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Phytoremediation Of Dairy Waste Water Using Aquatic Macrophytes

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ABSTRACT
*Dairy industries generate large volumes of nutrient-rich wastewater containing dissolved sugars, proteins, fats, and other organic matter. This effluent, characterized by high biochemical oxygen demand (BOD) and chemical oxygen demand (COD), cannot be discharged into the environment without treatment. Conventional treatment methods, although effective, are costly and require skilled operation. This study evaluates the phytoremediation potential of hydrophytes—Water Hyacinth (*Eichhornia crassipes*), Duckweed (*Lemnoidae*), Water Lettuce (*Pistia stratiotes*), Kariba Weed (*Salvinia molesta*), and* Azolla*—for dairy wastewater treatment. Wastewater samples were collected from a dairy plant and analyzed for pH, electrical conductivity, total solids, alkalinity, hardness, dissolved oxygen, BOD, and COD over a 28-day period under controlled laboratory conditions. Results indicated that* Azolla *achieved the highest reduction in most physicochemical parameters, particularly hardness, TDS, BOD, and COD, while Duckweed was most effective in turbidity reduction. The findings demonstrate that phytoremediation using floating aquatic plants is an eco-friendly, cost-effective, and sustainable alternative for dairy wastewater treatment.*
Keywords: *Phytoremediation, Dairy wastewater, hydrophytes,* Azolla, *BOD, COD*

INTRODUCTION

The dairy industry is one of the largest sources of wastewater for food processing in many countries. A significant amount of water is used during milk processing, resulting in the generation of high volumes of effluent containing dissolved sugars, proteins, fats, and other organic compounds. These effluents are primarily organic in nature and are characterized by high concentrations of organic matter, **high Biochemical Oxygen Demand (BOD)**, and **metal content**. Due to these characteristics, without proper treatment

Such wastewater cannot be used for land irrigation and cannot be started directly into public sewers or s urface water .

It is essential to treat dairy waste water properly before disposal., It can be effectively treated with conventional treatment methods like the **activated sludge process**, **trickling filters**, and **waste stabilization ponds** Since dairy wastewater is **readily biodegradable**. However, conventional treatment techniques have limitations, such as:

1. **High operational and maintenance costs**,
2. **Requirement of technically skilled labor**,
3. **Disposal issues for biological sludge**.

Given these challenges, there is a growing need to develop **low-cost** and **efficient treatment techniques** for dairy wastewater. This need has led to exploring alternative, cost-effective methods, such as **phytoremediation** and other sustainable technologies, to reduce the environmental impact and improve wastewater management in the dairy industry.

Phytoremediation is a natural wastewater treatment approach that utilizes plants to absorb, break down, or contain pollutants in water. Plant species are selected according to their ability to remove contaminants such as heavy metals, excess nutrients, and organic compounds through their roots, stems, and leaves.. This approach utilizes various techniques, including **specific planting arrangements**, **constructed wetlands (CW)**, **floating-plant systems**, and several other configurations to treat wastewater.

The removal of wastewater occurs through a range of mechanisms such as **sedimentation**, **filtration**, **chemical precipitation**, **adsorption**, and **uptake by vegetation**. Among these, the **floating-plant systems** technology, which integrates **phytoremediation** and microbial processes, is considered one of the most effective strategies.

In addition to improving water quality and contributing to **energy savings**, floating-plant systems offer several other **environmental benefits**, such as:

1. **Promoting biodiversity** by supporting a variety of plant and animal species,
2. **Providing habitat** for wetland organisms and wildlife,
3. Acting as a **climatic buffer** by helping regulate water temperature and humidity.

These advantages make floating-plant systems a promising solution for treating wastewater while also providing broader ecological benefits.

These **floating-plant systems** are generally **cost-effective**, **simple**, and **environmentally non-disruptive**, making them ecologically sound and offering **low maintenance costs** and **minimal land requirements**.

The fundamental principles of the **phytoremediation** system are to clean up contaminated water by identifying pollutants and implementing effective aquatic plant species that can uptake dissolved **nutrients** and **metals**. The process involves several key steps:

1. **Identification of contaminants** in the water,
2. **Selection of efficient aquatic plants** suited for remediation,
3. By growing plants **,**uptake of dissolved nutrients and metals .
4. From the remediation process **harvesting** and **beneficial use** of the plant biomass produced

The selection of an appropriate plant species is crucial to the success of the phytoremediation system. Ideal plants should have the following qualities:

- **It should have high uptake of both organic and inorganic pollutants**,
- Ability to **thrive in polluted water**,
- **Control and ease of propagation** to avoid uncontrolled spread.

The uptake and accumulation of pollutants can vary from plant to plant and even within species of the same genus and its important to note that. Therefore, selecting the right plant for specific contaminants and environmental conditions is critical for maximizing the effectiveness of phytoremediation systems.

