brno, 2025).

Duckweed production is 5 times higher than that of maize annually, and duckweed belongs to an assembly of monocot water plants. Reproduction usually occurs from the meristem by means of asexual budding, but in some species, sexual reproduction also occurs through flowering. The division depends on the factor of nutrient accessibility and slow movement, as they can even grow in eutrophic conditions. Duckweed is characterized by its fast growth and elevated level of nutrition, which also acts as a cover for many animals, including fish, tadpoles, and amphibians. A square meter of duckweed

plant is richer in minerals and vitamin B12 than soybean; due to this its cultivated as a food source in many countries like Thailand, as it usually takes 1.2 to 1.5 days to grow. Due to great resistance, they can be effortlessly grown in industrial and local wastewater, and this feature makes them best suited for various practical and laboratory experiments. (Bergman et al., 2012).

Climate change has affected food supply, causing a threat to food security. Duckweed has played an efficient role in some places by fulfilling the need, as it also consists of secondary metabolites, having great benefits, but alternatively, they are difficult to remove due to their speedy growth; therefore, aquatic herbicides or physical removal are used. It is also the smallest and fastest-increasing angiosperm (Brunner et al., 2009). The reproduction occurs due to rapid colonization and vegetative budding. It is rich in carbohydrates, essential minerals, lipids, and protein. It has single or multiple roots with a simple structure that helps to grow double in rate within less than 72 hours, depending on the environment and nutrients (Manière, Ziegler, Geillon, Featherstone, & Grosjean, 2016). The leaf-like shape is known as a frond that usually has two or fewer pockets where daughter fronds are formed. Depending upon the species, up to 18 daughters can be produced. Duckweed can be seen in almost every zone except extreme dry and intense cold zones (Ziegler et al., 2023).

It acts as an excellent aquatic plant for bioremediation due to its binding and accumulation capacity. The best species of duckweed for bioremediation include Lemna minor and Spirodela polyrhiza. Spirodela polyrhiza is a type of duckweed, usually called giant duckweed, having common names including duck meat, greater duckweed, common duck meat, and giant duckweed. It has a size of 0.5 to 1.0cm in width and is mostly seen in the form of condensed colonies that resemble a mat, as its upper layer is dark green with red sometimes, and the lower layer is typically red. It has both male and female flowers that die in the fall season, and new growth is seen in the winter season (Tackenberg et al., 2012). Spirodela polyrhiza can be used as a phytoremediator due to its adaptability to a broad pattern of environmental conditions and short doubling time (Sembada, Duteurtre, & Moulin, 2019). S. polyrhiza can be used to treat heavy metals such as lead, cadmium, and arsenic, and it also performs rhizofiltration that removes pollutants by adsorption through roots (Kanwar & Bauer, 2026). Duckweed-derived adsorbents contain an amino group, a carboxyl group, and a hydroxyl group as a base

functional group that performs adsorption of cationic dyes such as methylene blue by strong hydrogen bonding, electrostatic force of attraction, and π-π bonding.

1.5.1 Lemna Minor Duckweed-Derived Adsorbents

The aquatic plant Lemna minor, commonly referred to as duckweed, is a member of the Araneae family (subfamily Lamnidae). It features a highly reduced, 1.7 mm oval-shaped structural body known as a frond, from which a single root anchors it to the water surface using internal buoyant air chambers. Propagation occurs predominantly through rapid asexual budding, though sexual reproduction is occasionally documented (Constantin et al., 2025). Because of its accelerated growth rates, high protein expression profiles, and substantial resilience to chemical stressors, L. minor serves as an ideal biological instrument. It is widely employed across laboratory environments as both an efficient bioremediator for contaminant extraction and a sensitive indicator for evaluating ecosystem-level ecotoxicity.

It has a wide range of tolerance for factors like abiotic factors and pH, but in contrast, it is affected by pollutants, light, and temperature. Almost 22 tons of Lemna minor are produced annually in Europe itself. It also competes as a current source of vegetable protein due to its high adaptability and resilience. It can survive a wide range of pH and temperature, ranging from 5 to 9 pH and 6 to 33 degrees (Vulpe et al., 2023). Lemna minor not only acts as food for humans but also as a biofuel, fish feed, and phytoremediator. As an aquatic plant, many species consume L. Minor as a source of food naturally.

There was a visible growth seen in fisheries after adding 20 percent L. Minor to the fish's diet as a supplement. In contrast, it also acts as a helpful supplement for shrimp growth (Irabor, Adeleke, Pierre, & Nwachi, 2022). Lemna Minor consists of 30 percent protein in total, which also allows poultry farmers to use it as a source of food for animals as well. It is also well known as a secondary metabolic repertoire that makes it fit for human consumption as a supplement. It is monocotyledonous and has anti-inflammatory, antiradical, and antioxidant properties, which double in less than 48 hours in growth depending upon factors like pH, light, temperature, and nutrients (Al-Snafi, Majid, Talab, & Al-Battat, 2019).

Lemna minor can not only efficiently bioremediate but also bioaccumulate heavy metals, including copper, nickel, lead, and cadmium, and the removal efficiency is about 80 percent depending on the growth medium. It is also used as a biofuel as it is efficient for biochar and bioethanol production. It also has antimicrobial, immunomodulatory, and antiparasitic properties (Vinchurkar, Mane, Balekar, & Jain, 2017). On the other hand, Lemna minor has some environmental considerations, as it causes chemical release that can suppress other aquatic plant growth, and it absorbs more nutrients that affect nutrient cycling by becoming a reason for eutrophication, thus requiring proper checks and balances. By nature, Lemna minor also has -OH and -COOH functional groups that help methylene blue particles bind. Duckweed adsorbents have a nanocrystal structure, grooves, ridges, lignin, hemicellulose, minerals, and multiple adsorption sites that enhance adsorption capacity and maintain a strong attraction.

Lemna minor has gained attention due to its abundance, low cost, effectiveness, numerous active adsorption sites, and rapid growth rate. The effectiveness is successfully demonstrated in different forms, including phytoremediation (Imron et al., 2022, 2019), magnetic duckweed-based adsorbents (Zhang et al., 2026), and powdered adsorbent form (Jeffrey et al., 2025) for synthetic wastewater. All studies successfully achieved higher removal efficiencies through different processes and systems.

CHAPTER 2

MATERIALS AND METHODS

2.1 Preparation and Activation of Adsorbent Material

Lemna minor duckweed was collected from the Azolla Farming garden in Hafizabad, then rinsed with tap water till water runs clear. After washing with distilled water, it was sun-dried for three days and oven-dried at 80 degrees (Jeffrey et al., 2025) in a Memer Plus advanced oven for 24 hours, and then at 105 degrees for one to two hours for complete removal of moisture to ease grinding and can marginally strengthen the surface area without any major negative effect. The powder was sieved to a 2mm size and then stored for further processing (Jeffrey et al., 2025). A molar solution of citric acid was prepared by completely dissolving 10.5 grams of citric acid powder into 500mL of distilled water. Duckweed powder was washed with a 1:10 molar solution of citric acid by soaking it for 40 to 60 min. It was then filtered using Whatman high-efficiency 125 mm-sized filter paper, dried at room temperature, and stored in an airtight container. Citric acid was used as an activator (Mobin, Deloya, & Guo, 2025) for many reasons, including its capacity to prevent pigment leaching and washing, and to enhance surface chemistry, creating more negatively charged sites for adsorption. Additionally, it enhances porosity and does not interfere with methylene blue absorbance. Citric acid contains a carboxyl group with a negative charge on it that helps to attach methylene blue, which has a cationic nature. By nature, Lemna minor also has -OH and -COOH functional groups that help methylene blue particles bind. Additionally, the citric acid improves the surface of lignocellulosic biomass, making it more acidic to attach methylene blue. It is widely used for the modification of bio-based adsorbents as it is less toxic, cost-effective, environmentally friendly, and biodegradable in nature (Jeffrey et al., 2025).

2.2 Preparation of Methylene Blue Working Solution

Methylene blue dye was obtained from the laboratory of Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan, with Catalog No. S001056, supplier No. IS22075 and 99% purity grade, and a 1000 ppm stock solution was prepared, for which 1 gram of methylene blue was dissolved completely in distilled water by keeping the volume at 1000 mL. It is light-sensitive and has a strong color, for which an airtight container and a dark place were used for preservation. By using a 1000 ppm stock solution, 100 ppm, 50 ppm, and 10 ppm stock solutions were synthesized for further processing by using the $C_1V_1 = C_2V_2$ dilution equation for dilution. Methylene blue easily dissolves in water; it does not require vigorous shaking, which causes bubbles to form on the surface, so proper mixing with the help of a stirrer or gentle shaking is preferable (Adesina et al., 2021). The stock solution of 1000 ppm has an intense, deep blue color that has the capacity to give color to cloth, paper, and hands that come in contact (Khan et al., 2022). The intensity decreases with the formation of lower ppm stock solutions.

Figure 2.1: Methylene blue working solutions of 1000 ppm, 100 ppm, and 10 ppm.

Methylene blue dye was used under control conditions as a model pollutant to prepare synthetic wastewater because of its cationic nature. It is abundantly used in textile industries and as an exemplary pollutant due to its environmental significance and well-defined chemical properties in adsorption studies. The use of synthetic wastewater removes the impurities combined with actual wastewater, presenting a reproducible and controlled system that assists precise assessment of the adsorption performance of the adsorbent. The results obtained using synthetic wastewater are representative of dye-holding textile wastewater under a monitored laboratory environment (Jeffrey et al., 2025; Khan et al., 2022; Ramírez-Castillo, Guillén-Padilla, Méndez-Sandate, Guerrero-Barrera, & Avelar-González, 2024)

2.3 Evaluating the Effects of Physical Parameters on the Removal Efficiency of Methylene Blue

Duckweed-based biosorbents have a great influence of physical parameters on adsorption capacity, for which the effects of pH (Foo & Hameed, 2012; Zhang et al., 2025), adsorbent dose (Pandey et al., 2022), time of contact (Jeffrey et al., 2025), and the effect of contaminant concentration (Ramírez-Castillo et al., 2024)were deeply studied. These parameters were commonly used to demonstrate the optimum conditions for adsorption efficiency (Hamad & Idrus, 2022; Jeffrey et al., 2025).

pH plays a vital role by affecting the ionization state of methylene blue and by enhancing the surface charge of biosorbents, which helps to achieve optimal adsorption with maximum electrostatic force of attraction. Time of contact helps to demonstrate the adsorption equilibrium point, which is mostly observed initially due to the abundance of activation sites that gradually decrease with time due to saturation. Adsorbent dose and contaminant concentration help to identify the right amount for removal that helps to maintain equilibrium. If more concentration is added, it will affect the percentage removal and adsorption capacity. By applying the best of all physical parameters, the highest degree of removal efficiency can be achieved.

2.3.1 Effect of pH on Removal of Methylene Blue

A 10 ppm solution of methylene blue was taken, and the general pH of the solution was measured, which was slightly acidic. Based on the literature review, aquatic plants work best in basic conditions. 0.1 molar solutions of HCl and NaOH were prepared to alter the pH. Three sets of tests were run to check the effect of pH, where the solution size was taken as 100mL, and the pH was set to 3, 7, and 10 by using HCl and NaOH solutions. 1g amounts of duckweed powder were added separately after measuring them using a UniBlock measuring analytical balance. There was no use of any kind of activator according to the literature review, but citric acid was used, which speeds up the reaction. A total of 60 minutes of contact time was given to the sample sets during this time; samples were placed in the Labo tonics Ash Less shaker incubator at 36 degree temperature at 120 rpm. After 60 minutes, all three sets were separately filtered using Whatman high-efficiency 125 mm-sized filter paper. The solution was taken for UV

spectrometry, and the filtrate material was stored for desorption studies. Based on the literature, the UV was measured at 664 nm. UV of a pure sample of a 10 ppm solution of synthetic wastewater was scanned using the ORI Germany UV 4000 advanced spectrophotometer, which was measured as 0.069. After which, the UV of set 1, having samples 1, 2, and 3, was measured. At pH 3, the UV was measured as 0.056, at pH 7 it was 0.030, and at pH 10 it was measured as 0.023. Duckweed-based adsorbents are positively charged, which is the reason why at low pH they repel methylene blue. Deprotonation of the functional group of duckweed was seen at neutral pH, which causes a strong force of attraction, and at a very high pH, many sites become deprotonated or undergo hydrolysis. That was the main reason behind taking pH slightly acidic, slightly basic, and neutral.

