Development of Integrated Biological Reactor for the Treatment of Pulp and Paper Industry Effluent

By

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A thesis submitted in partial fulfillment of the requirements for the

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In

MICROBIOLOGY

By

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بسم الله الرحمن الرحيم

IN THE NAME OF ALLAH, THE MOST BENEFICENT, THE MOST MERCIFUL

Allah said in His Book:

«We made from water every living thing» (Anbiyya' 30)

«Say (O Muhammad): Tell me? if (all) your water were to sink away, who then can supply you with flowing water» (Mulk 30).

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List of Abbreviations

ABR	Anaerobic baffled reactor
BOD	Biological oxygen demand
BL	Black liquor
CFU	Colony forming unit
COD	Chemical oxygen demand
DO	Dissolved oxygen
EC	Electrical conductivity
EMB	Eosin metylene blue
EPS	Exopolysaccharide (matrix)
FBR	Fixed biofilm reactor
FC	Final clarifier
FTIR	Fourier transform infrared spectroscopy
GDB	Gravity driven bioreactor
HRT	Hydraulic retention time
IC	Intermediate clarifier
IVFCW	Integrated vertical flow constructed wetland
KgCOD/m^3/day	Kilogram of COD per cubic meter per day
Sp	Species
MPN	Most probable number
Conc.	Concentration
NA	Nutrient agar
PC	Primary clarifier
DNA	Deoxyribonucleic acid
IR	Infrared
OD	Optical density
Hr	Hours
PPI	Pulp and paper industry

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All praises to **Almighty Allah,** the Light of Heavens, and Earths, **The One** Who puts good thoughts in one's mind, turns them into determinations and then makes the way towards their fulfilment, showering all His Blessings throughout the journey. Best of the praises and Peace be upon all the Sacred Messengers, especially for the Last of them, **Hazrat Muhammad (SAWW),** who is the minarets of knowledge for all humanity.

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As time and calendar, as violin and melody, as life and what lies beyond the last breath, me and **my family.** It is impossible to start, to acknowledge. Words fail to express the intensity of my gratitude to my loving **father, *Abdul Sattar*,** whose prayers and inspiration gave me the courage to dream high and opened avenues for me to explore my abilities; my sweet and loving mother, ***Fatima***, whose self-sacrificing love will always be appreciated but can never be repaid—caring sisters and encouraging brothers ***Malik Habib Ullah (advocate)*** and ***Dr Shafqat Rasool*** for their extreme support and caring attitude.

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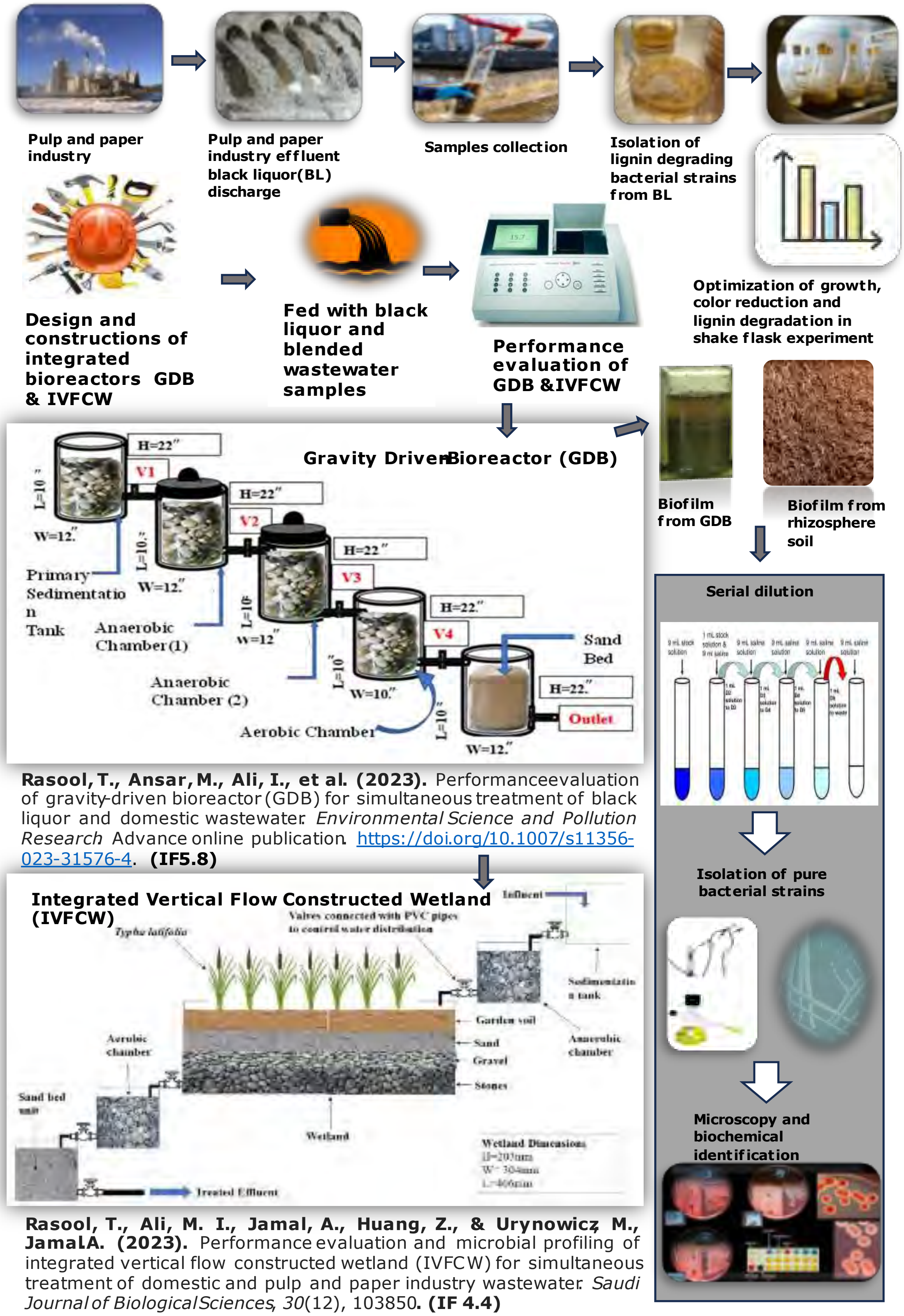
Tabassum Rasool

Graphical abstract



Abstract

Amidst rapid industrialization, like pulp and paper manufacturing, the resultant wastewater emerges as a pressing environmental contaminant. Effective treatment of wastewater generated by pulp and paper industry (PPI) is important to adhere to the stringent national environmental quality standards and to safeguard the ecosystem. In the present study, an integrated biological treatment system was designed for the PPI wastewater (black liquor, BL). The research was conducted in four phases. During the first phase, physicochemical characterization of the BL was carried out using different analytical methods. It was found that BL exhibited a pH of 8.2±0.15 with high chemical oxygen demand (COD) of 1750.56±35.50 mg/L and biological oxygen demand (BOD) of 1513.53± 32.35 mg/L. The other parameters such as sulfate, phosphate, total dissolved solids (TDS), total suspended solids (TSS), total nitrogen (TN) and lignin content were 4.2.21±0.117 mg/L, 3571.455±48.23 mg/L, 1890.411±57.28 mg/L, 160.911± 4.48 mg/L and 189.33±6.53 mg/L respectively which were beyond the permissible limits of EPA. In the second step, the bacterial strains were isolated from the BL samples using enrichment technique in MSM BL amended with 2 % lignin. Out of thirteen bacterial strains, three bacterial strains (TR-1, TR-2, & TR-3) were selected for their higher adoptability and lignin degradation traits. The optimum metabolic performance of these strains was found to be between pH (7-9) and temperature (37-41^{o}C). These bacterial TR-1, TR-2, and TR-3 were biochemically identified as *Bacillus subtilis*, *Bacillus cereus* and *Pseudomonas* respectively.

In the second phase of the research, two different bioreactors were designed i.e., gravity-driven bioreactor (GDB) and integrated vertical flow constructed wetland (IVFCW), to evaluate their performance for the treatment of black liquor. Both GDB and IVFCW were operated with an organic loading rate (OLR) of 1750 mg-COD/L-Day, flow rate of approximately 12 mL/minute, and HRT of 72 hours for ten samples (S1-S10) with a frequency of one sample per week under the natural temperature of workstation (35-40°C). The average results showed that the GDB achieved promising treatment efficiencies: 73.50% for COD, 67.46% for BOD_5, 177 times increase in dissolved oxygen content, and 54.31%. for TDS, 92.29% for TSS, 57.11% for EC, 54.39% for PO_4^{3-}, 53.94% for SO_4^{2-}, 55.87% for TN, and 87.99% for turbidity levels. Additionally, about a 51.35% reduction in lignin content was observed and the average change in pH was 11.66% after the treatment. Similarly, the average results showed that the IVFCW achieved a notable treatment efficiency: 76.50% for COD, 73.76% for BOD5, 188-time increase in dissolved oxygen content, 66.4%. for TDS, 100% for TSS, 67% for EC, 54.7% for PO_4^{3-}, 60.6522% for SO_4^{2-}, 72.9% for TN, and 88.4% turbidity levels. Likewise, a 59.88% reduction in lignin content was observed with 5% change in pH after the treatment through IVFCW.