The **economic viability** of **phytoremediation** largely depends on plant growth rates and the **photosynthetic efficiency**, as well as their ability to thrive in environments with **low to moderate pollution levels**. Various plant species have been researched for their potential in treating contaminated waters and effluents. Some of the most commonly used species include:

- **Water Hyacinth**
- **Water Lettuce** (Pistia stratiotes)
- **Duckweed** (Lemnoidae)
- **Azolla**
- **Bulrush** (Typha)
- **Vetiver Grass**
- **Kariba Weed**
- **Common Reed** (Phragmites australis)

These species have been used to treat different types of contaminated waters, including dairy wastewater, industrial effluents, and domestic sewage.

Based on the findings from various studies, plants like **water hyacinth**, **water lettuce**, **duckweed**, and **kariba weed** are often preferred as they have the ability to efficiently remove heavy metals and other pollutants. These species have the **high reproduction rate**, **tolerance to various ecological factors**, and **effective pollutant removal capacity** necessary for efficient wastewater treatment.

This paper analyzes the role of these selected plant species in the context of **dairy wastewater treatment**, highlighting their potential for reducing pollutants, improving water quality, and offering an eco-friendly solution to dairy wastewater management.

Objective Of The Study

- Toidentify local, reliable, simple and acceptable environmentally friendly water treatment technology..
- To identify how we can effectively utilize aquatic macrophytes for dairy waste water treatment.
- To identify the factors affecting Phytoremediation.
- To check the nature of the toxic metals

1.1 SCOPE OF THE WORK

This study explores the potential of **aquatic macrophytes** in treating **dairy wastewater**, with a particular focus on **phytoremediation** as an effective and sustainable approach. The scope of this research includes

- The study assesses the viability of using **floating plant systems** for the remediation of dairy wastewater. Phytoremediation using aquatic plants is considered a highly effective method for wastewater treatment, especially in cases where organic matter and nutrients are the primary pollutants.
- A significant focus is placed on the **economic aspects** of using aquatic macrophytes. The study aims to demonstrate that phytoremediation can be an affordable and cost-effective solution, especially when compared to traditional methods, which often involve high operational and maintenance costs.
- Phytoremediation not only treats wastewater but also contributes to environmental sustainability. The floating plant system improves **biodiversity** and offers ecological benefits by supporting various organisms and contributing to the stabilization of aquatic ecosystems.
- Traditional wastewater treatment methods are often **complex and costly**, which makes them less feasible for widespread use, particularly in rural or underdeveloped areas. This study highlights the limitations of these methods and emphasizes the need for **in situ alternatives** that are both efficient and economically viable.
- Investigating the role of aquatic plants in extracting **metals** from wastewater, focusing on the **bioavailable fraction** in the water. The effectiveness of plant species in extracting toxic metals and pollutants from the wastewater is a key area of interest.
- In light of the challenges associated with conventional wastewater treatment methods, this study advocates for the promotion and implementation of **low-cost**, **sustainable treatment technologies** like phytoremediation. The research aims to contribute to the development of alternative treatment methods that can be implemented at a **household or community level**.

MATERIALS AND METHODS

3.1MATERIALS

3.1.1 Dairy Waste Water

The dairy industry consumes significant amounts of water during milk processing, leading to the production of large volumes of wastewatercontaining dissolved sugars, proteins, fats, and other organic compounds. These effluents are typically rich in organic matter and have high concentrations of **Biochemical Oxygen Demand (BOD)** and **Chemical Oxygen Demand (COD)**. This makes the wastewater highly biodegradable but also potentially harmful if not properly treated before being discharged into the environment. The high organic load in dairy wastewater can lead to **eutrophication**, water pollution, and negatively affect aquatic ecosystems. Therefore, effective and sustainable methods for treating dairy wastewater are necessary to reduce its environmental impact and make it suitable for disposal or reuse. Dairy waste water is the water that is collected after all the process has been done in a dairy industry.

3.1.2 Kariba weed

Fig 3.1 Kariba weed

Salvinia is also known as Kariba Unskilled) is a free floating fern that forms a dense mat on the surface of the water. It consists of a branched horizontal stem. It is usually 1 mm in diameter floating directly be

low the surface of the water. Each knot or joint on the stem wears several floating, green, oval, hairy leav es. At each node, there is also a brown, hairy frond that looks and acts like a root, trailing in the water.
Salvinia's appearance changes as it matures.
Due to its low density, the plant has small floating waves (approximately 10 mm wide) and is flat on the surface of the water. However, in the case of dense invasions, the floating leaves fold themselves, folding up to 60 mm wide, packed in a concerto-
like arrangement. The surface of these floating leaves is covered with striking hair with egg fittings that r eject water. This helps buoyancy and plants float.Salvinia's rapid growth and vegetative reproduction make it a highly invasive species. It can double in size every two to three days, creating thick mats that cover large areas of water. These mats can choke waterways, block sunlight, and reduce oxygen exchange in the water. As a result, Salvinia infestations can create anoxic conditions that are unsuitable for fish and other aquatic life. Additionally, the dense mats can impair water flow and reduce the natural biodiversity and beauty of wetlands.
Due to its aggressive growth and ecological impact, Salvinia is considered an invasive species and a significant environmental concern in many areas, especially in water bodies with slow-moving or stagnant water.