2.3.2 Effect of Adsorbent Dose on Removal of Methylene Blue

A 10 ppm stock solution was taken, and the pH was set to 10 as it was giving the highest removal efficiency. Three sets of tests were run where the solution size was taken as 100mL, and 0.5 g, 1g, and 1.5g amounts of duckweed powder were added separately after measuring them using a UniBlock measuring analytical balance. A total of 60 minutes of contact time was given to the sample sets during this time; samples were placed in the Labo tonics Ash Less shaker incubator at 36 degree temperature at 120 rpm. Afterward, all three sets were separately filtered using Whatman high-efficiency 125 mm-sized filter paper. The solution was taken for UV spectrometry, and the filtrate material was stored for desorption studies. Based on the literature, the UV was measured at 664 nm. UV of a pure sample of a 10 ppm solution of synthetic wastewater was scanned, which was measured as 0.069. But after a general scan, the best peaks were observed at 580 nm and then 560 nm. The UV of the 10 ppm stock solution at 580 nm was 0.338. After this step, the UV of set 2, having samples 1, 2, and 3, was measured. The UV for 0.5g duckweed was 0.044, for 1g it was 0.056, and for 1.5g it was 0.048, after which the adsorption capacity was measured.

2.3.3 Effect of Contaminant Concentration on Removal of Methylene Blue

Different concentrations of the contaminants were studied to determine optimal concentrations. The adsorbent dose was taken as 0.5 g, and the pH was set to 10; both factors yielded the highest removal efficiency. Three sets of tests were run where the solution size was taken as 100mL, and the concentrations of the methylene blue solution were taken as 10 ppm, 50 ppm, and 100 ppm, which were formed by using the $C_1V_1 = C_2V_2$ dilution equation for dilution. A total of 30 minutes of contact time was given to the sample sets during this time; samples were placed in the Labo tonics Ashless shaker incubator at 36 degree temperature on 120 rpm. After which, all three sets were separately filtered using Whatman high-efficiency 125 mm-sized filter paper, and the filtrate material was stored for desorption studies. The UV was done before and after treatment for 10 ppm, 50 ppm, and 100 ppm solutions. The UV for the untreated methylene blue concentration 10 ppm stock solution was 0.309, for 50 ppm 1.413, and for 100 ppm 2.683, whereas for the treated sample, 3 tests 1, 2, and 3, it was 0.046 for 10 ppm, 0.094 for 50 ppm, and 0.425 for 100 ppm. The removal efficiency was then studied by using percentage removal analysis.

2.3.4 Effect of Time Period on Removal of Methylene Blue

A 10 ppm stock solution was taken, the adsorbate dose was taken as 0.5 g, and the pH was set to 10, as it was giving the best removal efficiency. Four samples of tests were run, where the solution size was taken as 100mL. For sample 1:30 minutes was given, for sample 2:60 minutes, for sample 3:90 minutes, and for sample 4, a total of 120 minutes contact time was given. During this time, samples were placed in a Labo tonics Ash less shaker incubator at 36 degree temperature on 120 rpm. The solution was filtered using Whatman high-efficiency 125 mm-sized filter paper after its respective time, taken for UV spectrometry, and the filtrate material was stored for desorption studies. The UV for the untreated methylene blue 10 ppm stock solution was 0.362, and for the treated set 4 sample 1 was 0.038, for 2 it was 0.041, for 3 it was 0.041, and for 4 it was 0.050.

2.4 Desorption and Reusability Studies of Duckweed-Derived Adsorbent

Desorption studies were conducted to measure the release of the adsorbed methylene blue from the duckweed surface, thereby determining the adsorbent reusability, binding mechanism, and contaminant recovery. The filtrate material from the best test of each test was collected separately, and agitation was performed. After a gentle wash, a molar solution of ethanol was taken, and a 1:1 desorption was done for 2 minutes. The best test filtrates were 0.5g from the adsorbent dose, pH 10 material from the pH effect, 30 minutes material from the time effect, and 50 ppm filtrate material from the contaminant concentration test. These filtrates were measured for 1:1 desorption using an analytical balance. After desorption, filtration was done, and particles of methylene blue were seen in the solution with the naked eye. The solution was taken for UV, and UV was done at 580 nm. The % removal analysis was done, and removal efficiencies after washing with ethanol solution ranged from 11% to 48.1%, depending on the type of parameter. The lowest removal percentage was seen for the filtrate material for the effect of pH, and the highest was seen for the factor of time. The filtrate material was washed with distilled water, and oven dried at 50 to 60 degrees for three hours for the reusability studies. Then this residual duckweed material was immense in the 10 ppm stock solution of methylene blue after activation. After 30 minutes, it was filtered using a Whatman high-efficiency 125 mm-sized filter paper, and the solution was taken for UV. The percentage removal was measured, and the time efficiency was 13.7 %. The material was then washed, dried, and activated for treatment for the third time. This time, efficiency dropped to 7.8 %. After adsorption for the first time, there was a sudden drop in efficiency as many sites became saturated with methylene blue, and bonding was strong; on the other hand, some material was also lost during ethanol washing.

2.5 Comparison of Physical Characteristics of DW Adsorbent Before and After Treatment

Table 2.1: Comparison of Physical Characteristics of DW Adsorbent Before and After Treatment

Physical Characteristics	**Adsorbent Before Treatment**	**Adsorbent After Treatment**
Color	Dark Green natural color	Strong Blue Color
Texture	Rough surface naturally	Slightly smooth surface due to saturation
Weight and Density	Lightweight and Low in Density	Slightly denser and Heavier
Surface Appearance	Tough, Coarse, and Parched surface	Darker, Saturated, Layered, and Smoother surface
Visual Clarification	Free Adsorptive Surface	Dye-Loaded Adsorptive Surface

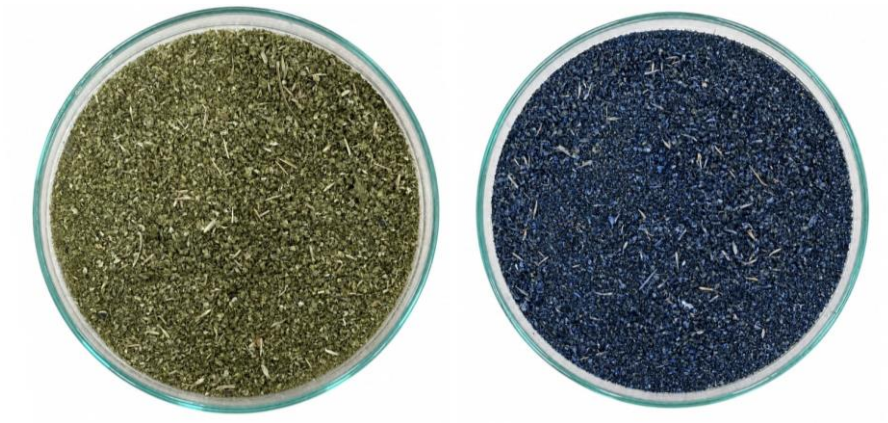
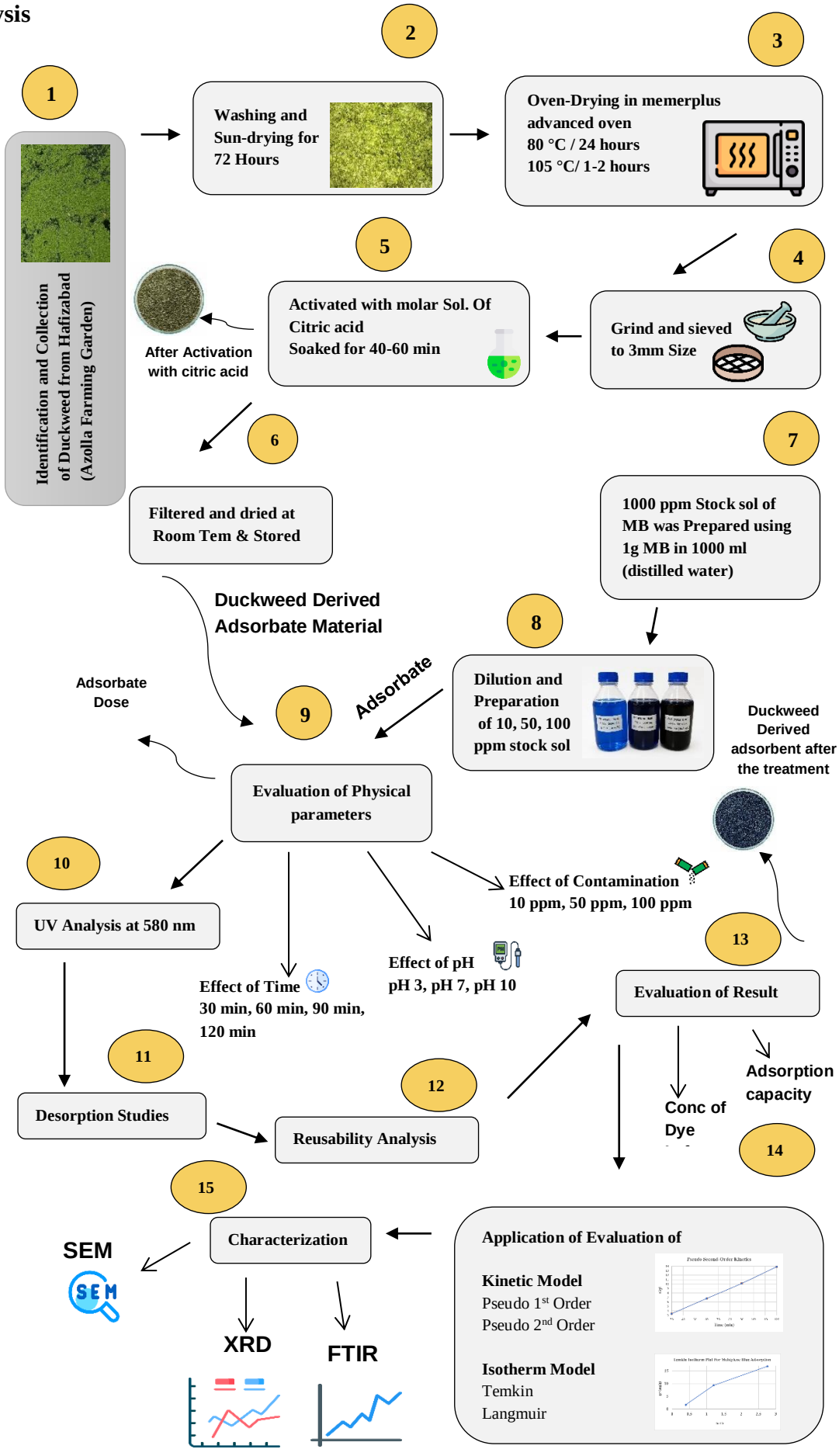


Figure 2.2: Comparison of DW Adsorbent Before and After Treatment based on Visualization.