In phase three, the treatment efficacy of GDB and IVFCW was improved using black liquor blended with domestic wastewater. The GDB and IVFCW were operated and maintained at an average organic loading rate of 1235 mg-COD/L-Day, at a flow rate of approximately 12 mL/minute and hydraulic retention time of 72 hours, for twelve samples (W1-W12) at a weekly frequency. The entire process was conducted within the workstation's ambient temperature range of 35-45°C from June 2022 to September 2022. The performance of the both reactors were evaluated in terms of various pollution indicators, including COD, BOD_5, lignin removal, TDS, TSS, EC, PO_4^{3-}, SO_4^{2-}, microbial load (CFU/mL and MPN index), total nitrogen and color reduction. The results showed that the GDB achieved promising treatment efficiencies: 84.5% for COD, 71.80% for BOD_5, 82.8% for TDS, 100% for TSS, 74.71% for E.C., 67.25% for PO_4^{3-}, 81% for SO_4^{2-}, and 69.36% for TN. Additionally, about 80% reduction in lignin content and 57% color reduction were observed after the treatment. The GDB substantially reduced microbial load in CFU/mL (77.98%) and MPN (90%). Similarly, the IVFCW were proved more promising in removing different contaminants, including total suspended solids (TSS) and total dissolved solids (TDS), with an estimated efficiency of 100% and 83%, respectively. Other parameters such as COD and BOD declined by 80% and 81%, respectively. Further to this, the IVFCW reduced sulfates (SO_4^{-2}), phosphates (PO_4^{-3}), and total nitrogen by 81%, 63%, and 61%, respectively. The treatment has led to lignin degradation up to 83%. The microbial load was also downsized to CFU/mL (67.98%) and MPN (93%). In the last and forth phase, the microbial profiling of aerobic biofilm developed on stone media in GDB and rhizosphere soil of *Typha latifolia* roots in IVFCW confirmed the presence of different bacteria genera.

The present study highlights the effectiveness of GDB and IVFCW, showed efficiency, in terms of lignin degradation, up to 83% and 81%, respectively and proved to be efficient in terms of removing COD, BOD and other pollutants from the pulp and paper industry effluent up to permissible limits approved by EPA. Therefore, GDBs and IVFCW have the potential for large-scale application for wastewater treatment as a cost-effective, environmentally friendly and efficient solution.

Publications

1. **Rasool, T., Ali, M. I., Jamal, A., Huang, Z., & Urynowicz, M., Jamal.A. (2023).** Performance evaluation and microbial profiling of integrated vertical flow constructed wetland (IVFCW) for simultaneous treatment of domestic and pulp and paper industry wastewater. *Saudi Journal of Biological Sciences*, *30*(12), 103850**. (IF 4.4)**
2. **Rasool, T., Ansar, M., Ali, I., *et al*. (2023).** Performance evaluation of gravity-driven bioreactor (GDB) for simultaneous treatment of black liquor and domestic wastewater. *Environmental Science and Pollution Research.* Advance online publication. https://doi.org/10.1007/s11356-023-31576-4. **(IF5.8)**

CHPTER 1

INTRODUCTION

1. Introduction

The availability of freshwater is linked to the dynamic processes of global water and biogeochemical cycles. The protection of natural water reservoirs is important due to escalating demands from the various sectors and increasing threats posed by pollution. Globally, the contamination of water resources is increasing, making huge volumes of water unsuitable for consumption. For example, a mere gram of heavy metal contamination can render 100,000 liters of water undrinkable (Buheji *et al*., 2023). Addressing water quality is not only inevitable for the sustainability of ecosystem but also important for the UN sustainable development objective 6 aims to guarantee universal access to water and sanitation underscore the urgent need for wastewater treatment initiatives, water recycling and participation in carbon credit scheme, to reduce the adverse effects of industrial operations on the environment (Cárcamo *et al*., 2022). At present, the global community faces a dual challenge: adequate supply of safe water and safeguard ecosystem from irreversible damage. The prevalence of water born, or water related illnesses further highlights the urgency and implementation of an effective wastewater treatment initiative (Qamar *et al*., 2022). Considering the socio-economic factors, public health and environmental conservation, collaborative efforts are required to ensure access to clean water. Recognizing the importance of wastewater treatment in this context is pivotal, underscores the need for sustainable, environmental friendly and technologically viable solutions to ensure water availability for current and future generations (Santos *et al*., 2023).

The ongoing disruption of the global water cycle presents a pressing challenge, impacting human societies and ecosystems alike (Mishra *et al*., 2023). The challenges are intricately linked to the escalating human population, urbanization, and intensified industrial activities. It has been highlighted in various international forums that the population increase has caused severe gaps in the demand for various commodities, with water at the top of the list (Rasool *et al*., 2018). The untreated wastewater, particularly in underdeveloped nations, is directly thrown into the freshwater bodies, leading to the deterioration of existing natural water reservoirs. The addition of contaminated water into the rivers, lakes and streams is causing biodiversity losses and acclamation of the containments in water, soil, plant, and animal tissues (Naz *et al*., 2017). This contaminated wastewater also causes serious damage to the agricultural soils and percolates into deeper soil layers carrying organic and inorganic containments, thereby damaging the underground water reservoirs. It is essential to mention that most countries rely on underground water for drinking. On the other side, this wastewater also has a severe, negative impact on the ecosystem, resulting in climate change, global warming, resource depletion, a decrease in agriculture yields, access to safe water and water scarcity (Rehman *et al*., 2022).

In Pakistan, about 4.43 billion cubic meters of wastewater being produced from domestic and industrial activities (Ali *et al*., 2018). This toxic wastewater discharges into the environment, leading to a number of ecological crises, including climate change, soil deterioration, species loss, and accumulation of inorganic and organic contaminates into food and agriculture products. Pakistan is facing severe challenges in the safe disposal of domestic and industrial wastewater due to a lack of treatment plant installations, unavailability of technical resources, reliance on forging technologies, energy, and economic crisis. These limitations make the conditions more adverse and demand cost-effective, environmentally friendly and efficient water treatment systems to manage domestic and industrial waste effluents. The myriad of factors discussed have led to a significant shift in Pakistan's status from a water surplus to a water-deficient country (Munir *et al*., 2022). This transformation intensifies water scarcity and inflicts environmental damage, contributing to the broader consequences of climate change. Addressing this multifaceted challenge necessitates urgently developing a sustainable, economically feasible, and ecologically viable indigenous wastewater treatment process to ensure the continued availability of safe water resources.

There are two main factors for water scarcity, i.e., increased usage of freshwater and contamination of the existing water reserves by domestic and industrial waste. The increased water consumption is primarily because of increased human population and industrialization. By 2025, water availability is projected to decrease to 700m^3 per capita, much below the worldwide guideline of 1500m^3 per capita (Martin *et al.,*2006). Another important reason for water scarcity in Pakistan is water pollution, as country is facing the deterioration of groundwater and freshwater reservoirs. The reason for quality deterioration is the discharge of untreated wastewater into the environment. In Pakistan, about 1.43x109m^3/year of untreated domestic wastewater and about 0.34x109m^3/year of untreated industrial effluent are directly discharged to nearby rivers (PWS, 2002). Similarly, 2000 million gallons of untreated sewage are directly discharged to surface bodies daily, which causes contamination of natural water resources, resulting in severe health, environmental and socio-economic concerns (Martin *et al.,*2006). The composition of industrial wastewater varies and depends on which industry it is produced. For example, the tannery industry contains a higher concentration of heavy metals than pharmaceutical industry effluent, which contains a higher concentration of drug precursors. Likewise, the PPI produces wastewater of dark brown color and a higher lignin concentration (Khan *et al.,*2022).

The PPI industry is among the largest sectors globally. Global production of paper and related products is about 390 million tons (Kumar & Verma, 2023) and Pulp and paper market size 2022 to 2023 is shown in Figure 1.1 This industry is one of the significant water resource industries ranked 3rd among the most water-consuming industrial units. According to Steephen *et al*. (2023), the production of 1 ton of pulp requires around 60 m^{3}of water. Pakistan is home to 100 industrial facilities dedicated to pulp and paper production, collectively capable of producing 650,000 tonnes per year (Munir *et al*., 2022). The PPI produces a type of wastewater known as black liquor (BL), which consists of lignin, cellulose, hemicellulose, alcohols, chlorates, heavy metals, sulphates, and inorganic salts. BL is composed of 15% solids, with 10% being inorganic chemicals and 5% being organic chemicals. The composition of organics in BL consists of around 40-45% soaps, 35-45% lignin, and 10-15% additional organic compounds, as reported by Wang *et al*. (2022).