Water Lettuce

Fig 3.2 water Lettuce

[3]**Pistia stratiotes**, commonly known as **water lettuce**, typically found in streams, lakes, and ponds and is a free-floating aquatic plant. This plant is well-adapted to marshland conditions and thrives in alkaline or lime-rich waters. It is a stoloniferous plant, meaning that it can anchor itself to the hydrosoil when water levels recede.
P. stratiotes forms dense mats on the water surface, which can disrupt the aquatic ecosystem by shading out other plants and reducing oxygen levels in the water. This can adversely affect the aquatic fauna, including fish and other organisms. Additionally, its mats can obstruct water flow, interfere with recreational activities such as swimming, boating, and fishing, and even hinder navigation.
In agricultural contexts, the plant can also become problematic when it enters rice fields, as its roots can establish themselves in the soil and compete with rice crops, particularly in shallow water conditions. Furthermore, the plant is known to replace native hydrophytes in ponds and other water bodies, altering local ecosystems.
The water lettuce has a unique look with bright green rosette style sitting leaves between 10 cm long an d 10 cm wide. The leaves are covered in round-up-
up rounded from the tip, and the underside is covered with whitish hair. The recognition of its ability t o remove nutrients and heavy metals from sewage sludge and drainage has made it an effective tool for plant-based mediation of waste, especially in tropical regions.Research has shown that water lettuce is capable of reducing various physicochemical parameters, such as turbidity, phosphates, total iron, sulfates, and suspended solids, when applied in influent and effluent ponds and is very efficient in removing heavy metals such as zinc (Zn), iron (Fe), copper (Cu), chromium (Cr), and cadmium (Cd) without causing harm to the plant itself. Due to its strong affinity for zinc absorption, water lettuce is considered an effective and eco-friendly option for the phytoremediation of wastewater, specifically for the removal of heavy

metals from industrial wastes. Its ability to tolerate and accumulate heavy metals makes it a promising plant for large-scale phytoremediation applications.

3.1.3 Water hyacinth

Fig 3.3 water hyacinth

[1] **Water Hyacinth** (Eichhornia crassipes) is an aquatic plant that was introduced to Egypt in 1879. Initially cultivated in ponds within public gardens, it eventually found its way into water canals and has since spread extensively throughout the country, particularly in the Nile Delta and regions like Upper Egypt, Damietta, and Rosetta branches of the Nile, as well as in drainage canals and El-Manzalla Lake. This plant is now a major concern due to its invasive nature, often forming large floating islands that disrupt local aquatic ecosystems.

The rapid growth and reproductive potential of water hyacinth are remarkable. In a span of 50 days, a single plant can produce up to 43 offsets, which in turn can generate 1,894 offsets in another 50 days. Over a period of 200 days, one plant can result in an astonishing 3,418,800 new offsets. This rapid multiplication makes water hyacinth highly productive, with some reports indicating that per hectare upto 20 dry tons can be harvested from standing water hyacinth plants. Harvesting can occur up to 5 to 8 times per year, depending on the conditions.

Water hyacinth's fast growth and high biomass production make it a useful resource, especially in the context of pollution control. It has been recognized for its ability to absorb and concentrate various plant nutrients from water, including nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), and potassium (K), with concentration factors ranging from 36 to 14,000 times the levels in the water. This nutrient-rich biomass can be used as compost and mulch, providing valuable organic material for soil enrichment.

Moreover, due to its rapid growth and high nutrient uptake, water hyacinth has garnered interest [23]as a solution for the removal of pollutants from domestic and industrial wastewater. Its ability to absorb and concentrate pollutants, combined with its ease of use for agricultural applications like green manure, makes it an attractive plant for wastewater treatment and environmental remediation. However, its invasive nature means that its use must be managed carefully to prevent it from causing ecological imbalances.