2.6 Steps of Formation of Duckweed-derived Adsorbent and MB Removal Analysis



CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Characterization of Duckweed Adsorbents

The FTIR, SEM, and XRD were used before and after physicochemical characterization of duckweed-derived adsorbents was done. The functional, morphological, and structural evaluations were done for structural modification. These technologies help in a better understanding of results and the bonding of the adsorbent with methylene blue, which helps to examine effectiveness.

3.2 Scanning Electron Microscopy Analysis of Duckweed-Derived Adsorbent

The SEM analysis was performed to demonstrate the physical characteristics and surface morphology of the adsorbent before and after the adsorption process. A high-resolution picture was engendered by using a JEOL IT100LA Scanning Electron Microscope, accelerating at 20kV voltage, which provides a detailed view of sponginess, texture, and physical variations through which the adsorbent exhibits changes. These characteristics are affected by the attachment of pollutants, which cause changes like clogged pores, evenness, and reduction in coarseness, based on which effective interaction is confirmed between the adsorbent and adsorbate material. Here, DW adsorbent was examined at 100μm, 50μm, 20μm, and 10μm, and by the resolution of 1600x,1000x, 950x, 750x, 550x, 300x, 220x, 100x, and 90x for a better understanding of the structural morphology.

3.2.1 SEM Interpretation Before Adsorption Analysis

An asymmetrical, porous, and uneven surface was observed before treatment based on a 220x resolution image at 100μm size. The surface structure was observed as less compact but having bulky interconnected pores that allow adsorption, demonstrating elevated

surface area. Many organic elements like Ca, N, C, O, Si, and Al were detected. Carbon and oxygen were predominant, which states DW adsorbent is an organic carbonaceous compound, whereas other elements are naturally occurring compounds. Carboxyl, cellulose, hydroxyl, and lignin functional groups were observed at 200µm in size with 90× resolution. Many available active sites with clean surfaces were visible. Carbon (43.95 wt.%) and Oxygen (53.54 wt.%) were usually present in high content, whereas Nitrogen (90 wt.%), Aluminum (0.19 wt.%), and Silicon (0.36 wt.%) were in low content before the treatment. Potassium was not detected before the treatment.

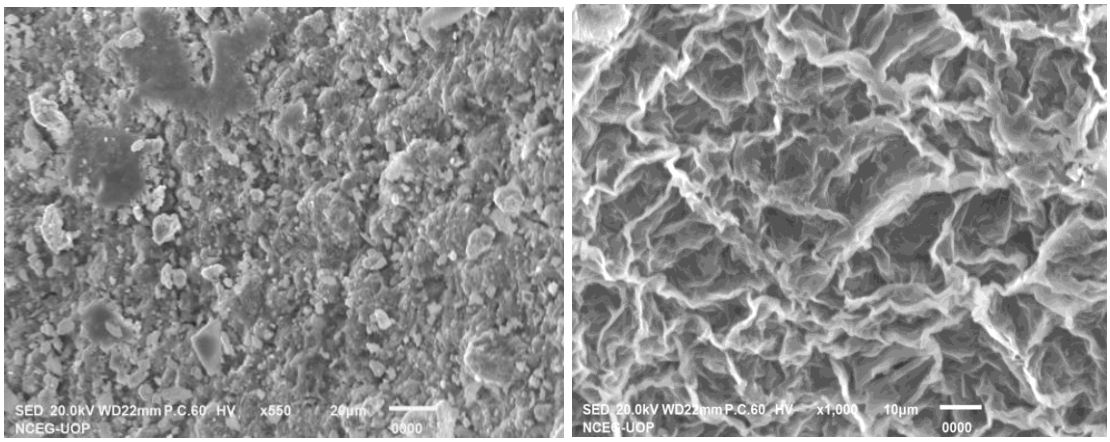


Figure 3.1: SEM Analysis of Duckweed-Derived Adsorbent Before the Treatment at 10µm with 1000x and 20µm with 550x Resolution

3.2.2 SEM Interpretation After Adsorption Analysis of Duckweed-Derived Adsorbent

The structure becomes denser and more compact based on a 140x resolution image at 100 µm. The surface was rougher, but in comparison with the sorbent before treatment, it became more aggregated. This proves the existence of molecular dye at sites. The pores observed at 1000x resolution with a 10 µm size were partially covered and reduced. Here, organic elements like Ca, N, C, O, Si, and Al were also detected, but there was a slight decrease in Carbon (42.01 wt.%) and Oxygen (52.31 wt.%), and an increase in minerals

like Al (0.37 wt.%) and Si (1.72 wt.%) was seen. K (0.42 wt.%) was also detected after the treatment.

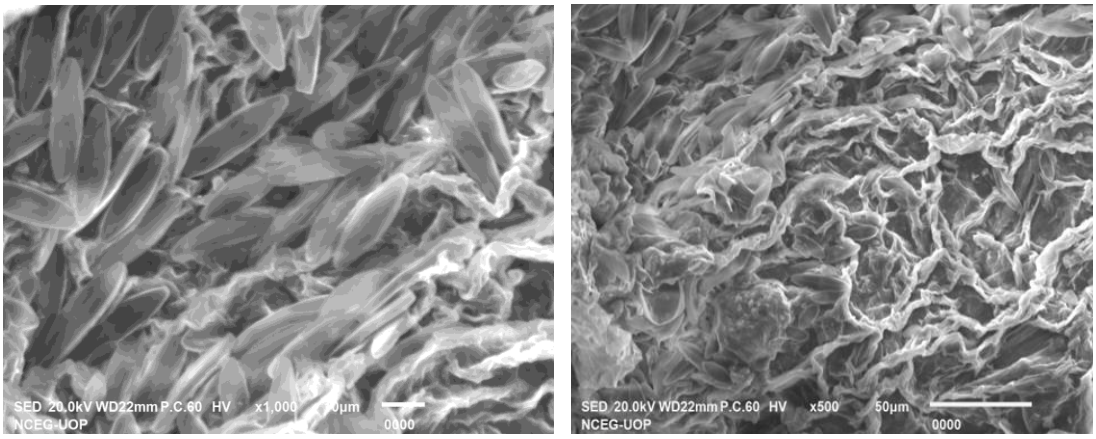


Figure 3.2: SEM Analysis of Duckweed-Derived Adsorbent After the Treatment at 20 µm with 1000x and 50µm with 500x Resolution

3.2.3 Comparison of Adsorbents Based on SEM Analysis

Table 3.1: Comparison of SEM Interpretation Before and After Treatment

Parameter	**Interpretation Before Treatment**	**Interpretation After Treatment**
Surface Structure	Asymmetrical Extremely spongy	More Combined Denser
Surface	Hollow	Aggregated
Consistency	Irregular	Partially shielded
Pore Visibility	Visible	Reduced pores
Adsorption spots	More uncovered sites	Partially engaged
Mineral content	Higher In Total The carbon and oxygen content were lower in comparison	Lower In Total The carbon and oxygen content were higher in comparison

In conclusion, the SEM analysis shows the availability of active sites before the treatment that can allow pollutants to attach, and a great number of saturations at the adsorption site after treatment, which reflects the removal efficiency of the DW adsorbent.

3.3 X-Ray Diffraction Analysis of Duckweed-Derived Adsorbent

The XRD analysis was done using Panalytical X-Ray Diffraction with X'Pert HighScore analytical software to study the crystalline structure and composition of atomic arrangements. The overall observed pattern was similar, which indicates stable dye removal. It reveals a predominantly amorphous crystalline peak with a slight change after that, indicating successful interaction of dye and adsorbent.

3.3.1 XRD Before Adsorption Analysis

The crystalline structural peaks were visible and sharp, which indicates the availability of active sites for adsorption. Surface composition shows a more open structure that defines a high intensity of amorphous peaks. The surface chemistry was overall porous and irregular.

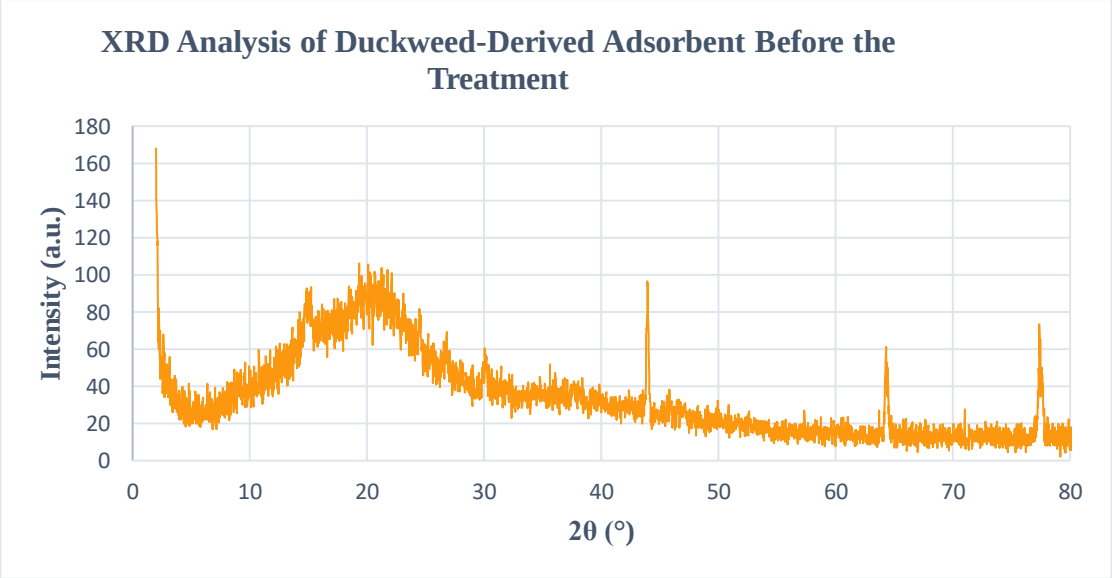


Figure 3.3: XRD Analysis of Duckweed-Derived Adsorbent Before the Treatment.

3.3.2 XRD After Adsorption Analysis

The crystalline structural peak was slightly shifted on the reduction side, which indicates the presence of a dye that interacts with the surface. Baseline becomes flatter, reduces irregularity, and indicates successful interaction.

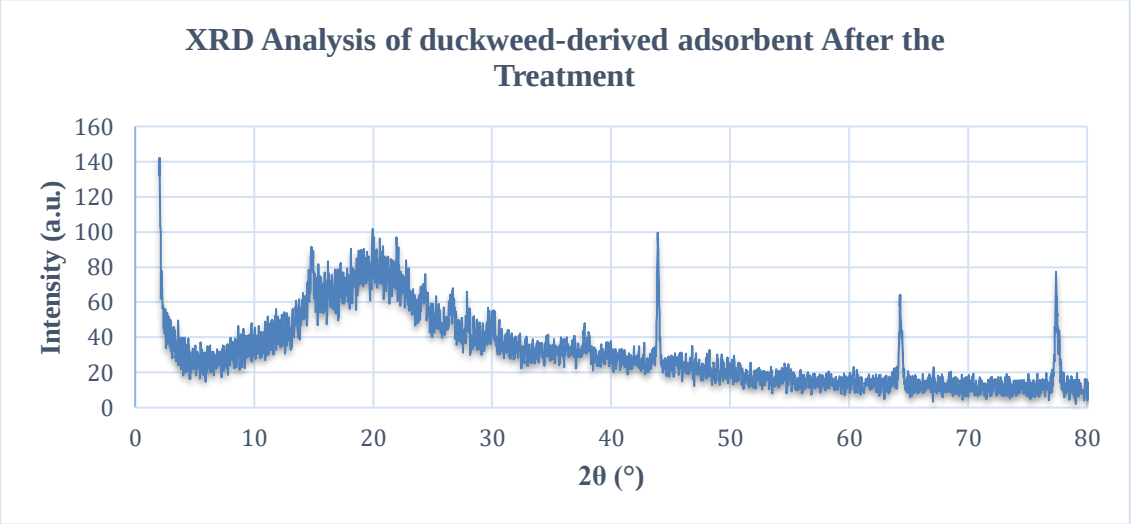


Figure 3.4: XRD Analysis of Duckweed-Derived Adsorbent After the Treatment

3.3.3 Comparison of Adsorbents Based on XRD Analysis

Both samples show a broad hump at an intensity between 15 and 30 degrees. The amorphous nature of the wide peaks indicates the existence of lignocellulosic and hemicellulose biomass. There was no major peak shift observed, which indicates stability of the adsorbent surface after treatment and proves no structural damage. Small peak shifts with broader intensity indicate possible chances of hydrogen bonding, interaction, and electrostatic force of attraction between the adsorbent and the dye molecule.