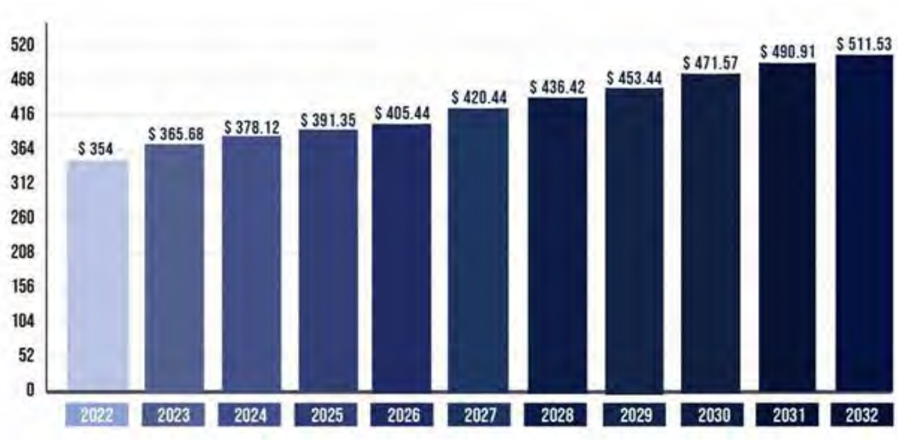


Figure 1.1 Pulp and paper market size 2022 to 2023 in USD Billion. (source: www.precedence research.com).

In Pakistan, most of this wastewater (industrial and domestic) is discharged to the sewage system, a nearby field, or an open drain without any treatment (Amen *et al*.,2020). It results in the pollution of natural water bodies and increased utilization of fresh water. Only a limited number of wastewater treatment plants (WWTPs) are present in major cities, and these plants are responsible for treating around 8% of the whole wastewater (Martin *et al.,*2006). However, an established provision for reusing treated water still needs to be established. There is no concept of WWTP's at the secondary and tertiary levels in the country (UN Water, 2008). The lack of wastewater treatment technology is due to additional operational costs, maintenance of the WWTP, and low performance of the existing wastewater treatment technology (Azizullah *et al.,*2011). Therefore, the most crucial aspect to consider when choosing a suitable WWTP in technologically less advanced nations is price. There is an urgent requirement to create cost-effective solutions for wastewater treatment in developing nations in order to guarantee environmental sustainability and safeguard human health.

Several physical and chemical techniques have varying degrees of effectiveness for wastewater treatment (Ali *et al.,*2012). However, these physicochemical methods have certain environmental and economic disadvantages (USEPA, 2000). At the same time, there is a growing interest in biological wastewater treatment methods because of their cost-effectiveness, ease of use, and lack of negative impact on the environment.. In biological wastewater treatment technologies, the key players are microbes, which are used in two reactors: suspended growth and attached growth bioreactors (Verma *et al.,*2006). In suspended growth bioreactors, oxidations ponds are environmentally friendly, cost-effective, and simple in operation, mainly involved in removing organic contaminants from the wastewater (Portela *et al.,*2022). However, oxidation ponds require a continuous supply of oxygen/air, extensive land area, and prolonged retention time. Furthermore, these ponds produce a vast quantity of solid sludge which requires proper disposal (Das *et al.,*2022). Activated sludge is another essential method of biological treatment of wastewater. This process not only removes carbonaceous contaminants but also removes suspended solids and pathogenic microorganisms (Kim *et al.,*2010). Nevertheless, the effectiveness and productivity of this procedure are susceptible to variations in retention time and other physicochemical parameters (Wu *et al.,*2023). Furthermore, settling suspended solids may require treatment costs; otherwise, the discharge of a large amount of solid particles in water can potentially endanger the aquatic ecosystem downstream and public health (Das *et al.,*2022).

In attached growth bioreactors, microbes attach to some solid surface or a packing media and form biofilm by secreting different polymeric substrates (Loupasaki & Diamadopoulos, 2013). Packing media of different materials such as polystyrene, plastic cubes, stones, or pebbles can be used, and these materials act as biofilters in the bioreactor (Dey & Raghuwanshi, 2021). The criteria for selecting a particular packing or filter media type depends on several factors. It must have a large surface area, be readily available across the globe, resistant to decay by microbial communities, be inert, and provide attachment sites to the microbial communities (Rehman *et al.,*2021). Attached growth bioreactors encompass several types such as trickling filters and bed biofilm bioreactors (Akratos *et al.,*2020). These attached growth bioreactors have some advantages over suspended growth bioreactors like these bioreactors provide high retention time because of high active biomass; thus, they can deal with wastewater of high organic loading rate (Rehman *et al.,*2021).

Furthermore, these bioreactors require low space, less operational or operational cost, and less energy requirement (Ali *et al.,*2017). These advantages make these bioreactors attractive for treating varying natures of wastewater. However, these bioreactors have drawbacks, like clogging due to prolonged feeding at a high organic loading rate (Naz *et al.,*2017). Thus, it may require time-to-time washing or replacement of the filter media. In attached growth bioreactors, the biofilm plays a key role as they have an enhanced role in the biodegradation reaction with a diverse and dynamic population of microbes (Deng *et al.,*2022). Therefore, solid media provides better attachment sites in attached growth bioreactors, accelerating microbial concentrations and contaminant degradation. The biofilm contains a wide range of microorganisms, which allows for operational flexibility, increased efficiency in situations with limited hydraulic retention time, resilience to changes in the external environment, high concentrations of active biomass, and less sludge production due to the slower growth of microbes (Arya *et al.,*2016). The biofilm in the attached growth bioreactors involves various complex mechanisms for the removal of the contaminants, such as biomineralization, biodegradation, bioaccumulation, and biosorption (Harb *et al.,*2016).

Among cost-effective, eco-friendly, and attached growth wastewater technology is constructed wetlands (CWs) (Thamke *et al.,*2021). CWs are artificial with vegetation and are used to remove organic content from wastewater. They offer cost-effective water treatment solutions with a focus on maintaining excellent quality. Due to their engineered nature, they utilize natural biodegradation processes efficiently by utilizing microbial inhabitants of plant root and substrate interactions (Sudarsan *et al.,*2015). In CW's wastewater is treated by different mechanisms, including physical processes (precipitation, filtration, and adsorption), biochemical processes (nitrogen cycle, carbon cycle, etc.), and biological processes (plants root uptake mechanism and microbial degradation) (Sayadi *et al.,*2012).

Constructed wetlands (CWs) are classified into two primary categories: free-water surface systems and subsurface flow systems (SFCW). The SFCW can be categorized into two types: vertical subsurface constructed wetlands (VSFCW) and horizontal subsurface flow constructed wetlands (HSFCW), depending on the direction in which water flows. In vertical flow, the enhanced aerobic conditions favor nitrification and inhibit excessive denitrification. These systems have the capacity to achieve a maximum removal of pollutants (Tanner *et al.,*1995). Such systems are efficient for removing phosphorus due to excessive phosphorus uptake by plant roots and removing TSS due to efficient filtration through the filter media. Inaccessible water, surface wetlands are shallow basins, having plantation and firm edges to prevent overflow of water (Dunne *et al.,*2012). They are primarily used for secondary or tertiary treatment units to improve water quality. These wetlands have another advantage of a higher growth rate of plants with an extensive surface for attaching microbes (Abdelhakeem *et al.,*2016).

Though CW's offers the potential to treat wastewater of different origins, they cannot withstand all types of wastewaters (Vymazal, 2013). In order to avoid choking or destruction of wetlands, influents sometimes need a pretreatment. This pretreatment also influences the quality of the final treated water. To remove scum and sludge from wastewater, the pretreatment process can be utilized (Ali *et al.,*2018). In combined sewage systems, a grit chamber often removes sand and soil particles from the influent. Pretreatment chambers physically removing the containments also require regular cleaning and monitoring to produce high-quality treated wastewater (Saif *et al.,*2021). Pakistan, which used to have an excess of water, is now classified as a water-stressed country. It requires a wastewater treatment system that is ecologically sound, economically viable, and environmentally friendly to handle both industrial and domestic wastewater. Previous research has yet to be reported for the Concurrent treatment of household wastewater and industrial wastewater (pulp and paper) effluent. The present study focused on the design, construction, and performance evaluation of a suitable biological system for treating pulp and paper industry effluent blended with domestic wastewater. In the present study, two different models, i.e., gravity-driven bioreactor (GDB) and integrated vertical flow constructed wetlands (IVFCW) were designed, constructed, and their efficiencies were monitored.

Significance and national relevance of the research

PPI is one of the largest industrial sectors of Pakistan with about 100 units scattered in different locations of Pakistan, as depicted in Figure 1.2. On the opposite side, this industry is also rated as a major contributor to environmental pollution because its effluent contains hundreds of different contaminants. The one site treatment of pulp and paper industry demands huge capital investment, foreign based technologies, and electricity to operate the treatment plants. Because of these limitations and the loss of the grip of environmental regulations, most of the black liquor is thrown out without any treatment. In addition, this waste stream mixes with domestic wastewater and causes severe environmental pollution and damage of natural water resources and soil. Considering these pertinent issues, development of cost effective, efficient, ecofriendly, indigenous and easy to operate treatment systems is a need of the time.

Figure 1.2 Locations of pulp and paper industry across Pakistan

The present work was an attempt to design and optimize different integrated systems having capability to treat BL alone and in combination with domestic wastewater. It is likely that providing comes of the present research would be a cost effect, self-driven, energy independent, easy to operate and ecofriendly integrated biological treatment system to cope some of the important challenges of Pakistan. Further, these bioreactors will enable recycling and protection of the natural freshwater reserves.

Aim and objectives.