3.1.4 Duckweed

Fig 3.4 duckweed

,[5] **Duckweed** (Lemnoidae), also known as water lens or bay root, refers to small, floating aquatic plants that thrive in still or slow-moving freshwater bodies and wetlands. These plants belong to the Araceae family, specifically the subfamily Lemnaceae (formerly considered a separate family, Lemnaceae, before the late 20th century). Duckweed plants are distinctive due to their simple structure, lacking obvious stems or leaves.
Instead,They are primarily composed of small, thin, leafy or leaf structures. These are cells that are only a small thickness and often contain air pockets (elentim), which help to float accurately below or above t he surface.
Duckweed thrives in nutrient-rich, often eutrophic environments, where there is an abundance of minerals, especially nitrogen and phosphorus. These plants are highly adaptable and can be spread by water flow, small mammals, or even inadvertently carried on animals' feet or bodies. In areas with constant water movement, duckweed does not proliferate significantly, but during periods of lower water flow, such as during dry spells, duckweed can rapidly grow and cover water surfaces. Once rainfall returns, these plants may be washed away, resetting their growth cycle.
One of duckweed's most remarkable traits is its high protein content, which surpasses that of soybeans, making it an important food source for waterfowl and even humans in some parts of Southeast Asia. Additionally, duckweed provides valuable ecological services, offering shelter and shade for aquatic species like fish and bullfrogs, and serving as cover for fry (juvenile fish). It also plays a [27]an essential role in controlling the growth of light-dependent algae by reducing light penetration in the water.
From a bioremediation perspective, duckweed is highly valued for its ability to remove excess nutrients from water, particularly nitrogen and phosphorus. When cropped or harvested, it can significantly reduce the levels of these minerals, helping purify the water. Given its rapid growth rate and nutrient uptake, duckweed is increasingly recognized as a potential water purifier, making it a promising tool for environmental remediation and water quality management.

3.1.5 Azolla

Fig 3.5 Azolla

Azolla is a fast-growing floating aquatic fern that can quickly spread across the surface of lakes or ponds, often covering entire water bodies within a few months. Each individual plant is small, typically 1–2 cm in diameter, and is green with tinges of pink, orange, or red at the edges. As it grows, it branches freely and can break into smaller sections. Azolla is sensitive to cold temperatures and typically dies back in temperate regions during winter, surviving by means of submerged buds.
One of the standout characteristics of Azolla is its ability to fix nitrogen from the atmosphere, a trait it shares with other species in its genus. This nitrogen-fixing capability has made Azolla valuable in agricultural practices, particularly in rice paddies, where it enhances crop growth by providing a natural source of nitrogen. It is often used as a green manure, contributing to the fertility of the soil when it decomposes.
Azolla is also known as "Mosquito Fern" because it can form dense mats over the water's surface, which is believed to prevent mosquito larvae from developing and hatching. This natural method of mosquito control makes Azolla a potential environmentally friendly solution for managing mosquito populations.
In addition to its agricultural uses, Azolla has gained attention for its role in **phytoremediation**. The plant is effective in purifying water by removing pollutants such as heavy metals, nitrogen, and phosphorus from wastewater. As it grows, Azolla absorbs these contaminants, and regular removal of its excess biomass

takes these pollutants out of the system. The harvested plant material can then be repurposed, such as being used as nitrogen-rich fertilizer or compost, making Azolla a valuable tool for both water purification and sustainable agriculture.

3.2METHODS

3.2.1 Collection of waste water sample

Dairy wastewater approximately 30 litres of raw effluent was collected from dairy plant, Edapally. The waste water collected was the water which is left to the pits after all the processes have been done and the waster which is ready to be disposed to drainage.

Approximately 30 litres of raw effluent collected from dairy plant was brought to the laboratory and stored in laboratory conditions.

3.2.2 Collection of materials

The aquatic plants such as water lettuce, water hyacinth, Kariba Weed, duck weed, Azolla etc were collected freshly from natural ponds.

These plants were cleaned properly to remove dirt and dust and stabilized in laboratory conditions for 1 week to normalize their growth in fresh water. The wastewater after necessary dilution in the ratio 1:1 were checked its initial values. Then it was poured into 4 rounded plastic troughs equally and similarly with domestic waste water also.

In this experiment, plastic troughs with a capacity of about 20 liters were used to maintain the aquatic plants. The plants that were initially kept in stock tanks were carefully collected and introduced into the experimental tanks. These tanks were kept under controlled laboratory conditions, ensuring that factors such as temperature, light, and water quality remained consistent throughout the experimental period. The experiment lasted for one month, providing enough time to observe the effects of the aquatic plants in treating the wastewater.. The samples were collected on 7th day, 14 th days, 21st day and 28 th days respectively. After observing the samples for 28 days, the best absorbing plant sample was selected for dairy samples and the water sample of that plants were collected

Fig 3.6 collected materials in sample

3.3TEST CONDUCTED

Parameters analyzed were:

- pH
- Electrical Conductivity
- Total Solids
- Total Alkalinity
- Total Hardness
- Dissolved Oxygen
- BOD
- COD

3.3.1 PH

The pH of a solution is determined by measuring the electromotive force (emf) of a cell composed of two electrodes: an indicator electrode and a reference electrode. The indicator electrode is typically a glass electrode that is responsive to hydrogen ions (H+), while the reference electrode, often a calomel electrode, provides a stable reference potential. These electrodes are placed in the test solution, and a contact is established between them through a liquid junction which is part of the reference electrode.
When the pH meter measures the emf, it quantifies the potential difference between the indicator and reference electrodes. This voltage is then related to the hydrogen ion concentration, and the pH is calculated based on this measurement. The resulting value provides an accurate representation of the solution's acidity or alkalinity.