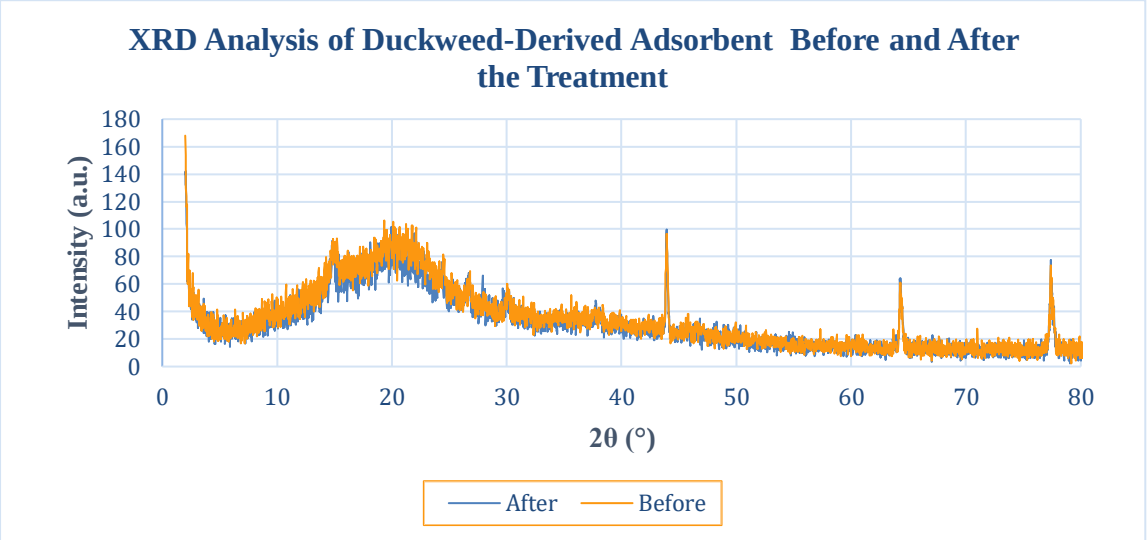


Figure 3.5: XRD Analysis of Duckweed-Derived Adsorbent Before and After the Treatment

3.4 Fourier Transform Infrared Spectroscopy of Duckweed-Derived Adsorbent

The FTIR spectroscopy was done by using a Thermo Fisher Scientific spectroscope to analyze the peaks of absorbance for the identification of surface chemistry, functional groups, and chemical composition of DW derived adsorbent before and after the treatment. The results strongly indicate the existence of carboxyl (-COOH) group and hydroxyl (-OH) group through electrostatic force of interaction, hydrogen bonding, and π-π interaction.

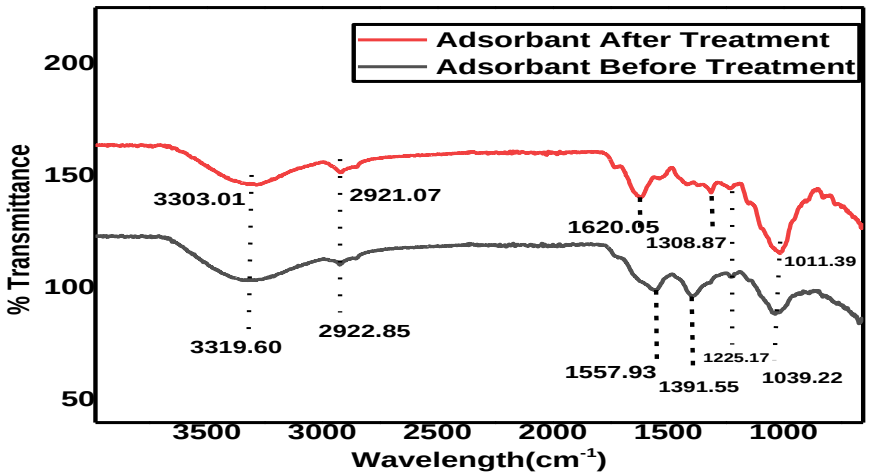


Figure 3.6: FTIR Analysis of Duckweed-Derived Adsorbent Before and After the Treatment

3.4.1 FTIR Interpretation Before Adsorption Analysis of Duckweed-Derived Adsorbent

Before the treatment, the main peaks of the wavelength were shown at 3320cm^{-1}, 2922 cm^{-1}, 1556 cm^{-1}, 1395 cm^{-1}, 1230 cm^{-1}, and 1036 cm^{-1}. These peaks show the presence of a hydroxyl group, polysaccharides, alkanes, alcohols, phenol, organic matter, and a carboxyl group having a negative charge that favors the adsorption of cationic methylene blue dye. The presence of O-H bonding at 3320, C-H bonding at 2922, N-H and C=C bonding at 1556, C-H bonding at 1395, C-O bonding at 1230, and C-O-C bonding at 1036 was evaluated by FTIR.

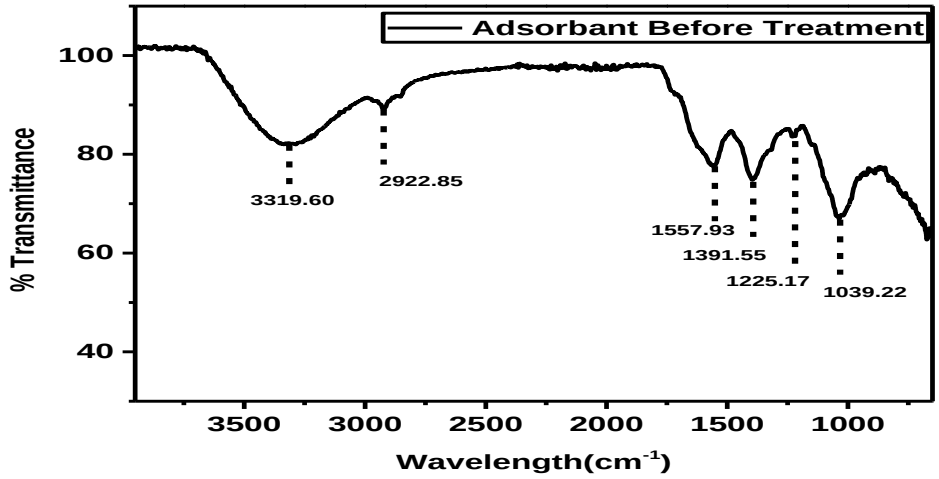


Figure 1: FTIR Analysis of Duckweed-Derived Adsorbent Before the Treatment.

3.4.2 FTIR Interpretation After Adsorption Analysis of Duckweed-Derived Adsorbent

After the treatment, a shift of peaks was studied as O-H bonding was shifted to 3283 from 3320 that signifies interface between hydroxyl group and methylene blue dye, some remained the same, like 2922 and many new peaks were form like 1729 that indicates C=O bonding, 1617 that indicate C=C bonding due to the presence of methylene blue, 1546 that indicates N-H bonding, 1418 that indicates C-N bonding, 1314 and 1299 that indicates amine C-N bonding and 660 that indicates dye fingerprint as C-H aromatic bonding. This also reflects an increase in complexity as the surface becomes saturated with dye molecules. Based on the evaluation, there was a strong existence of electrostatic

force of interaction between dye and adsorbent, hydrogen bonding between methylene blue and dye -OH group, and π-π bonding between adsorbent and aromatic ring of dye.

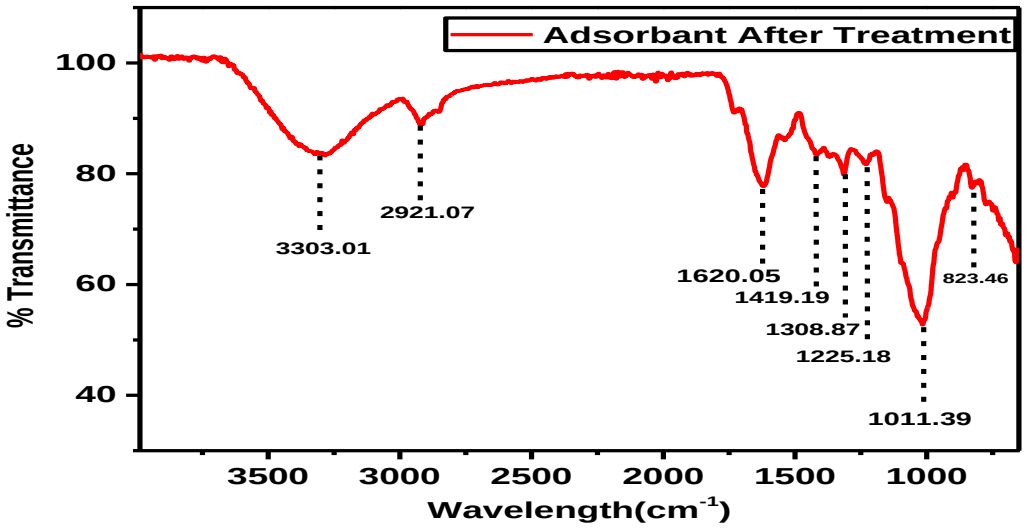


Figure 3.8: FTIR Graph Analysis of Duckweed-Derived Adsorbent After the Treatment

3.5 Adsorption Analysis Results

The adsorption test measures the removal capacity of the known adsorbent material from a solution. Under controlled conditions, a known concentration of input is introduced into the dye solution, and adsorption is performed. For this, the adsorption capacity is measured first, followed by percentage removal capacity, which is further used in kinetics and isothermal studies. Additionally, the remaining dye concentration is also known by this test. Numerous functioning parameters that were discussed above were analyzed to determine their effects on the adsorption rate. This also validates that the adsorbent possesses the capability of being a low-cost, efficient, and eco-friendly material for the elimination of the methylene blue pollutant.

3.5.1 pH Effect on Removal of Methylene Blue

1. Percentage Removal

Percentage removal efficiency is the measure of the pollutant percentage being removed, with a formula:

$$\%\ Removal = \frac{A_o - A_t}{A_o} \times 100$$

Here, V (volume) is 100mL, Time taken is 60 minutes, m (Adsorbent dose) = 1g, A_o (initial absorbance) is 0.069 at 664 nm, C_o is 10 mg/L, and A_t (final absorbance) at 3 is 0.056, at 7 is 0.030, and at 10 is 0.023.