The present study is designed considering the local conditions of Pakistan, i.e., electricity/power shortage and industries close to the urban population. There is a need to develop a cost-effective, energy-independent, and in-situ treatment facility to treat pulp and paper industry effluent. The aim of present study is development of integrated biological reactor for the treatment of pulp and paper industry effluent and specific objectives are:

1. Physicochemical analysis of wastewater and characterization of lignin degrading bacterial strains isolated from black liquor.
2. Design, construction, and performance evaluation of lab-scale gravity-driven biological reactor (GDB) and integrated vertical flow constructed wetland (IVFCW) for the treatment of paper and pulp industry effluent (black liquor)
3. Efficiency monitoring for GDB and IVFCW for the treatment of black liquor blended with domestic wastewater.
4. Study of biofilm developed on stone media in GDB and rhizosphere of IVFCW.

CHPTER 2

LITERATURE REVIEW

2. Literature review

Paper and pulp industry (PPI) is among the most growing industrial sectors of Pakistan owing to the increasing use of different commercial products such as paper, packing materials and chip boards. There are about 100 industrial units with 1 million employes and significant contribution in the national GDP (Munir *et al.,*2019). Most importantly, PPI is a resource intensive sector and consumes huge amounts of fresh water, different chemicals during the pulping process and energy for different operations. To produce one ton of the final product, about 250 – 300 m^3 of water is required during different steps (Chaudhry & Paliwal, 2018). Once water is discharged from this industry, the effluent constitutes lignin-based compounds in excess, such as chlorinated lignin, chlorinated organic acids, and metal ions. If left untreated, black liquor can have severe consequences, including water pollution, ecosystem disruption, and health hazards. Therefore, effective treatment processes are necessary to mitigate these risks and ensure sustainable practices within the industry. This study contributes to understanding treatment technologies, particularly microbial treatment of BL, promoting environmental protection and sustainable industrial practices.

2.1.Pulp and paper industry (PPI).

PPI is widely acknowledged as the preeminent and oldest in the world. The paper began to be utilized daily at about 3000 B.C., particularly in the ancient Egyptian era. The Egyptians employed papyrus manufactured from Nile reeds to create scrolls and records that preserved their learning and civilization for future generations. This early innovation established the groundwork for the paper and pulp industry's long and illustrious history. Nowadays, the pulp and paper industry reach an impressive 100 metric tons per day, underscoring its substantial scale and influence within the global industrial landscape (Sharma *et al.,*2014). According to the International Energy Agency (IEA), the pulp and paper industry is the third largest consumer of water, behind the textile industry. Additionally, it is the third largest consumer of energy globally. This industry consumes a significant 6% of the worldwide energy supply while emitting around 2% of global carbon dioxide emissions (Quadrelli & Peterson, 2007). Such numbers highlight the industry's critical role in the global energy landscape and its environmental impact.

2.2.Pulp and paper industry (PPI) in Pakistan

Approximately one hundred divisions of the PPI are operational in Pakistan. with a total annual water consumption of around 650,000 tons (Munir *et al*.,2019). Wastewater containing different contaminants from pulp and paper manufacturing facilities is released without treatment. A variety of contaminants, including organic and inorganic substances of exceptionally high intensity, get released directly into the environment. These pollutants are harmful in two ways: they harm the ecosystem by polluting receiving bodies of water, and they harm human health (Liu *et al.,*2011; Munir *et al.,*2019; Erhardt *et al.,*2021). In Pakistan, the agriculture sector provides a large portion of the raw materials used by the PPI, accounting for 46%, other sources for paper production consist of 29% imported pulp and 10% derived from wastepaper, rice straw, cotton linter, and wheat straw (Shabbir & Mirzaeian, 2016). However, analyzing the distribution of these paper products is critical, with 4% corresponding to kraft products, 32% corresponding to writing papers, 61% corresponding to the board, and 3% corresponding to various other specialist products. This massive industrial setup needs much electricity, much water for process parameters, a lot of raw materials, and many people. Energy consumption related to the PPI accounts for 7% of overall energy consumption in Pakistan. It is worth noting that the industry supports various pulp and paper enterprises across the country, actively employing approximately 30% of young people (Munir *et al.,*2019).

2.3.Black liquor treatment processes

Black liquor is a complex and significant waste produced during the pulping process and contains a wide range of inorganic and organic contaminants. Its treatment and disposal continue to concern the environment, demanding the development and execution of suitable treatment techniques. This chapter thoroughly overviews the various physicochemical and biological treatment procedures used for black liquor remediation, offering insight into their mechanisms, advantages, and limitations. In recent decades, environmental regulations have become increasingly stringent, compelling the PPI to seek innovative and sustainable solutions for managing black liquor waste. Physicochemical treatment methods include various techniques such as coagulation and precipitation, electrocoagulation, adsorption, and membrane technologies. Biological treatment processes, on the other hand, use plants, algae, or microorganisms to degrade the organic fraction of black liquor, resulting in less negative environmental impact of black liquor. A comprehensive examination of these processes can lead to a more environmentally friendly and productive future for the PPI (Smith *et al.,*2020; Johnson and Brown, 2018; Patel and Jones, 2019).

2.3.1. Physico-chemical methods for black liquor treatment

Different methods are used to remove lignin from the BL. These processes include coagulation, precipitation, adsorption, advanced oxidation, and membrane-based technologies.

2.3.1.1.Coagulation and precipitation

PPI effluent treatment process via coagulation and precipitation involves using metal salts are added to increase the aggregation of smaller particles by enhancing interaction between them, ultimately resulting in more extensive flock formation. Salts are added to the steam of effluent while it flows out. To effectively treat wastewater, Wang *et al*. (2011) utilized starch graft and $AlCl_3$ as flocculants and coagulants, respectively. The study's outcomes stated that the best conditions for removing lignin and water recovery occurred at pH 8.3 with flocculant dosages of 22.3 and 871 mg/L of coagulant. According to Li *et al*. (2018), pre-treatment of corn stover using metal catalysts such as NiO, MgO, CuO, Fe2O3, and ZnO reduced sugar depletion and increased lignin removal. For control purposes, liquid hot water (LHW) was used to cure corn stover. Among metal oxides, MgO is considered as better catalyst for biomass pre-treatment. The benefit of this procedure is the reduction of a step which is washing the inhibitor or acid, as no by-products are formed. In addition, such processed liquids (biomass) can directly undergo hydrolysis and fermentation.

A significant quantity of sludge production is an unfortunate consequence of treating BL with coagulation and precipitation. Getting rid of this sludge can be challenging and might cost more to cleanse or dispose of (Tawfik *et al.,*2022). Furthermore, if heavy metals are not adequately handled, using metal salts as coagulants may introduce heavy metals into wastewater, raising certain environmental concerns (Jagaba *et al.,*2022). Finally, differences in the properties of the effluent can affect the effectiveness of coagulation and precipitation, necessitating modifications to the treatment process that would reduce its consistency (Esmaeeli *et al.,*2023).

2.3.1.2.Electrocoagulation

Approaches based on physicochemical methods are more practical and feasible on larger scales. This is due to the increased need to treat water and effluent remediation through electrochemical methods (Soloman *et al.,*2009). In the electrocoagulation process, a soluble anode is suspended in the effluent for the treatment. Once the process begins, floc formation results in effluent due to dissolving of metal hydroxides on the soluble anode. A clear distinction from other procedures, electrocoagulation is considered a more practical and workable electrochemical approach (Lewis *et al.,*2013). According to Ugurlu *et al*. (2008), aluminum electrodes have more incredible BOD and lignin removal capacity from the BL. Shankar *et al*. (2013) also utilized the same electrode to remove lignin and observed the similar results. Electrocoagulation works well to clean wastewater from the pulp and paper sector, but it has some significant drawbacks. The procedure requires significant electrical energy, which raises operating expenses, especially for large-scale applications. The electrocoagulation process's electrodes deteriorate with time and require frequent maintenance or replacement, raising overall costs (Das *et al.,*2022). By-products from this process, including sludge and leftover metals from the electrodes, may also be produced; these must be carefully managed and disposed of to prevent environmental problems. Maintaining the ideal pH level in the effluent during electrocoagulation can also be a challenging process that frequently calls for extra chemicals, adding to the complexity of operations and expenses (Sahu *et al.,*2014; Kuokkanen *et al.,*2013).

2.3.1.3.Adsorption

Adsorption techniques are commonly employed for efficient removal of chemical substances and coloration from the BL. Pokhrel & Viraraghavan (2004) and Kamali & Khodaparast (2015) demonstrated acceptable color and lignin removal BL. The adsorption onto charcoal, activated carbon, coal ash, and fuller's earth removes 90% of the color (Murthy *et al.,*1991). Shawwa *et al*. (2001) reported adsorption on activated coke to eliminate 90% of color and COD from bleached wastewater. According to Das and Patnaik's study from 2000, lignin was removed by adsorption using a blast furnace, dust, and slag, achieving removal rates of 80.4% and 61% respectively. Another work by Xilei *et al*. (2010) utilized cost-effective adsorbent bentonite (450 mg/L) and poly aluminium silicate chloride (400 mg/L) for the purpose of eliminating 41.38% of color and 60.87% of COD via a tertiary adsorption and coagulation procedure. Adsorption presents a distinct set of difficulties in treating wastewater from the pulp and paper sector. Initially, the expenses associated with the operation might be high, mainly because vast amounts of expensive adsorbent materials are required, which can be problematic for large-scale operations (Duan *et al.,*2010). Secondly, the process becomes complicated and expensive when regenerating or disposing of wasted adsorbents loaded with pollutants unique to pulp and paper effluent (Pokhrel *et al.,*2004). Careful waste management is therefore required to prevent possible environmental problems. Furthermore, depending on the effluent's particulars, adsorption's efficacy may vary, making it less reliable and necessitating customized optimization for various effluents. Last but not least, the limited ability of adsorbents to adsorb specific compounds necessitates routine replacement or regeneration, increasing the maintenance and operating costs for BL treatment (Hou *et al.,*2020).