3.3.2 Electrical Conductivity:

The method of measuring conductivity is used to assess the ability of a solution or water to conduct electricity, which is influenced by the presence of dissolved ions. The conductivity value, usually measured in millisiemens per centimeter (mS/cm), can provide a rough estimation of the charged particles in the water sample.
To estimate the dissolved ionic content more specifically, the measured conductivity is multiplied by an empirical factor, which typically ranges from 0.55 to 0.90. This factor depends on the specific soluble components present in the water and the temperature at the time of measurement. For example, higher conductivity values usually indicate higher concentrations of dissolved minerals or salts.
Conductivity measurement is a practical and rapid method for detecting variations in the dissolved mineral contents of water, offering valuable insights into water quality. It can be particularly useful for monitoring changes in water chemistry, such as increased salinity or contamination by pollutants.

3.3.3 Total Alkalinity

To estimate the alkalinity of a water sample, a titration method using standard sulfuric acid (0.02N) is employed at room temperature.The phenolphthalein indicator and methyl orange indicator were used to identify different stages of neutralization during the titration process.
Here's how the process works:
1. **Phenolphthalein Indicator:**
   - The sample is first titrated with the sulfuric acid until the phenolphthalein indicator turns from pink to colorless. This color change indicates the neutralization of hydroxide ions (OH^-) and half of the carbonate ions (CO_3^{2-}).
   - This is the **first endpoint** of the titration, representing the neutralization of OH^- and part of CO_3^{2-}.
2. **Methyl Orange Indicator:**
   - After reaching the first endpoint, the methyl orange indicator is added to the sample.
   - The titration continues until the solution shows a sharp color change from yellow to orange. This change indicates the neutralization of the remaining carbonate ions (CO_3^{2-}) and bicarbonate ions (HCO_3^-).
   - This is the **second endpoint**, representing the total alkalinity of the sample.

The volume of acid used in each stage of the titration can then be used to calculate the concentration of different components contributing to the alkalinity, such as hydroxide, carbonate, and bicarbonate ions. This titration method gives an accurate estimate of the water's alkalinity.

3.3.4 Total Hardness

The **EDTA method** for determining water hardness involves a chelation reaction in an alkaline condition where **EDTA (ethylenediaminetetraacetic acid)** and its sodium salts form soluble complexes with divalent metal ions, specifically **calcium (Ca^{2+})** and **magnesium (Mg^{2+})** ions. Here's how the procedure works:
Key Points of the EDTA Hardness Determination Method:
1. **Indicator Used:**
   - **The indicator used is Eriochrome Black T** . At a pH of 10.0 ± 0.1, Ca^{2+} and Mg^{2+} ions in the water will form a wine-red complex with this indicator.
2. **Titration:**

o **EDTA** is used as the titrant. When added to the solution, it reacts with and **complexes the calcium and magnesium ions**. This binding causes a **sharp color change** from wine red (indicating the presence of metal ions) to blue (indicating that the metal ions have been complexed with EDTA and are no longer free).

o The endpoint of the titration is the color change from **wine red to blue** , which corresponds to the point where all the calcium and magnesium [28]ions have been complexed by EDTA.

3. **Addition of Magnesium Salt:**

o Magnesium ions (**Mg^{2+}**) are required for a sharp and clear endpoint. To ensure this, [19]a small amount of **magnesium salt of EDTA** is added to the buffer. This ensures that **magnesium ions are present** in the solution for accurate titration.

4. **pH Control:**

o The **pH is maintained at 10.0 ± 0.1** using a buffer. This pH range is a compromise between the increased sharpness of the endpoint at higher pH and the precipitation of magnesium ions at higher pH levels (around pH 12).

o At **higher pH levels (around 12)**, **magnesium ions precipitate out**, leaving only calcium ions in solution. At this higher pH, the titration would only measure **calcium hardness**.

o However, at pH **10.0 ± 0.1**, both **Ca^{2+}** and **Mg^{2+}** are in solution, allowing for the accurate measurement of **total hardness**.

5. **Murexide Indicator (at pH 12):**

o If the pH is raised further (around pH 12), a different indicator called **murexide** (or **ammonium purpurate**) may be used to titrate calcium ions, as the magnesium ions would have precipitated out by this point. This can help determine **calcium hardness** separately.

Summary of the Procedure:

- **pH 10.0 ± 0.1** is ideal for the EDTA titration method as it keeps both calcium and magnesium in solution.

3.3.5 Dissolved Oxygen

Using the **Winkler method the determination of dissolved oxygen is done** . This technique is based on the oxidation of manganese in the presence of oxygen, followed by titration with sodium thiosulfate ($Na_2S_2O_3$). Here's a breakdown of the process:

Steps Involved in the Winkler Method for DO Determination:

1. **Oxidation of Manganese:**

o The **manganous hydroxide** (Mn^{2+}) usually in the form of **$MnSO_4$** (manganese sulfate) is initially present [25]in the sample,.

o The **oxygen** (O_2) present in the water sample oxidizes the manganous hydroxide to **manganese (III)** in the form of **manganic oxide (MnO(OH))**. This is an oxidation reaction in which oxygen rapidly oxidizes **Mn^{2+}** (manganous ion) to **Mn^{3+}**.

o The manganese is oxidized to its higher valency state, precipitating as a brownish hydrated manganese oxide.