At 3 pH

$$\%\ Removal = \frac{0.069 - 0.056}{0.069} \times 100 = 18.8\ \%$$

At 7 pH

$$\%\ Removal = \frac{0.069 - 0.030}{0.069} \times 100 = 56.5\ \%$$

At 10 pH

$$\%\ Removal = \frac{0.069 - 0.023}{0.069} \times 100 = 66.7\ \%$$

2. Concentration Of the Dye Left in Solution

To find the value of C_t that tells us how much dye is left in the solution, we use the following formula:

$$C_t = C_o \left(1 - \frac{\%\ Removal}{100}\right)$$

For pH 3

$$C_t = 10\ (\ 1 - 0.188\ ) = 8.12\ mg/L$$

For pH 7

$$C_t = 10\ (\ 1 - 0.565\ ) = 4.35\ mg/L$$

For pH 10

$$C_t = 10\ (\ 1 - 0.667) = 3.33\ mg/L$$

3. Adsorption Capacity

Adsorption capacity is the measure of dye adsorbed by per unit mass (1 gram) of adsorbent from the medium, which is measured by using the following formula:

$$q_t = \frac{(C_o - C_t) \times v}{m}$$

At 3 pH

$$q_t = \frac{(10 - 8.12) \times 0.1}{1} = 0.188$$

At 7 pH

$$q_t = \frac{(10 - 4.35) \times 0.1}{1} = 0.565$$

At 10 pH

$$q_t = \frac{(10 - 3.33) \times 0.1}{1} = 0.667$$

The highest adsorption capacity of methylene blue by duckweed was observed at pH 10, the lowest in acidic conditions, and moderate at neutral pH.

Table 3.2: Analysis of percentage removal and adsorption capacity with residual dye based on pH.

pH	% Removal	C_t (Residual Dye)	q_t (Adsorption capacity)
3	18.8 %	8.12 *mg/L*	0.188 *mg/g*
7	56.5 %	4.35 *mg/L*	0.565 *mg/g*
10	66.7 %	3.33 *mg/L*	0.667 *mg/g*

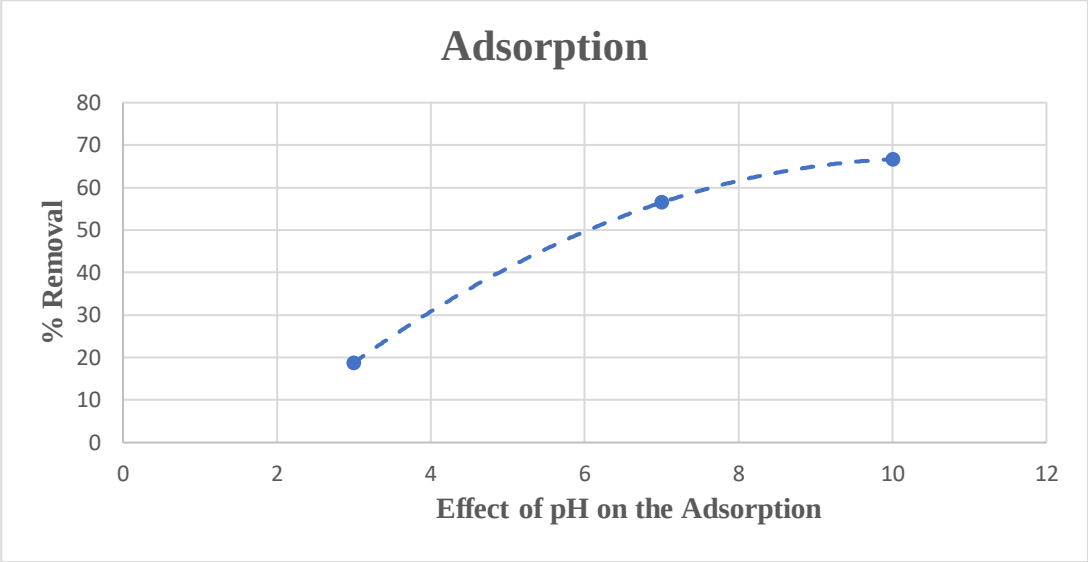


Figure 3.9*:* Analysis of percentage removal and adsorption capacity with residual dye based on pH.

3.5.2 Adsorbent Dose Effect on the Removal of Methylene Blue

1. Percentage Removal

Percentage removal efficiency is the measure of the pollutant percentage being removed, with a formula:

$$\%\ Removal = \frac{A_o - A_t}{A_o} \times 100$$

Here, V (volume) is 100mL, Time taken is 60 minutes, A_o (initial Absorbance) is 0.362, and A_t (Final Absorbance) is 0.044 for 0.5g, 0.056 for 1g, and 0.048 for 1.5g, where the initial concentration C_o is 10 ppm.

With 0.5g of Adsorbent

$$\%\ Removal = \frac{0.362 - 0.044}{0.338} \times 100 = 87.85\ \%$$

With 1g of Adsorbent

$$\%\ Removal = \frac{0.362 - 0.056}{0.362} \times 100 = 84.53\ \%$$

With 1.5g of Adsorbent

$$\%\ Removal = \frac{0.362 - 0.0480}{0.362} \times\ 100 = 86.74\ \%$$

2. Concentration of the dye left in solution.

To find the value of C_t that tells us how much dye is left in the solution, we use the following formula:

$$C_t = C_o\ \left(1 - \frac{\%\ Removal}{100}\right)$$

For 0.5g of Adsorbent

$$C_t = 10(\ 1 - 0.8785) = 1.21\ mg/L$$

For 1g of Adsorbent

$$C_t = 10(\ 1 - 0.8453) = 1.55\ mg/L$$

For 1.5g of Adsorbent

$$C_t = 10(\ 1 - 0.86674) = 1.33\ mg/L$$

3. Adsorption Capacity

Adsorption capacity is the measure of dye adsorbed per unit mass (1 gram) of adsorbent from the medium, which is measured by using the following formula:

$$q_t = \frac{(C_o - C_t) \times\ v}{m}$$

For 0.5g of Adsorbent

$$q_t = \frac{(10 - 1.21) \times\ 0.1}{0.5} = 1.758\ mg/g$$

For 1g of Adsorbent

$$q_t = \frac{(10 - 1.55) \times\ 0.1}{1} = 0.845\ mg/g$$

For 1.5g of Adsorbent

$$q_t = \frac{(10 - 1.33) \times 0.1}{1.5} = 0.578\, mg/g$$

The highest adsorption of methylene blue by duckweed was observed with 0.5g of adsorbent, which was also good at 1g and 1.5g.

Table 3.3: Analysis of percentage removal and adsorption capacity with residual dye based on Adsorbent Dose

Adsorbent Dose	% Removal	C_t (Residual Dye)	q_t (Adsorption Capacity)
0.5g	87.85 %	1.21 *mg/L*	1.758 *mg/g*
1g	84.53 %	1.55 *mg/L*	0.845 *mg/g*
1.5g	86.74 %	1.33 *mg/L*	0.578 *mg/g*

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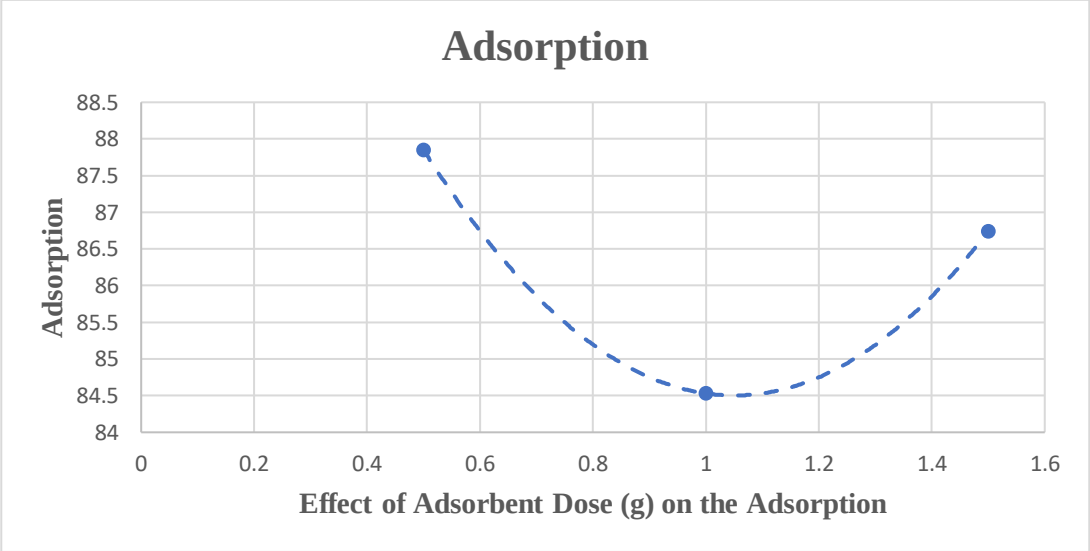

Figure 3.10: Analysis of percentage removal and adsorption capacity with residual dye based on Adsorbent Dose.

3.5.3 Contaminant Concentration Effect on The Removal of Methylene Blue

1. Percentage Removal with Concentration of Dye Left

Percentage removal efficiency is the measure of the pollutant percentage being removed, with a formula:

$$\%\, Removal = \frac{C_o - C_t}{C_o} \times 100$$

Here, V (volume) = 100mL, m (Adsorbent dose) = 1 g, where absorbance for pure mb concentration (C_o) of 10 ppm was 0.309, for 50 ppm it was 0.094, and for 100 ppm it was 0.425. Absorbance after treatment was 0.046 for 10 ppm, 0.094 for 50 ppm, and 0.425 for 100 ppm, but we don't know the value of C_t, so we apply the formula based on absorbance:

$$C_t = \left(\frac{A_{after}}{A_{before}} \times C_o \right)$$

For 10 ppm

$$C_t = \left(\frac{0.046}{0.309} \times 10 \right) = 1.49\ ppm$$

$$\%\ Removal = \frac{10 - 1.49}{10} \times 100 = 85.1\ \%$$

For 50 ppm

$$C_t = \left(\frac{0.094}{1.413} \times 50 \right) = 3.33\ ppm$$

$$\%\ Removal = \frac{50 - 3.33}{50} \times 100 = 93.34\%$$

For 100 ppm

$$C_t = \left(\frac{0.0425}{2.683} \times 100 \right) = 15.84\ ppm$$

$$\%\ Removal = \frac{100 - 15.84}{100} \times 100 = 84.16\ \%$$

2. Adsorption Capacity

Adsorption capacity is the measure of dye adsorbed by per unit mass (1 gram) of adsorbent from the medium, which is measured by using the following formula:

$$q_t = \frac{(C_o - C_t) \times v}{m}$$

At 10 ppm:

$$q_t = \frac{(10 - 1.49) \times 0.1}{0.5} = 1.70\ mg/g$$

At 50 ppm:

$$q_t = \frac{(50 - 3.33) \times 0.1}{0.5} = 9.33\ mg/g$$

At 100 ppm:

$$q_t = \frac{(100 - 15.84) \times 0.1}{0.5} = 3.71\ mg/g$$

The highest adsorption of methylene blue by duckweed was observed at 50 ppm, which was also good at 10 ppm and 100 ppm.

Table 2.4: Analysis of percentage removal and adsorption capacity with residual dye based on contaminant concentration.