2.3.1.4.Membrane technologies

For decades, membrane-based technological methods have also been used for BL treatment. The expansion of this treatment method is not solely feasible due to technological constraints but also due to substantial manufacturing expenses (Greenlee *et al.,*2010). For their application on a larger scale and to maintain their effective water treatment, an extra step is required, which is to use pre-treatment techniques to enhance the potential performance of this system. According to Li and Zhang's (2011) study, flocculants can cut the COD in wastewater from PPI by up to 75%. BL lost color, COD, BOD, and TDS after membrane electrochemical reactor treatment, according to Chanworrawoot & Hunsom (2012). Sulphate, COD, total hardness and conductivity were measured using ultrafiltration membranes to treat BL at pH 10 (Gönder *et al*., 2012).

Integration of membrane technologies with other treatment procedures has shown great potential for treatment on a large scale. Membrane Bioreactor (MBR) technology is used, in which the membrane works as a physical barrier for solid separation, and reactors aid in the degradation of compounds. This integrated system resulted from the fusion of membrane units with bioreactors. Researchers have already documented using the MBR method to treat wastewaters from multiple origin (Izadi *et al.,*2018). MBR system has proven to have high potential by producing treated quality effluent, which is clarified widely. MBR technology-based treatments have advantages over other methods, resulting in less sludge production and membrane presence and solid separation (Neoh *et al.,*2016). The water treated through MBR can be reused in various agricultural and industrial applications (Krzeminski *et al.,*2017; Patel & Patel, 2020). MBR technology has a drawback of pore-clogging; it results in forming a foulant layer, jeopardizing the system's efficiency. Pore-clogging reduces the performance of membrane treatment, and solid separation is affected. Tiny pores allow low filtration capability, and due to this reason, it has considerable suction time. Both factors limit the functionality of the MBR system (Scholes *et al.,*2019). Integration of different systems with membrane technology has contributed to the stability of treatment, and routinely backwashing pores also solves the foulant layer problem. Qu *et al*. (2012) decolorize the effluent using a integrated MBR technology, reducing COD of 88.6 1.9 to 92.3 0.7%. Merayo *et al*. (2013) reported that MBR coupled with AOP was followed by ozonation to remove 90% COD from pulp mill effluent. In 14 hours, the sequence batch reactor, using a combination of bacteria, decreased the levels in the paper mill's effluent by 72.3%, BOD by 91.1%, color by 55% (Kumar *et al.,* 2014). The combined MBR-photo electrooxidation (MBR-PEO) approach was designated by Giacobbo *et al*. (2015) for treating tannery wastewater. They used the MBR-PEO reactor to reduce COD and BOD by 97% and 87.8%, respectively. More than 90% of metals can be removed by combining MBR with electrocoagulation, i.e., Zn and Cu (Vijayakumar & Balasubramanian, 2015). Fusion of the MBR system with advanced oxidation process and electrocoagulation methods aided in removing metals and recalcitrant, colored chemicals and solved the pore-clogging problem. Microbial bioreactors introduces additional carriers into the MBR system, reducing suspended solids concentrations, maintaining the efficacy of the process, and preventing the foulant membrane problem (Neoh *et al.,*2016). According to Gao *et al*. (2016), a submerged anaerobic membrane bioreactor removed 83% of COD from the pulping effluent (SAnMBR). Izadi *et al*. (2019) reported the FBMB reactor and achieved significant reductions in COD and nitrogenous compounds. Likewise, Izadi *et al*. (2020) created HAMB reactor and effectively decreased COD, ammonium, and total nitrogen. Poojamnong *et al*. (2020) reported a submerged polyvinylidene fluoride membrane bioreactor (MBR) achieved a 73% COD and 79% color removal efficiency in treating BL.

Although membrane technologies provide efficient ways to treat BL they also have some inherent drawbacks: i)They require frequent cleaning and maintenance, which can be time-consuming and expensive because they are prone to fouling and clogging because of the debris in the effluent, ii)because these procedures frequently call for high hydraulic pressures, raising energy consumption and operating costs, iii)membrane materials can be susceptible to mechanical and chemical harm, which would shorten their lifespan and necessitate replacement (Obotey *et al.,*2020; Zhou *et al.,*2006). The composition of the effluent can also affect treatment efficiency, making membrane technologies less consistent in their performance and necessitating changes for various effluent characteristics. This complicates the application of membrane technologies in BL treatment (Hubbe *et al.,*2016).

2.3.1.5.Ozonation

Ozonation is employed for the treatment of BL. Several studies enlist ozonation to remove color, harmful chemicals, and COD from industrial effluent (Yamamoto, 2001). Ozone-based water treatment lowers effluent toxicity, COD, and TOC (Yeber *et al.,*1999). Wastewater from bleached mill wastewater is treated with photocatalysis and ozone, reduces different pollutants (Torrades *et al.,*2001). A 95-97% loss of color is visible when ozonation is done for up to 15 minutes at a high ozone dose (Sevimli & Sarikaya, 2002). Manttari *et al*. discovered that ozonation and activated sludge treatment efficiently cope with nano-filtered effluents (2006). By increasing the ozone dosage from 800, 900, to 1100 mg/L, a more than 50% fall in turbidity was observed, lignin content was reduced, and color removal was observed. The application of ozone to treat BL resulted in an improvement of water quality by reduction in COD, BOD, and lignin (Ruas *et al*., 2007), but it was ineffective at removing color. According to Michniewicz *et al*. (2012), lignin degradation depends on the amount or dosage of ozone used to treat alkali lignin solution. At an ozone dose of 0.1 and 3.6 mgO3/mg COD, respectively, the lignin content is expected to be reduced by approximately 40 to 96.6%, corresponding to COD in the 8.8 to 69.6% range. Extensive exploration has been done by Hermosilla *et al*. (2015) and Kamali *et al*. (2019) on the treatment of BL using ozonation methods. Although ozonation is an efficient restorative procedure for BL, there are certain downsides. First, it can be costly because maintaining the equipment and producing ozone needs much electricity. Additionally, by raising capital expenses, the process can be needed to install complex ozone contactors (Covinich *et al*., 2014). The complex and unpredictable composition of BL can also affect the effectiveness of process, making its performance less predictable. Aldehydes and organic acids, two by-products of ozone therapy that may be hazardous, need extra processing or disposal. Additionally, ozone can be limited to removing color and some organic compounds, necessitating additional treatment procedures for complete effluent remediation (Hermosilla *et al.,*2015).

2.3.1.6.Advanced oxidation process

The usage of advanced oxidation processes has proved to be of excellent efficacy in eliminating the lignin and color along with other compounds from the effluent of the paper bleach industry when it is used BL treatment (Hermosilla *et al.,*2015; Abedinzadeh *et al.,*2018). This process is effective when microbes are unable to degrade compounds into simpler forms. They oxidize large and complex organic compounds in wastewater (Merayo *et al.,*2013; Lindholm-Lehto *et al.,*2015). Oxidation results in the formation of free radicals, which are highly reactive species, in this case, the generation of hydroxy radicals. These radicals are nonselective in their action and constitute the fundamental mechanism of AOPs (Al-Rasheed, 2005).

Wastewater treatment from the industrial sector is done by different methods, either physical, chemical, or biological methods (Azadi *et al.,*2016). Every method has some benefits and drawbacks as well. The compositional elements of wood are broken down into simpler forms by various natural processes, but they are not prone to degradation. It is because of the presence of xenobiotic chemicals. These chemical makes the biochemical treatment of such effluent difficult (Kalyani *et al.,*2009). Wastewater has been treated by using different methods and procedures. The removal of such compounds that give color to the water, chromophoric compounds, is done using absorbing media (Sell *et al.,*1994). For the reduction of total organic carbon (TOC), photocatalytic and ultrafiltration is used to reduce AOX by 99%. (Yao *et al.,*1994). For efficient removal of AOX, salt, color reverse osmosis, and nanofiltration techniques combined with membranes are used (Savant *et al.,*2006). On treatment with ozone, followed by the degradation process in a biofilm reactor, AOX, salt, and other substances are also reduced (Mobius & Helble 2004). Coagulation and flocculation are widely used procedures as they produce the suspensions of dissolved materials, which are later separated (Toczyowska-Mami'nska, 2017). The limitation of these procedures to be used at large scale is that they are costly. The physical, chemical, and electrochemical methods have high production costs. The coagulation method leads to the formation of substantial amounts of toxic sludge, which is unsafe to dispose of. Metals are added to remove or lower the toxicity, which impacts human health (Toczyowska-Mami'nska, 2017). Furthermore, these treatments are done at extreme pH. After treatment, they must be neutralized for safe usage. The pH is returned to the standard or neutral range to be recycled and reused (Hubbe *et al.,*2016; Bajpai, 2018). Oxidations are costly, and chlorine-based oxidation forms secondary pollutants that pose an environmental threat. Additionally, these physicochemical processes contribute to greenhouse gas emissions. Membrane fouling in membrane-based technologies contributes to the flow drop process (Lin *et al.,*2012). Therefore, procedures for eliminating dangerous substances must be developed that are affordable and environmentally friendly.