2. **Formation of Precipitate:**

o After the oxidation, an alkaline solution is created by adding a base such as **NaOH** or **KOH** along with **KI** (potassium iodide).

o This forms a brown precipitate of **manganese hydroxide**.

3. **Acidification and Reversion to Divalent Manganese:**

o The sample is then acidified (often with **H_2SO_4** or **HCl**), which causes the manganese to revert to its **divalent state** (Mn^{2+}) from the higher oxidation state.

o As the manganese returns to the divalent state, it **liberates iodine (I_2)** from the added **KI** (potassium iodide) in proportion to the amount of dissolved oxygen that was originally present in the sample.

4. **Titration with Sodium Thiosulfate:**

o The iodine released is then titrated with **sodium thiosulfate ($Na_2S_2O_3$)**, a reducing agent.

o The titration is done until the iodine color disappears, which indicates the end point of the titration.

o **Starch** is added as an indicator in the final stages of the titration to help identify the endpoint, as the iodine forms a blue complex with starch, which disappears when all iodine has been reduced by sodium thiosulfate.

5. **Calculation of Dissolved Oxygen (DO):**

o The volume of sodium thiosulfate solution used in the titration is directly related to the amount of iodine liberated, and this, in turn, is proportional to the amount of **dissolved oxygen (DO)** present in the sample.
o By knowing the normality of the sodium thiosulfate and the volume used, the DO concentration in the water sample can be calculated.
. The chemical reactions involved in the method are given below:

1. MnSO4 + 2KOH ® Mn (OH) 2 + K2SO4 (white ppt)

2. 2 Mn (OH)2 + O2 ® 2 MnO (OH)2 (Brown ppt)

3. MnO(OH)2 + 2H2SO4)2 + 3H2O

4. Mn(SO4)2 + 2 Kl ® MnSO4 + K2SO4 + l2

5. 2Na2S2O3.5H2O + l2 ® Na2S4O6 + 2NaCl + 10 H2O

6. 2NaN3 + H2SO4 ® 2HN3 + Na2SO4

7. HNO2 + HN3 ® N2 + N2O + H2O

3.3.6. Total Solids

Total Solids refer to the total amount of solid material (both organic and inorganic) present in a given volume of water or wastewater. This is determined by evaporating the water from the sample and measuring the remaining solid material.

- **Procedure**:

1. 20 ml of the water sample is placed in a clean, pre-weighed dish (such as a glass or porcelain dish).
2. The sample is then evaporated, typically by placing it in an oven at a temperature of **103-105°C** until all the water is evaporated, leaving behind the solids.
3. The dish with the residual solids is then weighed again.
4. The weight of the solids is determined by subtracting the initial weight of the dish from the final weight of the dish plus solids. This gives the **Total Solids** (TS) content.

3.3.7. Biological oxygen demand

Biochemical Oxygen Demand (BOD) test, which is widely used to measure the amount of oxygen consumed by microorganisms while degrading organic materials in water. This test is essential for assessing the level of organic pollution in water, which helps determine the pollution load and the efficiency of wastewater treatment.

The **BOD** test measures the amount of oxygen consumed by microorganisms as they break down organic materials in water over a specified incubation period (typically 5 days at 20°C). The test is based on the principle that organic substances require oxygen for microbial degradation. The more oxygen consumed, the higher the BOD, indicating a greater amount of biodegradable organic material in the water.

Procedure:

1. **Sample Preparation**:

o Collect a sample of water that is to be tested. For accurate results, at least **1.5 L** of the sample is required.
o The sample should be thoroughly mixed to ensure homogeneity before testing.

2. **Initial Dissolved Oxygen (DO) Measurement**:

o Before incubation, measure the initial **Dissolved Oxygen (DO)** of the sample using an **iodometric titration** method or a DO meter. This is the starting DO level.

3. **Incubation**:

o The sample is [19]then incubated at a fixed temperature, typically **20°C**, for a **5-day** period (this is known as the **5-day BOD test**). The purpose of the incubation is to allow microorganisms to degrade the organic material in the sample and consume oxygen.

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4. **Final DO Measurement**:
o After the 5-day incubation period, the final DO of the sample is measured again, using the iodometric titration method or DO meter.
5. **Calculation of BOD**:
o The **BOD** is determined by computing the difference between the initial DO and the final DO:

3.3.8. Chemical oxygen demand

The **open reflux method** is a widely used procedure for measuring the **chemical oxygen demand (COD)** of a water sample. COD measures the amount of oxygen required to oxidize organic matter with the help of a chemical agent in water, providing an indicator of the level of pollution in wastewater or natural water bodies.