Contaminant Concentration	Absorbance Before	Absorbance After	% Removal	C_t	q_t
10 ppm	0.309	0.046	85.1 %	1.49 *mg/L*	1.70 *mg/g*
50 ppm	1.413	0.094	93.3 %	3.33 *mg/L*	9.33 *mg/g*
100 ppm	2.683	0.425	84.16 %	15.84 *mg/L*	3.17 *mg/g*

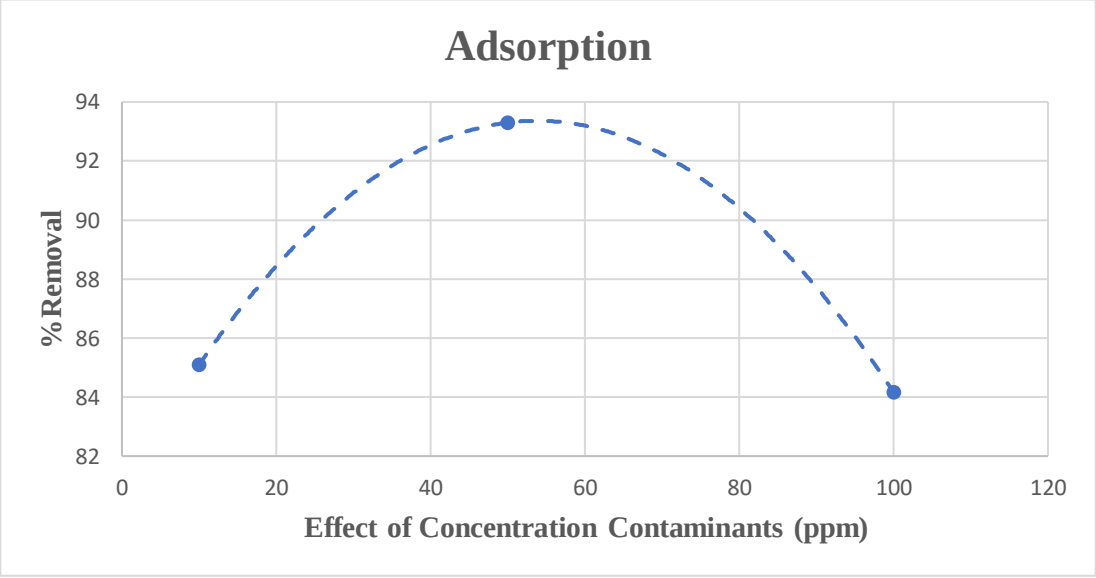

Figure 3.11: Analysis of percentage removal and adsorption capacity with residual dye based on contaminant concentration.

3.5.4 Time Period Effect on The Removal of Methylene Blue

1. Percentage Removal

Percentage removal efficiency is the measure of the pollutant percentage being removed, with a formula:

$$\%\ Removal = \frac{C_o - C_t}{C_o} \times 100$$

Here, C_o (initial concentration) = 50, V (volume) = 100 mL, m (Adsorbent dose) = 0.5g, where absorbance for pure MB concentration of 10 ppm was 0.362, after 30 min of removal = 0.038, 60 min = 0.041, 90 min = 0.041, and for 120 min = 0.050.

For 30 minutes:

$$\%\ Removal = \frac{0.362 - 0.038}{0.362} \times 100 = 89.5\ \%$$

For 60 minutes:

$$\%\ Removal = \frac{0.362 - 0.41}{0.362} \times 100 = 88.7\ \%$$

For 90 minutes:

$$\%\ Removal = \frac{0.362 - 0.41}{0.362} \times 100 = 88.7\ \%$$

For 120 minutes:

$$\%\ Removal = \frac{0.362 - 0.050}{0.362} \times 100 = 86.5\ \%$$

2. Concentration of the dye left in solution

To find the value of C_t that tells us how much dye is let in the solution, we use the following formula:

$$C_t = C_o \left(1 - \frac{\%\ Removal}{100}\right)$$

For 30 minutes:

$$C_t = 50 \left(1 - \frac{89.5}{100}\right) = 5.25\ mg/L$$

For 60 minutes:

$$C_t = 50 \left(1 - \frac{88.7}{100}\right) = 5.65\ mg/L$$

For 90 minutes:

$$C_t = 50 \left(1 - \frac{88.7}{100}\right) = 5.65\ mg/L$$

For 120 minutes:

$$C_t = 50 \left(1 - \frac{86.5}{100}\right) = 6.85\ mg/L$$

The highest concentration of dye left in the solution was at 120 minutes, where the adsorption is lowest.

3. Adsorption Capacity

Adsorption capacity is the measure of dye adsorbed by per unit mass (1 gram) of adsorbent from the medium, which is measured by using the following formula:

$$q_t = \frac{(C_o - C_t) \times\ v}{m}$$

For 30 minutes:

$$q_t = \frac{(50 - 5.25) \times 0.1}{0.5} = 8.95\ mg/g$$

For 60 minutes:

$$q_t = \frac{(50 - 5.65) \times\ 0.1}{0.5} = 8.87\ mg/g$$

For 90 minutes:

$$q_t = \frac{(50 - 5.65) \times 0.1}{0.5} = 8.87\ mg/g$$

For 120 minutes:

$$q_t = \frac{(50 - 6.85) \times\ 0.1}{0.5} = 8.63\ mg/g$$

The highest adsorption capacity of methylene blue by duckweed was observed at 30 minutes, which gradually decreased over time.

Table 3.5: Analysis of percentage removal and adsorption capacity with residual dye over time

TIME	% REMOVAL	C_t (Residual Dye)	q_t (Adsorption capacity)
30 min	89.5 %	5.25 *mg/L*	8.95 *mg/g*
60 min	88.7 %	5.65 *mg/L*	8.87 *mg/g*
90 min	88.7 %	5.65 *mg/L*	8.87 *mg/g*
120 min	86.3 %	6.85 *mg/L*	8.63 *mg/g*

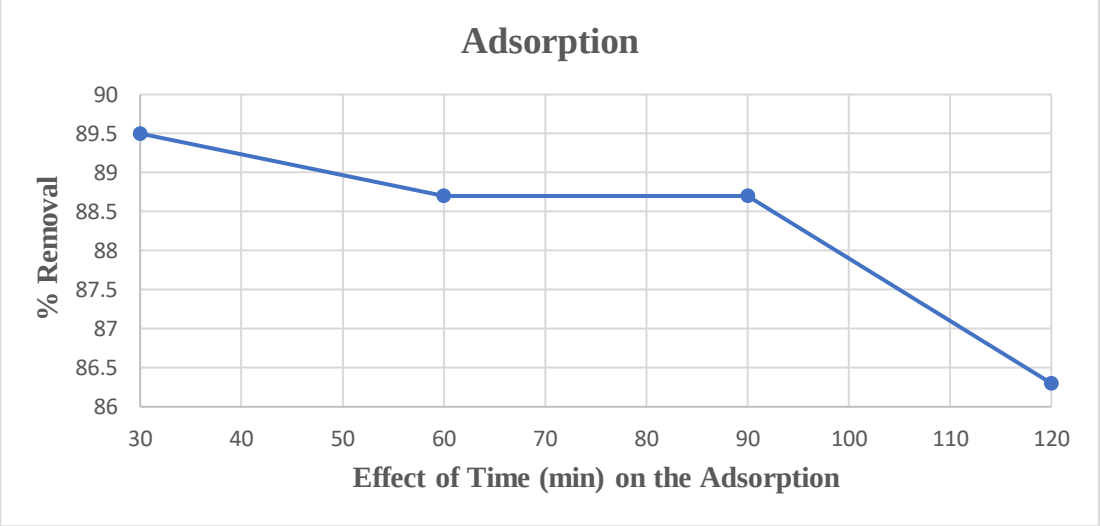

Figure 3.12: Analysis of percentage removal and adsorption capacity with residual dye over time

3.6 Comparison of Physical Parameter Efficiencies in percentage

The following graph represents the overall efficiencies of physical parameters, including pH, Adsorbent Dose, Time Analysis, and contaminant concentration.

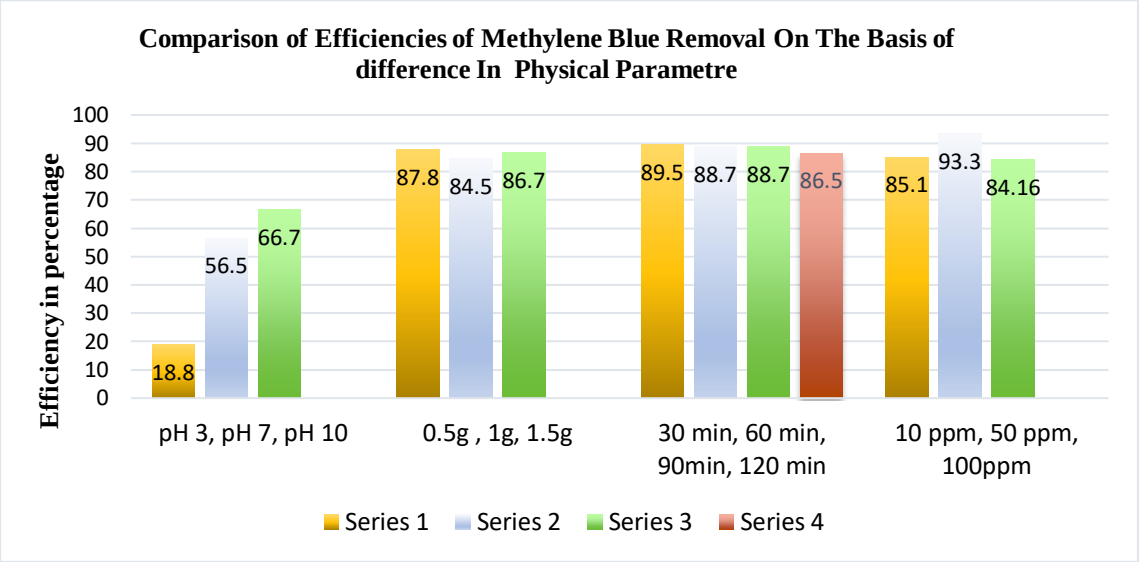

Figure 3.13: Comparison of Physical Parameter Efficiencies in percentage

3.7 Kinetic Studies of The Removal Analysis of Methylene Blue

The kinetic studies are applied to study the rate of reaction or the occurrence of biological, chemical, and physical processes, and the factors affecting the rate of reaction. These types of studies are widely used to understand adsorption behavior, reaction processes, efficiency, dynamics, and microbial activities. The basic concept of the rate of reaction is the change in concentration over time. Kinetic studies also explain how long a reaction takes to achieve the equilibrium state, which helps explain the efficient or poor interaction between adsorbate and adsorbent sites. It helps in understanding the reaction speed and feasibility and provides critical knowledge about pollutant removal.

3.7.1 Pseudo First Order Reaction Kinetic Model

The pseudo-first-order kinetic model is extensively used to determine the adsorption rate in the liquid-solid medium. Commonly, it estimates the rate of transfer of adsorbates from the liquid phase to the solid surface that acts as an adsorbent. It mainly

describes the first stage of adsorption and states that the number of available unoccupied sites is directly proportional to the rate of occupation of sites. In a pseudo-first-order reaction, adsorption predominantly occurs through physical interactions rather than chemical interactions. This type of reaction supports a good match for different dye-based adsorbents during the first phase, as a substantial number of sites are accessible. The experimental data fits well in the first stage when numerous vacant sites are available, as adsorption does not cause a considerable change in the adsorbate overtime. However, when the reaction goes ahead towards the equilibrium state, multilayers are formed, and the surface becomes heterogeneous, which can cause a significant change in the predictive power of the model. This model also aids in time-dependent concentrations of remaining adsorbate in pollution removal analysis. For the application of the pseudo-first-order reaction kinetic model, the following formula is used:

$$ln\ (q_e - q_t) = ln(q_e) - k_1 t$$

According to the measured data, C_o is 50 mg/L, V is 0.1L, m is 0.5g, and % Removal is 89.5 for 30 minutes, 88.7 for 60 and 90 minutes, and 86.3 for 120 minutes, where the total time for the test was also 120 minutes. Here, q_t is the adsorption at time, qe is the adsorption at equilibrium, k is the rate constant, and t denotes the time. As qe is the adsorption capacity at equilibrium, it denotes the maximum amount of dye the adsorbent adsorbs per gram without any change. After 30 minutes, there was a change in the adsorbent, so:

$$q_e \approx 8.9\ mg/g$$

After calculating the $q_e - q_t$ and its log, the following values were obtained:

Table 3.6: Pseudo-First Order Kinetic Reaction Model

TIME	$\boldsymbol{q_t}$	q_e-$\boldsymbol{q_t}$	ln(q_e-$\boldsymbol{q_t}$)
30	8.95	8.95 - 8.95 = 0	Undefined
60	8.87	8.95 - 8.87 = 0.03	-3.51
90	8.87	8.95 - 8.87 = 0.03	-3.51
120	8.63	8.95 - 8.6 = 0.27	-1.31

Now, applying a linear equation and calculating the slope, the following formula was used:

$$k_1 = -\frac{\Delta\, ln(q_e - q_t)}{\Delta t}$$

For 60 and 120 minutes

$$k_1 = -\frac{-1.31 - (-3.51)}{120 - 60}$$

$$k_1 = -\frac{2.20}{60}$$

$$k_1 = 0.037\ min^{-1}$$

The results are qe = 8.9 mg/g and k_1 = 0.037 per minute. At 30 minutes, the q_t is almost equal to q_e, which indicates that this model is not a good fit because absorption was fast and equilibrium was reached early. The calculated qe did not correspond to the experimental value, which shows a sign of weak linearity.