Table 2. 1Physicochemical methods for paper and pulp wastewater effluent treatment

Methods	Advantages	Limitations	References
Membrane Filtration	It involves several methods such as ultrafiltration, Nano-filtration and reverse osmosis.	High cost. Recurrent membrane fouling. Need for a pre-treatment method.	Valderrama *et al.,*2021
Adsorption	A range of adsorbents can be used.	Excessive maintenance costs. Safe disposal of the spent adsorbent. Need for pre-treatment methods.	Rashid *et al.,*2021 Duan *et al.,*2010
Coagulation	Very effective for Sulphur and dispersing dyes. Give better results in the reduction of colourization. Additives include: alum, lime, ferric chloride, and activated charcoal	Very in-effective for acidic, direct, reactive and vat dyes. Use of excessive chemicals. Disposal of sludge containing a huge quantity of reactive azodyes is problematic	Mehmood *et al.,*2019
Ultrasound	Reduce colour, turbidity, and chemical oxygen demand (COD) of effluent.	Unavailable disinfection power, Maintenance/replacement of ultrasound probe Energy usage, Maintaining and replacing ultrasonic-damaged devices increases operating costs.	Hassani *et al.,*2022 Shaw *et al.,*2009 Fetyan & Salem, 2020
Photocatalytic systems	Degradation of organic matter, COD & BOD reduction. Cost-effective process.	Photocatalysis is significantly more effective on pretreated waters than on raw or untreated wastewater.	Nguyen *et al.,*2020 Kansal *et al.,*2008
Oxidations	At pH 5, Fenton's reagent (H2O2/Fe2+) removes 85% color, 88% TOC, and 89% AOX in 30 min	Hydrogen peroxide alone removes TOC, AOX and color at an extreme pH of 11. UV treatment alone fails to remove color, TOC, and AOX.	Mainardis *et al.,*2020 Catalkaya & Kargi, 2007

2.3.2. Biological methods

Biological wastewater treatment is also employed for the removal of contaminants and color in the treatment of BL. It includes using different microbes recognized to degrade lignin (Chandra & Singh, 2012; Abhishek *et al.,*2015). Lignin is the primary cause of the color and intensity of the effluent, and it is harmful to the environment (Raj *et al.,*2014; Haq *et al.,*2016a, b; Haq *et al.,*2017). Two processes, namely (a) the action of enzymes and microorganisms and (b) biosorption, the binding of contaminants onto the cellular surfaces which makes their removal easy, are involved in the treatment process (Brown & Chang, 2014; Rybczyska-Tkaczyk & Korniowicz-Kowalska, 2016). The white-rot fungus from Basidiomycotina and some species from Ascomycotina are responsible for the lignin breakdown (Haq *et al.,*2016a). Wood decay fungus has a distinctive trait as they have the potential to degrade lignin in a shorter duration than any other living creature present in nature. They use lignin as their food source. In addition to this, they use cellulose and hemicellulose as carbon sources (Blanchette, 1995). White-rot fungi break the lignin by its ligninolytic activity and use it as a food source (Asina *et al.,*2016). The ligninolytic activity or delignification can either be selective or nonselective. In nonselective delignification, each essential and non-essential component is destroyed, while selective delignification selectively destroys the lignin compound only (Blanchette, 1995). The transformation of waste products from effluent into an essential resource for growth and metabolic activities is the most unique and remarkable characteristic of plants and microbes (Ghosh & Thakur, 2017).

Biological-based wastewater treatments are less expensive and more environmentally friendly than physicochemical methods (Esmaeeli *et al.,*2023). Typically, biological procedures were used following the initial treatment for clarity. Biological treatment uses various traditional procedures such as aerobic, anaerobic, and combination treatments, to eliminate organic pollutants that are rich in lignin (Pokhrel & Viraraghavan, 2004; Bajpai, 2018; Chaudhry & Paliwal, 2018). Table 2.2 provides a summary of the numerous microbial species that have been documented for the treatment BL. These biological methods lead to a healthier environment by reducing pollution. Because cellulose, lignin residue, and hemicellulose components in BL have aromatic groups with larger molecular weights than other organic substances, microbial breakdown of these compounds is challenging (Azadi *et al.,*2016).

Microorganisms have evolved a distinctive approach to get across this barrier, and they have the potential to penetrate the compounds of lignin.

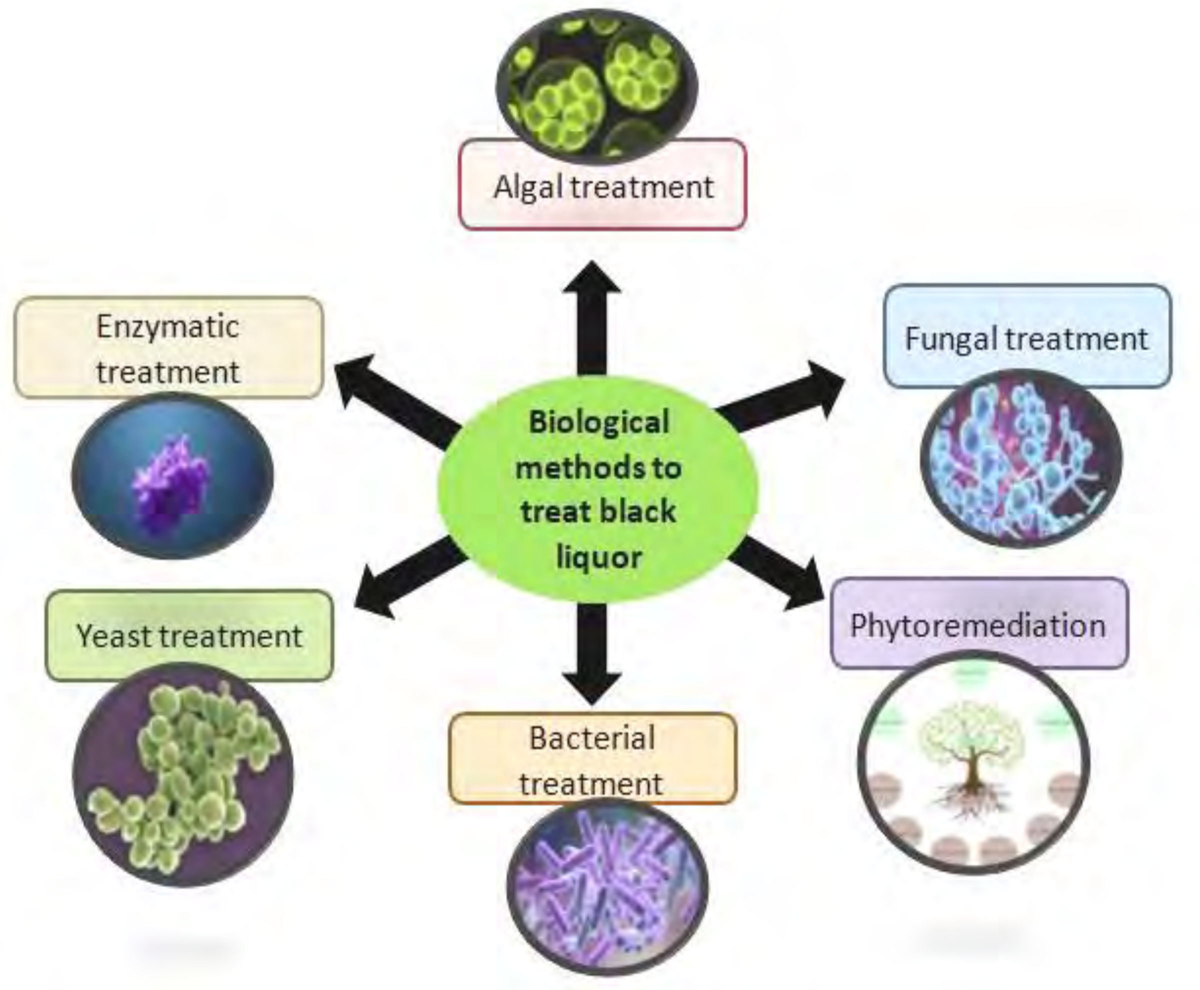


Figure 2. 1 Biological methods for paper and pulp wastewater effluent treatment

2.3.2.1.Aerobic process

Aerobic biological wastewater treatment techniques offer a durable and effective means of removing nutrients and organic contaminants from wastewater. When oxygen is present, these processes exploit microbes' metabolic processes to change pollutants into carbon dioxide and water and play an important role in promoting safer and cleaner water supplies for a rapidly increasing world. An abundance of research exists on aerobic WWT process on a large scale, as well as in small-scale laboratory settings (Ren *et al.,*2022).