In this method, organic matter in the sample is oxidized by **potassium dichromate ($K_2Cr_2O_7$)** in an acidic medium, often in the presence of a **silver sulfate (Ag_2SO_4)** catalyst. The oxidation reaction converts organic compounds into carbon dioxide (CO_2) and water (H_2O). The remaining excess potassium dichromate is then titrated to determine how much oxygen was consumed in the reaction.

Procedure:
1. **Sample Preparation**:
o 20 ml of the water sample is placed in a **refluxing apparatus** (such as a round-bottom flask).
o The sample is combined with a measured amount of **potassium dichromate** ($K_2Cr_2O_7$), which acts as the oxidizing agent.
o **Concentrated sulfuric acid (H_2SO_4)** is added to provide an acidic medium necessary for the oxidation reaction.
o **Silver sulfate (Ag_2SO_4)** is used as a catalyst to enhance the oxidation of organic compounds, particularly those that are difficult to oxidize.
2. **Refluxing**:
o The sample is heated to a boil and maintained under reflux conditions for a specific time (typically 2–3 hours). During this period, the potassium dichromate oxidizes the organic matter in the sample.
3. **Cooling**:
o After the refluxing period, [26]the solution is allowed to cool to room temperature.
4. **Titration**:
o The excess potassium dichromate that did not participate in the oxidation reaction is determined by titrated wiith ferrous ammonium sulphate

The dichromate consumed gives the oxygen (O2) required for oxidation of the organic matter. The chemical reactions involved in the method are as under:

- . 2K2Cr2O7 + 8 H2SO4 ® 2 K2SO4 + 2Cr2(SO4)3 + 8H2O + 3O2
- C6H12O6+ 6O2® 6CO2 + 6H2O
- Cr2O7-- + 6Fe^{++} + 14H+ ® 6Fe^{++} + 2Cr3^{+}+ 7 H2O

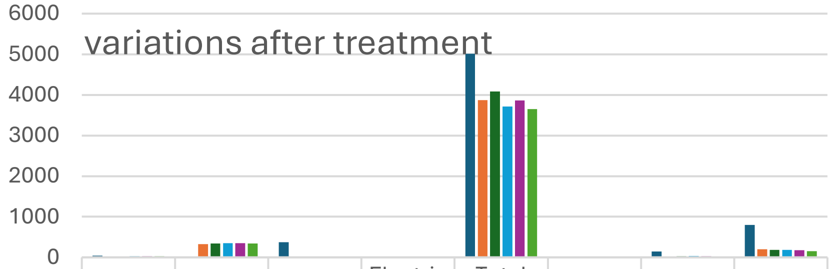
RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Initial Parameters

The initial parameters obtained after conducting various tests on the samples Table.4.1: Initial reading of the diary waste water sample

Sl.no	Parameters	Units	Initial values
1	Alkalinity	mg/L	44
2	pH	-	8.2
3	Hardness	mg/L	376
4	Electrical Conductivity	mg/L	1.5
5	Total Dissolved Solids	mg/L	5010
6	Dissolved Oxygen	mg/L	0
7	BOD	mg/L	145
8	COD	mg/L	796.6



	Alkalinity	Ph	Hardness	Electrical conductivity	Total dissolved solids	Dissolved oxygen	BOD	COD
Before treatment	44	8.2	376	1.5	5010	0	145	796.6
Treatment using duck weed	7.5	322.5	7.3	0.5	3870	0	22.5	197
Treatment using kariba weed	22	342.8	7.93	0.6	4085	0	28	183
Treatment using water hyacinth	27	351.7	7.62	0.6	3710	0	37	185.2
Treatment using water lettuce	30	348.6	7.93	0.7	3865	0	24.9	174.6
Treatment using azolla	23	340	7.3	0.4	3650	0	12	152

4.1.2 Alkalinity
In the Alkalinity test conducted for dairy wastewater it was observed that the sample with duckweed showed the best absorbing property

4.1.3 Hardness
In the Hardness test conducted for dairy wastewater it was observed that the sample with duckweed showed the best absorbing property.

4.1.4 PH
In the pH test conducted for dairy wastewater it was observed that the sample with duckweed showed the best absorbing property.

4.1.5 Electric Conductivity
In the EC test conducted for dairy wastewater it was observed that the sample with duckweed showed the best absorbing property.

4.1.6 Total Dissolved Solids
In the TDS test conducted for dairy wastewater it was observed that the sample with water hyacinth showed the best absorbing property

4.1.7 Dissolved Oxygen
In the DO test conducted for dairy wastewater it was observed that the entire sample showed a nill reading. This may be because of the high content of waste in the sample.

4.1.8 BOD
In the BOD3 conducted for duckweed sample in both dairy and domestic waste water, it was observed that first both sample is having a low BOD3 before treatment and it was observed that it is been then increased after the treatment.