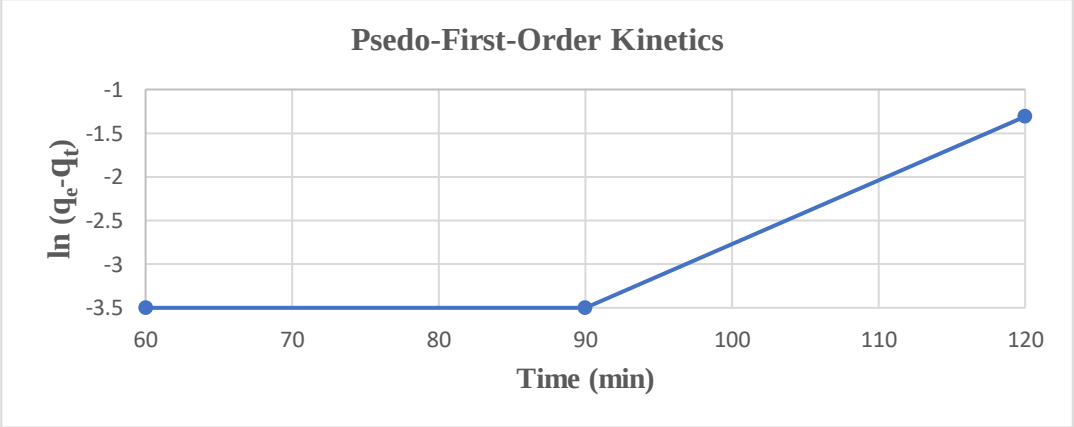

Figure 3.14: Pseudo-First Order Kinetic Reaction Model

3.7.2 Pseudo Second-Order Reaction Kinetic Model

Pseudo-second-order reaction is used to evaluate the rate of adsorption in the solid-liquid medium. It is one of the most broadly used models for studying the rate of adsorption. Mainly applied to pollutants like heavy metals, dyes, and adsorbents like biochar, nanoparticles, and biomass. The reaction rate depends on the square of the number of unoccupied adsorption sites in chemisorption. The adsorption is mostly

chemical rather than physical, where the rate is dependent on the adsorption capacity. It is a time-based analysis that is better than a pseudo-first-order reaction due to the domination of chemical bonding. Here, it was applied to illustrate the adsorption performance of methylene blue dye on Duckweed-based adsorbent and the effect of physical parameters. The linear formula for the pseudo-second-order reaction is:

$$\frac{t}{q_t} = \frac{1}{k^2\, q_e^2} + \frac{t}{q_e}$$

According to the measured data, C_o is 50 mg/L, V is 0.1L, m is 0.5g, and % Removal is 89.5 for 30 minutes, 88.7 for 60 and 90 minutes, and 86.3 for 120 minutes, where the total time for the test was also 120 minutes, q_e = 8.9 mg/g, and k_1 = 0.037 per minute. In the formula, q_t is the adsorption at time, qe is the adsorption at equilibrium, k is the rate constant, and t denotes the time.

Calculating $\frac{t}{q_t}$ at the given times:

At 30 minutes

$$\frac{t}{q_t} = \frac{30}{8.85} = 3.35$$

At 60 minutes

$$\frac{t}{q_t} = \frac{60}{8.87} = 6.77$$

At 90 minutes

$$\frac{t}{q_t} = \frac{90}{8.87} = 10.15$$

At 120 minutes

$$\frac{t}{q_t} = \frac{30}{8.63} = 13.90$$

Finding Slope

As a straight line of the linear equation of a pseudo-second order reaction is

$y = mx + c$, where y is equal to t by q_t, x is t, and slope(m) is equal to 1 by qe. So, for slope, we apply its formula, which is:

$$Slope = \frac{y_2 - y_1}{x_2 - x_1}$$

We use two points, 30 minutes and 60 minutes, for calculation:

$$Slope = \frac{13.90 - 3.35}{120 - 30} = 0.117$$

Now q_e becomes 8.55 mg/g as q_e is equal to 1 by slope. That is close to the experimental value.

Calculating k_2 using 30 mins

$$3.35 = \frac{1}{k_2(8.55)^2} + \frac{30}{8.55}$$

$$3.35 = \frac{1}{k_2\,(73.1)} + 3.51$$

$$K_2 \approx 0.085 g/mg.\ min$$

The Adsorption of methylene Blue on duckweed was well defined by a pseudo-second order reaction. The plot of t/q_t shows a good linearity that signifies chemosorption as a leading mechanism that tells the reaction rate by including electron exchange between active sites of the adsorbent and methylene blue.

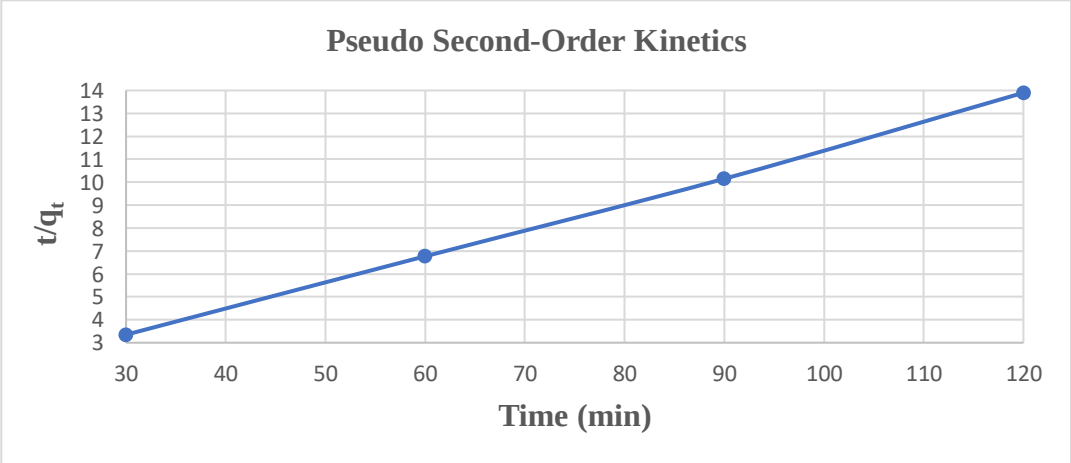

Figure 3.15: Pseudo-Second Order Kinetic Reaction Model

3.8 Isotherm Studies of The Removal Analysis of Methylene Blue

Isotherm studies were conducted to demonstrate the graphical and mathematical relation between the adsorbent and the adsorbate on a compact surface at an equilibrium application of adsorbate in the neighboring solution. This study tells us the nature, strength, and how much duckweed-derived adsorbent material can hold.

3.8.1 Langmuir Isotherm Model

The Langmuir isotherm model was applied to study the adsorption rate at active sites. Here, each adsorption site binds to one or more adsorbate molecules, forming a monolayer that indicates the existence of homogeneous active sites. It was observed that adsorption occurs via a monolayer where each site can adsorb only a single pollutant molecule. The linear equation of Langmuir is:

$$\frac{C_e}{q_e} = \frac{1}{Q_{max}{}^{b}} + \frac{C_e}{Q_{max}}$$

Here, C_e is the equilibrium concentration, b is the Langmuir constant, q_e is the adsorption capacity, and Q_{max} is the monolayer adsorption capacity. Based on the previous evaluation, the volume in 100mL, the adsorbent dose is 0.5g, and the initial concentrations are 10 ppm, 50 ppm, and 100 ppm. The % Removal calculated was 85.1%, 93.34 %, and 84.16 %. Where C_e was 1.49 mg/L for 10ppm, 3.33 mg/L for 50 ppm, and 15.84 mg/L for 100 ppm; q_e for 10 ppm was 1.70 mg/g, at 50 ppm it was 9.33 mg/g, and at 100 ppm it was 16.83 mg/g.

To calculate the $\frac{C_e}{q_e}$ value:

At 10 ppm

$$\frac{C_e}{q_e} = \frac{1.49}{1.70} = 0.876$$

At 50 ppm

$$\frac{C_e}{q_e} = \frac{3.33}{9.33} = 0.357$$

At 100 ppm

$$\frac{C_e}{q_e} = \frac{15.84}{16.83} = 0.941$$

Table 3.7: Langmuir Isotherm Reaction Model

Initial Concentration	% Removal	Equilibrium Concentration	Adsorption Capacity	C_e / q_e
10 ppm	85.1	1.49 *mg/L*	1.70 *mg/g*	0.876
50 ppm	93.34	3.33 *mg/L*	9.33 *mg/g*	0.357
100 ppm	84.16	15.84 *mg/L*	16.83 *mg/g*	0.941

Finding Slope

As a straight line, the linear equation of the reaction is $y = mx + c$, where y is equal to C_e by q_e, x is C_e, and the slope(m) is equal to 1 by Q_{max}. So, for the slope, we apply its formula, which is:

$$Slope = \frac{y_2 - y_1}{x_2 - x_1}$$

$$Slope = \frac{0.941 - 0.876}{15.84 - 1.49} = 0.00453$$

Other Factors

The Q_{max} is 220.75 mg/g, as it is the reciprocal of the slope, and the intercept (c) is 0.869. Now, to calculate the Langmuir constant (b), we use the intercept $\frac{1}{Q_{max}{}^{b}}$ formula and get 0.0052 L/mg as a value. For the separation factor (R_L), the following interpretations were made using C_o as 100mg/L:

$$R_L = \frac{1}{1 + bC_o}$$

$$R_L = \frac{1}{1 + (0.0052)(100)} = 0.658$$

The R_L greater than 1 is unfavorable, equal to one becomes linear, and equal to zero is irreversible. The Langmuir isotherm model predicts that the adsorption occurred through a monolayer. The maximum adsorption capacity indicates a strong adsorption hypothetical value of the adsorbate for the adsorbent material. It also suggests that duckweed-derived adsorbents are promising and effective adsorbents for dye removal.