The activated sludge process is an essential aerobic biological method for treating wastewater, which effectively decreases contaminants in the BL. It employs a multichambered reactor teeming with microbes to break down organics and improve effluent quality, leading to reasonable removal rates, as demonstrated by numerous studies (Jagaba *et al.,*2022). A multichambered reactor with a high microbe concentration reduces BOD, COD, TSS, TOC, AOX, and chlorinated compounds from BL in the activated sludge process. They degrade organic compounds and remove contaminants, improving effluent quality (Ashrafi *et al.,*2015). An assessment of the potential of an activated sludge system is done by Ghoreishi and Haghighi (2007) to treat BL and found that the AS system removes efficiently removed pollutants up to 97% (TSS). Post-treatment was done based on the Fenton oxidation process for removing COD and color. It increased the quality of treatment and made the process more effective. Aerated stabilization basins (ASBs) efficiently eliminated 67% of COD and 90% of BOD from BL, according to Bryant (2010). All aerobic processes need a continuous supply of biological oxygen, which performs oxidation. This requirement of high oxygen demand limits the usage of aerobic process-based treatments. (Toczyowska - Mami'nska, 2017).

2.3.2.2.Anaerobic process

Anaerobic treatments are one of the latest approaches used to treat BL. Contrary to aerobic processes, anaerobic ones rely on microbes' capacity to degrade complex organic compounds without oxygen and generate valuable byproducts like biogas. Anaerobic treatment processes provide long-term solutions for treating pulp and paper industry effluent, thus mitigating environmental issues caused by the BL (Esmaeeli *et al.,*2023). Commonly used methods for the anaerobic treatment of BL include the renowned technique of fluidized-bed reactors (FBR) (Ashrafi *et al.,*2015). This anaerobic method holds the potential for maximum COD removal in restricted activity. Chinnaraj and Venkoba Rao (2006) reported the UASB reactor effectively decreased the COD levels in the effluent from the agro-based pulp and paper business by 80-93%. The UASB reactor has another advantage of fuel and biogas production. Buzzini and Pires (2007) employed the UASB reactor for the treatment of BL These experiments demonstrated the removal of 71–99% of chlorinated derivatives and 78–82% of COD from BL. Adding ligninolytic fungi to an FBR increases the removal efficiency of COD and color, as Ortega-Clemente and Poggi-Varaldo (2007) claimed. Deshmukh *et al*. (2009) effectively removed COD (50%) and AOX (50%) from BL using an up-flow anaerobic filter (UAF). Different anaerobic techniques provide different benefits in different aspects. FBR-based processes are of great significance in removing pollutants when used to treat BL. On the other hand, fixed-film reactors offer lower capital costs, and UASB reactors require small amounts of energy to carry out the treatment (Rajeshwari *et al.,*2000).

2.3.2.3.Role of bacteria

Since bacteria are efficient in breaking down intricate pollutants, they are crucial to treat BL. Several well-known bacterial genera, including *Pseudomonas*, *Alcaligenes*, *Nocardia*, *Bacillus*, *Arthrobacter*, and *Streptomyces*, have demonstrated the capacity to degrade lignin and other pollutants of BL. Their many enzymatic processes allow them to remove color, break down lignin, and degrade organochlorines, all of which significantly impact the BL remediation (Chandra *et al.,*2007). Lignin breakdowns involve the mineralization and solubilization process. They are like the enzymatic action of lignin-degrading bacteria; this requires extensive research for this relationship to get more precise (Chen *et al.,*2012). Bacterial enzymes are functional in different environmental settings, and as they are versatile, their metabolic system outperforms in various environments. Bacterial development and multiplication are quicker than fungi (Harms *et al.,*2011). Bacterial ligninolytic systems are more active, tolerable, and stable for salt, pH, and thermostability than fungal enzyme systems (Bugg *et al.,*2011; Haq & Raj, 2019). According to certain researchers, the following species of bacteria are associated with the process of delignification. They include *Pseudomonas*, *Arthrobacterium*, *Xanthomonas*, *Aeromonas*, *Flavobacterium*, and *Streptomyces*. There are few investigations on which specific bacterial species that are either thermophilic or anaerobic are also involved in lignin breakdown. Marchand (1978) found that *Pseudomonas*, *Corallina*, *Nocardia*, and *Torula* used alkali lignin from sulfate effluent. Microorganisms need a carbon food source. They even take KL from the paper mill effluents as their carbon source and acquire needed nutrients by breaking it down (up to 98%) into simple forms (Raj *et al.,*2007). According to studies by Ojha and Markandeya (2016), lignin was degraded to a maximum of 40.19% in wastewater from the paper industry when three bacterial isolates were used in consortia. The rate of lignin breaks and color reduction of 50-97% is achieved through the application of *Bacillus* and *Pseudomonas* in investigations of batch scale (Raj *et al.,*2007; Tiku *et al.,*2010; Tyagi *et al.,*2014). Bacteria degrade lignin in diverse ways. Several papers have mentioned that bacteria cause the disassembly of the structure of lignin. Only a limited number of strains have the ability to break down intricate lignin derivatives that are formed during the pulping procedure (Chandra *et al.,*2007; Chandra & Bharagava, 2013). For bacteria, the degradation of lignin either aerobically or anaerobically is not an easy task to accomplish. Some bacteria, like *Streptomyces viridosporous*, are actively involved in lignin breakdown, but the principal action mechanism is unknown. However, it was revealed that bacterial consortia and protozoa found in sand-receiving paper mill effluent broke down lignin (Hossain & Ismail, 2015). Two bacterial species, *Serratia liquefaciens* and *Paenibacillus sp.*, were reported to produce or induce enzymes for lignin breakdown. The enzymes LiP and laccase performed the restoration as they were used in the bioremediation of BL (Raj *et al.,*2014; Haq *et al.,*2016a, 2017). Laccase enzyme efficiently handles cellulose and hemicellulose-based crude products, converting them into marketable products with added value from pre-hydrolysis liquid (PHL). Wang *et al*. (2015) saw lignin removal from PHL following the modeling of laccase induction in kraft pulp.

Bacteria undergo significant and substantial modifications in several conditions, enabling them to fulfil a crucial function in the treatment of BL. Bacterial genera, listed in Table 2.2 have been shown to have the ability to decolorize, remove lignin, and degrade organochlorines from BL. In the bleach kraft effluent, the decolorizing abilities of the different bacterial strains were recorded (Raj *et al.,*2007; Tiku *et al.,*2010; Chandra & Singh, 2012). Besides decolorizing black liquor, bacteria can depolymerize lignin based on its unique enzymatic activity (Tyagi *et al.,*2014). In a study a single bacterial strain reduced color and lignin from the BL by 67% and 54%, respectively (Zainith *et al.,*2019). Keharia and Madamwar (2003) demonstrated that bacterial strains are capable of breaking down organochlorine compounds found in the BL. *Ancylobacter* has been documented and witnessed to decrease the AOX levels in softwood effluents. Bacteria produce ligninolytic enzymes to a limited degree This volume of enzymes produced is not significant for lignin degradation. Due to this reason, the ligninolytic activity by bacteria is constrained (Paliwal *et al.,*2012). The fungus attacks lignin, penetrates it, and forms low-molecular-weight lignin components, further degraded by several bacteria. A single bacterium cannot degrade lignin and treat BL due to a deficiency of lignin-degrading enzymes. Therefore, a bacterial combination of at least two different strains or coaction of a fungal species is needed to remove lignin and effectively treat BL.