4.1.9 COD
In the COD conducted for duckweed sample in dairy waste water, it was observed that first both sample is having a low COD before treatment and it was observed that it is been then increased after the treatment.

4.2. DISCUSSIONS
In this study, an investigation was conducted to assess the efficiency of various aquatic weeds—**water hyacinth**, **water lettuce**, **duckweed**, and **Kariba weed**—in treating **dairy wastewater** under controlled laboratory conditions. The main objective was to examine how these aquatic plants influence the physicochemical properties of dairy wastewater during a treatment period of **28 days**. The periodic changes in properties like **pH**, **alkalinity**, **conductivity** (EC), **total solids**, **dissolved oxygen** (DO), and **hardness** were analyzed to evaluate the treatment effectiveness.

1. **pH**:

o The study observed a significant decrease in **pH** values during the treatment period. Raw dairy wastewater was acidic in nature at the outset, and the phytoremediation process led to a reduction in acidity, bringing the pH closer to neutral.

o The reduction in pH is beneficial because it promotes microbial activity, which helps reduce **biochemical oxygen demand (BOD)** and **chemical oxygen demand (COD)** in the wastewater.

2. **Conductivity (EC)**:

o The **EC** values were initially high in the untreated effluent, indicating a high concentration of soluble salts.

o The presence of aquatic plants, particularly **duckweed** and **Kariba weed**, led to a reduction in EC, which was attributed to the absorption of salts by the root systems of the plants. This uptake of ions and salts likely improved the water quality.

3. **Turbidity and Total Solids**:

o **Duckweed** was found to be the most effective in reducing the **turbidity** of dairy wastewater compared to other plants. This could be due to the plant's fine root structure, which has the ability to trap both coarse and fine particulate matter.

o **Total solids** (TS) are a significant indicator of the quality of water. A decrease in total solids was noted in the treated samples, which indicates an improvement in water quality. This reduction is likely a result of the filtration and absorption capabilities of the aquatic plants.

4. **Hardness**:

o **Total hardness** was significantly reduced in the presence of **Azolla** compared to other plants. Hardness is often related to the presence of calcium and magnesium ions, which can be absorbed by the plants. A reduction in hardness means a decrease in these ions, reflecting an overall improvement in the water's suitability for other uses.

5. **TDS (Total Dissolved Solids)**:

o A decrease in **TDS** was observed in the treated samples, indicating a reduction in the dissolved salts and minerals in the wastewater. This reflects the effectiveness of the aquatic plants in improving water quality through the uptake of dissolved solids from the effluent.

6.BOD

The sharp decrease indicates the efficient uptake of organic compounds by azolla.Reduction of biodegradable matter showing improved water quality

7.COD

The substantial reduction suggests azollas capability to directly absorb or aid in the degradation of a wide range of organic compounds.

The study demonstrates that hyd**rophytes** like **water hyacinth**, **water lettuce**, **duckweed**, and **Kariba weed** are highly efficient in treating **dairy wastewater**. Key improvements were observed in various physicochemical parameters, especially **pH**, **conductivity**, **hardness**, **total solids**, and **TDS**. Among the plants tested, **duckweed** emerged as particularly effective for reducing **turbidity**, while **Azolla** showed the highest efficiency in reducing **hardness**. These findings suggest that phytoremediation using aquatic plants can be a sustainable and effective approach to wastewater treatment, contributing to the improvement of water quality in dairy and other industrial effluents.

CONCLUSION

Among the tested species Azolla demonstrated the highest phytoremediation potential for dairy wastewater achieving maximum reductions in BOD,COD,TDS and hardness while Azolla achieved the most substantial reductions in organic load due to its high surface coverage and nutrient uptake efficiency. The study confirms that **phytoremediation** using floating aquatic plants is an economically viable method for wastewater treatment. It is low-maintenance and can enhance **biodiversity** in the treated ecosystems.

Various researchers have explored the use of plants like water hyacinth, water lettuce, duckweed, and Kariba weed for wastewater treatment**.** However, the effectiveness of these plants in contaminant removal varies depending on factors such as climate, contaminants' concentration, temperature, and plant growth rate. Moreover, the **hydraulic retention time** and the specific type of plant selected significantly influence the plant's efficiency in contaminant removal.

While the efficiency of contaminant removal may differ among plants, **Azolla** stands out as a highly promising candidate for **phytoremediation** due to its ability to grow rapidly across a wide range of pH levels and its tolerance to cold temperatures, which allows it to be cultivated throughout the year. Additionally, **Azolla** has the unique capability of accumulating heavy metals more effectively than other aquatic plants, making it an excellent option for phytoremediation of polluted water bodies. This study suggests that **Azolla** can be a valuable tool for wastewater treatment, particularly for **heavy metal removal**, and could be instrumental in further research and **phytoremedial approaches** to address water pollution. Thus, **Azolla** proves to be a highly efficient, sustainable, and cost-effective solution for dairy wastewater treatment and could potentially be applied in large-scale environmental clean-up initiatives.

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