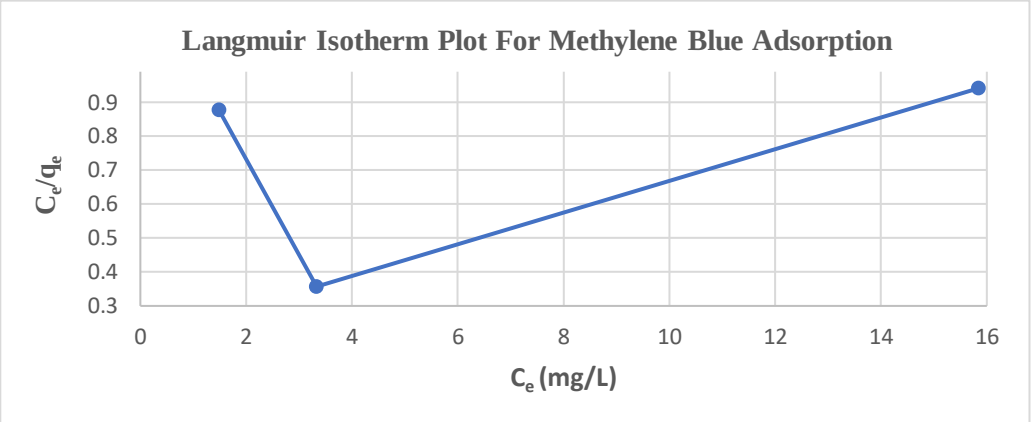

Figure 3.16: Langmuir Isotherm Plot for Methylene Blue Adsorption

3.8.2 Temkin Isotherm Model

The Temkin Isotherm model was applied to study the heat of adsorption that was linearly reduced with surface treatment. It helps in understanding the interaction between methylene blue and duckweed and states that the adsorption energy was not constant. Energy was not constant, and the heat of adsorption gradually decreased based on the reaction rate, which indicates heterogeneity of adsorbent surfaces and strength. The linear form of the Temkin Isotherm is:

$$q_e = B \ln A + B \ln C_e$$

Here, volume V is 100mL, adsorbent dose is 0.5g, time T is 30 minutes, and the initial concentrations are 10 ppm, 50 ppm, and 100 ppm. The % Removal calculated was 85.1%, 93.34 %, and 84.16 %. C_e was 1.49 mg/L for 10ppm, 3.33 mg/L for 50 ppm, and 15.84 mg/L for 100 ppm. The q_e for 10 ppm was 1.70 mg/g, at 50 ppm it was 9.33 mg/g, and at 100 ppm it was 16.83 mg/g. After calculating ln (C_e) for 1.49 we got 0.399, for 3.33 it was 1.203, and for 15.84 it was 2.762.

Table 3.8: Temkin Isotherm Reaction Model

Equilibrium Concentration	Adsorption Capacity	In C_e
1.49 *mg/L*	1.70 mg/g	0.399
3.33 *mg/L*	9.33 mg/g	1.203
15.84 *mg/L*	16.83 mg/g	2.762

To find the value of Temkin constant B, we use the slope formula:

$$Slope = B = \frac{y_2 - y_1}{x_2 - x_1}$$

$$B = \frac{16.83 - 1.70}{2.762 - 0.399} = 6.40\ mg/g$$

Calculating the intercept using the $y = mx + c$ formula

$$1.70 = (6.40)\ (0.399) + C$$

$$C = -0.85$$

Here, B ln A is also equal to -0.85. Temkin Constant A can be known by keeping B as a substitute for 6.40.

$$6.40 \ln A = -0.85$$

$$\ln A = -0.133$$

$$A = e^{-0.133}$$

$$A = 0.875\ L/g$$

The Temkin isotherm model states that the heat of adsorption gradually decreases with an increase in surface coverage, which involves a moderate interaction between adsorbate and adsorbent material. It also states that the adsorption process is not uniform, and surface chemistry plays an important role in adsorption.

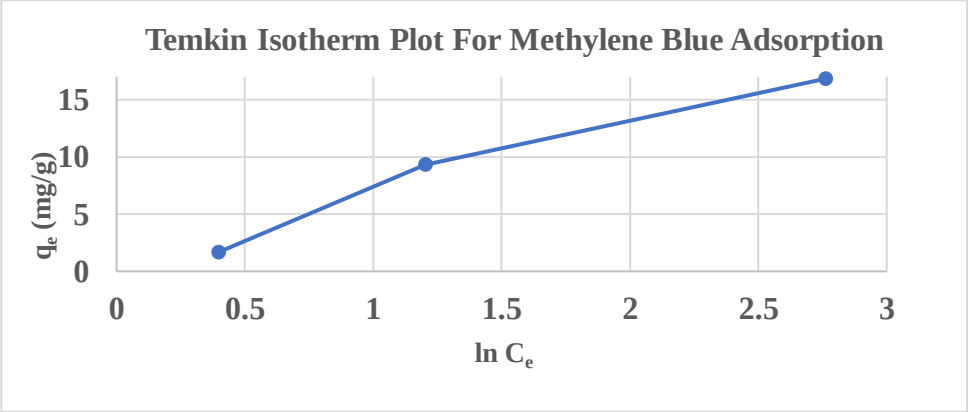

Figure 3.17: Temkin Isotherm Plot for Methylene Blue Adsorption

3.9 Interpretation of Adsorption Kinetics Model

The reaction kinetic models were examined to recognize the removal mechanism of methylene blue dye by duckweed-based adsorbents. The Pseudo-first order and pseudo-second-order reaction kinetics were evaluated as adsorption kinetics. Based on the data interpreted, the pseudo-second-order reaction kinetic model offers the best fit, as the plot of t/q_t shows good linearity, indicating chemosorption as a leading mechanism that determines the reaction rate by including electron exchange between active sites of the adsorbent and methylene blue. The R^2 value of the pseudo-second-order model was greater than that of the pseudo-first-order reaction model, as there were large deviations between the experimental and calculated capacities in the pseudo-first-order reaction model. The duckweed biomass contains compounds like lignin, cellulose, and hemicellulose, followed by carboxyl and hydroxyl groups as abundant oxygen containing function groups, which was confirmed in an FTIR analysis study. The pseudo-second-order reaction kinetic model also provides the best fit for other plant-based bioadsorbents with similar adsorption behavior (Hamad & Idrus, 2022; Jeffrey et al., 2025; Pandey et al., 2022)

3.10 Discussion

This study validates that the removal efficiency of Methylene Blue by Duckweed-derived adsorbents can be improved using an activator, and a supreme removal efficiency of 93.34 percentage was accomplished using citric acid as an activator under the optimal condition of 0.5g of adsorbent, 50 ppm MB solution, and at pH 10 in 30 minutes. Based on the different literature, the highest removal efficiency was achieved at pH 10, although many articles do not provide a removal percentage number; the highest removal efficiency in percentage was 80.44 percent in 24 hours (Imran et al., 2018). Furthermore, an author states that the electrostatic force of attraction is improved by a negatively charged adsorbent surface due to an increase in the deprotonation of oxygen-containing functional groups (Jeffrey et al., 2025). All three datasets signify that the best medium for MB removal with maximum efficiency is an alkaline medium, irrespective of the activation process. As compared to the different literature sets, the maximum removal efficiency was achieved by using 20mg of adsorbent for 60 minutes, as the experiment was run in a small-scale laboratory (Jeffrey et al., 2025), and 50 ppm of adsorbent was used, keeping the sample size for 24 hours (Cwalinski et al., 2022). The present study uses a 50 mg dose of adsorbent for 30 minutes due to the different experimental design.

Few literature sets indicate an increase in removal efficiency by increasing the adsorbent dose, as their main focus was on biological uptake because of the existence of more active sites, but the current study and other sets using duckweed derived adsorbents indicate an increase till it reaches the state of equilibrium, after which it decreases even after adding more adsorbent due to diffusion limitation, particle aggregation, and reduced concentration gradient. Living Lemna Minor was used as a phytoremediation process (Castillo-Suárez et al., 2023; Imran et al., 2018), and a magnetic Duckweed adsorbent was synthesized using Fe_3O_4 and nanoparticles co-precipitation (Hamad & Idrus, 2022); however, the recent study uses the duckweed-derived adsorbents that are artificially synthesized in the laboratory only using citric acid as an activator, so the elimination was completed by the adsorption process rather than biological uptake. A relative analysis of kinetic studies shows that the pseudo-second-order model provides the best fit results, as it has the highest value for the coefficient of determination, which justifies that the rate of adsorption was mainly governed by molecular interaction between adsorbent and adsorbate. Furthermore, similar findings were studied in literature, where the pseudo-

second-order model consistently provides a better understanding of adsorption analysis of methylene blue (Hamad & Idrus, 2022; Jeffrey et al., 2025; Pandey et al., 2022).

The effect of physical parameters, including pH, contaminant concentration, adsorbent dose, and time effects, was studied further using XRD, FTIR, and SEM analysis for the study and comparison of the characterization of the adsorbent before and after treatment. Alternatively, many studies do not explain a deep comparison, especially those using living Lemna minor as their focus, which was only biological removal. The absorbance was studied at 665 nm in the literature, whereas the current study uses 580nm wavelength for measuring results, as the highest peak was observed at this wavelength. On comparison, the current study achieved maximum results as compared to the literature review in a shorter time due to differences in experimental design and use of citric acid as an activator but produced a residue of duckweed-based adsorbent with methylene blue dye in contact. Alternatively, those using living Lemna minor do not produce waste, but they do not provide a realistic relationship between methylene blue and Lemna minor with scientific evidence.

RECOMMENDATIONS

This study provides physical evidence of duckweed material as an eco-friendly, cost-effective, and biodegradable alternative for methylene blue removal from synthetic wastewater. Furthermore, there is a need to enhance its efficiency for practical applications at a large scale in industrial wastewater treatment. The accurate bond between adsorbate and adsorbent is still unknown. Only the Lemna Minor type was used as an adsorbent; deeper study is required to use other types like Spirodela, Wolffia, etc. Other activators, especially green activators, can also be used to enhance removal efficiency. There is a need to study the treatment process in different media, such as basic, acidic, and solvent-based media. Further studies can be done to prepare activated biochar and to make the adsorbent more sustainable. The application of duckweed adsorbent is limited to methylene blue removal, so there is a requirement for expansion towards other contaminants.

CONCLUSION

This study validates the potential of duckweed-based adsorbents as a cheap, eco-friendly, and biodegradable material for methylene blue dye removal from synthetic wastewater. The powdered adsorbent was prepared so that it can accumulate methylene blue into its available sites and make it less toxic. This method can also help improve the water quality of effluent discharged from industries. The separation, preparation, and regeneration of duckweed-derived adsorbents confirm their durability due to the existence of a porous structure and cationic functional group that favors dye adsorption.

The effect of physical parameters was assessed to improve the adsorption process. The adsorbent works best at low temperatures, and factors such as adsorbent dose, pH, initial dye concentration, and contact time play important roles in determining the rate of adsorption. A maximum number of efficiencies, including 93.3 %, 88.7 %, and 86 %, was attained depending on these factors. Additionally, desorption studies were done, and reusability was experimented with, where percentage removal analysis was done to measure efficiency in percentages using different amounts of stock solution wavelengths as input and results wavelengths as an output. Citric acid was also used as an activator due to its capability of preventing pigment leaching, washing, and enhancing surface chemistry, with more negatively charged sites for adsorption.

The removal efficiency of the adsorbent was determined using reaction kinetics and reaction isotherm models. The adsorption is well defined by the pseudo-second-order kinetic model, which reveals chemisorption as an influential mechanism. Temkin and Langmuir isotherm models define the equilibrium actions of adsorption, including graphical and mathematical relations and molecular attachment. XRD, FTIR, and SEM tests were conducted to study the characterization of duckweed adsorbent, which evaluates new peaks, a strong cellular structure, and durable attachment of methylene blue dye to duckweed on a molecular level. Despite all these outcomes, there is still a need for improvement in large-scale applicability and reusability efficiency that enhances adsorption performance and helps to treat wastewater commercially. As duckweed is inexpensive and abundantly available, it also has some limitations, including a limited surface area, a small root system, sites becoming saturated over time, the molecular structure of the methylene blue dye, and its inability to be used more than twice due to a decrease in efficiency over time.

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DUCKWEED DERIVED ADSORBENT FOR EFFICIENT METHYLENE BLUE REMOVAL FROM SYNTHETI WASTEWATER

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5	Submitted to Higher Education Commission Pakistan Student Paper	
6	Dinesh Arora, Amit Arora, Asha Singh, Rashmi Agarwal, Sunil Kumar. "Usability of Brachiaria mutica (para grass) and Cyperus rotundus (nut grass) as bioadsorbents for t removal of methylene blue from aqueous solution: isotherms, kinetics, and thermodynamics studies", Sustainable Water Resources Management, 2022 Publication	
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