2.3.2.4.Role of fungi

Fungi play a pivotal role in treating BL, with their unique ability to break down multiple pollutants due to extensive enzyme systems. They use enzymes like laccase, LiP, and MnP to depolymerize lignin as part of their non-specific effect against lignin degradation. Fungi are incredibly effective in breaking down lignin because they possess extracellular enzymes that bacteria do not possess. In high-effluent load situations, fungi are more robust than bacteria, outperforming them. Further supporting their appropriateness for biodegrading BL is their capacity to adapt to harsh and inhibiting conditions, such as extreme temperatures or hazardous chemicals. Fungi are non-specific in their action against lignin degradation, causing its depolymerization. These all are extracellular enzymes that are only found in fungi. Under high effluent load, fungi are capable of surviving. They have a high percentage of survival (Kamali & Khodaparast, 2015). Bacteria are vulnerable to inhibitory chemicals, while fungi have a high tolerance against inhibitory chemicals. The presence of extra-polysaccharide cell walls prevents the inhibition action by inhibitory chemicals in the adsorption process. In addition, fungi contain several genes that allow them to bear the action of inhibitory substances and chemicals and even make them more adaptable to harsh and hazardous environments (Gupta & Gupta, 2019). All these characteristics of the fungus make it a strong contender for the biodegradation of effluent from paper and pulp companies. One of the earlier investigations revealed that a particular fungus could penetrate and degrade organochlorine's sinuous complexes and readily take up metals from the aqueous solution (Bajpai, 2018). Numerous fungi are important lignin degraders, listed in Table 2.2. Among white-rot fungi, specifically, *Versicolor* and *Chrysosporium* are well-known fungal species with decolorizing and degenerative properties. In a small-scale preliminary study, these white-rot fungi are examined for their degradation properties in various techniques, including percolating filters, ultralight reactors, and liquid desiccant reactors (Bajpai, 2018). A thorough investigation of *P. chrysosporium* showed that the fungal mycelia contribute to the removal of color and the degradation of organochlorine compounds (Pointing, 2001). The BL was reported to be decolored by different fungal strains by Apiwattanapiwat *et al.,*2006. *Pleurotus* and related genera were discovered to be effective in the biodegradation of organic compounds in BL (Fonseca *et al.,*2015; Singh & Arya, 2019). White-rot fungus is mainly responsible for the lignin breakdown and belongs to the kingdom *Basidiomycotina*. A few species from *Ascomycotina* are also involved in ligninolytic activity (Haq *et al.,*2016). The wood decay fungus is another creature that has more potential and is quicker in its action than any other living organism. They target cellulose, its derivatives, and hemicellulose masses and utilize them as their carbon source and lignin (Blanchette, 1995). White-rot fungi employ their ligninolytic activity to break down lignin and related chemicals, resulting in a break used for growth and metabolic activity (Asina *et al.,*2016). This delignification can be of a selective or nonselective nature. Selective-based lignin breakdown targets the lignin only, while the nonselective method destroys each component of the cell (Blanchette, 1995). The extracellular enzymes of white-rot fungi known for lignin breakdown include Lac, LiP*,* and MnP (Blanchette, 1995). Some supplemental enzymes are also involved, but their function is unknown. Conversely, MnP, LiP, and Lac are essential in the degradation of lignin (Srinivasan *et al.,*1995). Multiple fungal cultures are employed to degrade the lignin component found in BL (Dashtban *et al*., 2010; Kamali & Khodaparast, 2015). 90% color removal efficiency has been recorded for *Schizophyllum* commune under ideal conditions during a 2-day incubation period (Saritha *et al.,*2010). *Gliocladium virens* can decolorize BL up to 42% and reduce its level by up to 52%. When Gomathi *et al*. (2012) inoculated effluent from the paper industry with immobilized *P. chrysosporium*, they reported an 83% drop in lignin, phenol, and COD. According to studies by Lokeshwari *et al*. (2013), after eight days of incubation, the fungus Aspergillus flavus effectively biodegrades 94% of the lignin used in the pulp sector and 45% of the COD levels. Fungi are more successful decomposers of lignin than bacteria. Their ligninolytic activity is based on certain parameters and factors that ensure the maximum efficiency of fungal degrading enzymes. When the environment is not conducive, and conditions are extreme, for example, high temperature, low pH, and harmful and inhibitory chemicals, the rate of lignin degradation reduces, and efficiency is compromised. Fungal ligninolytic systems are less successful in treating BL (Amr *et al.,*2009: Haq *et al.,*2017).

2.3.2.5.Role of algae

Algae-based treatment techniques are becoming increasingly well-known for their effectiveness in treating effluent from the pulp and paper sector. The primary contaminant, lignin, may be effectively decolored by the metabolism of algae, which eventually turns the effluent colorless. *Planktochlorella nurekis* and *Chlamydomonas reinhardtii*, two types of algae, have shown they are capable of removing color from BL as well as heavy metals, nitrate, phosphate, COD, and other pollutants. Algae are a viable option for the remediation of BL since it has been discovered that combining different algal strains rather than a single species can improve treatment effectiveness and shorten processing time. The algal metabolic activity can result in the decolorization of chromophoric molecules of lignin. It can convert the effluent into a colorless state (Chandra & Singh, 2012). Various species of algae as they can remove heavy metals and kraft lignin from wastewater of the paper industry are listed in Table 2. According to reports, the algae species *Planktochlorella* introduction of bacterial strain combination, it has been discovered that algal species must also be introduced into the effluent in mixed form instead of single algal species. The presence of more than one algal strain for wastewater treatment increases the treatment efficiency and reduces the time duration. By employing a hybrid algal method, Tarlan *et al*. (2002) could remove 75% COD. To clean wastewater, Usha *et al*. (2016) used a mixed culture of *Scenedesmus sp*. in a lab-scale investigation and discovered the maximal elimination of BOD and COD. *Nurekis* and *Chlamydomonas reinhardtii* may successfully remove metals, nitrate, phosphate, COD, and other contaminants from wastewater used in pulp and paper production (Sasi *et al.,*2020).

2.3.2.6.Role of enzymes

Specific microbial enzymes can regulate waste, laden with lignin and other contaminants. Specific enzymes regulate the organic waste generated by the paper and pulp industries. The microbial enzymes responsible for this process include cellulases, xylanases, laccases, peroxidases, catalases, amylases, proteases, and lipases. Bioremediation of any organic matter depends upon the type of microbial or species present and environmental parameters. Favorable and optimum conditions aid in remediation and promote the microbe's activity. The white-rot fungus produces ligninolytic enzymes more than one in number of ligninolytic enzymes that are capable of breaking lignin into a more straightforward form of compounds and degrading xenobiotic chemicals (Deshmukh *et al.,*2016). LiP and MnP are two classes of ligninolytic enzymes generated by a white-rot fungus (He *et al.,*2015). It is well known that white-rot fungus has laccase and class II peroxidases that may break down persistent organic contaminants (Ikehata, 2015). Laccase could oxidize phenolic compounds. The *Versicolor* has laccase activity that is more than 20 times higher than other bacteria (Margot*et al.,*2013). The enzymatic treatment effectively eliminates or partially eliminates contaminants from the BL (Shankar *et al.,*2020).

Table 2. 2Biological methods for paper and pulp wastewater effluent treatment

Biological method	**Strain**	**Color reduction (%)**	**Lignin degradation (%)**	**BOD Reduction (%)**	**COD Reduction (%)**	**Reference**
Algal method	*Chlorococcum, Pandorina, Eudorina, Nitzschai, Cyclotella, and Anabaena*	75	-	-	85	Tarlan *et al.* (2002)
	Microcyctis, Chlorella, Chlamydomona	75	-	-	85	Fayyad *et al.,*2020 Tarlan *et al.* (2002) Sharma *et al.* (2014) Kamali & Khodaparast (2015)
	Scenedesmus sp.	-	-	82	75	Usha *et al.* 2016
Fungal method	*Merulius aureus and Fusarium sambucinum*	78	79	-	89	Malaviya & Rathore (2007)
	Cryptococcus sp.	27	24	-	-	Singhal and Thakur (2009)
	Phanerochaete Chrysosporium	78	79		89	Saritha *et al.,*2010
	Trametespubescens	-	-	-	-	Gonzalez *et al.,*2010
	Phanerochaetechrysosporium	86	71		56	Chopra & Singh (2012)
	Trametesversicolor	80	-	-	82	Pedroza-Rodríguez & Rodríguez-V´azquez (2013)
	Gliocladium virens	-	52	-	-	Kamali & Khodaparast, 2015
	Bierkanderaadustaand Phenarochetecrysosporium	-	74-97	35(as TOC)	-	Costa *et al.* (2017) Aftab *et al.* (2011)
	Pleurotusostreatus	-	37.7-46	-	-	Li *et al.* (2019)

	PleurotusostreatusEB 016	-	-	-	92	Heinz *et al.* (2019)
	Trametes versicolor	98	-	-	-	Goksel Demir *et al.* (2007), Senthilkumar *et al.* (2014),
	Tinctoporiaborbonica	+	+	+	+	Kamali & Khodaparast (2015) [i] Shankar *et al.,*2020
	Phanerochaetechrysosporium MTCC No. 787	86	71	-	-	Khatami *et al.,*2019
	Pleurotusostreatus	-	37-46	-	-	Li *et al.,*2019
	Sordariamacrospora k-hell and M. thermophile	-	15-20	-	-	Yang *et al.,*2020
	Schizophyllum commune	90	-	-	-	Saritha *et al.,*2010
	Phanerochaete chrysosporium	37	56 (Phenolic compounds)		65	Diaz *et al.,*2022
	Aspergillus *uvarum*	81	67 (Phenolic compounds)		61	Diaz *et al.,*2022
	Aspergillus niger	43	-	-	60	Liu *et al.,*2011
	Rhizopus oryzae	-	-	-	82	Freitas *et al.,*2009
	A consortium of *Merulius aureus*, an unidentified genus and *Fusarium sambucinum*	78.6	-	-	89.4	Malaviya & Rathor, 2007
Bacterial method	*Pseudomonas ovalis*	97		87	94	Tyagi *et al.* (2014) Raj *et al.* (2007)
	Aeromonas formicans	86	78		71	Gupta *et al.* (2001)
	Pseudomonas fluorescens	*75*	45		79	Chauhan & Thakur (2002)
	Paenibacillus sp., Aneurinibacillusaneurinilyticus, and Bacillus sp.	*39-61*	28-53	65-82	52-78	Raj *et al.* (2007)
	Pseudomonas aeruginosa	-	-	-	-	Chandra & Bharagava (2013), Kamali & Khodaparast (2015)

	Serratia marcescens, Citrobacter sp., and Klebsiella Pneumonia	*85*		74	83	Chandra *et al.* (2011)
	Paeni bacillus sp. strain LD-1	*68*	54	-	-	Raj *et al.,*2014
	Pseudomonas fluorescens DSM 50,090 and Rhodococcusopacus DSM1069	-	80	-	-	Ravi *et al.,*2019
	Serratia liquefaciens	*84*	72	-	-	Haq *et al.,*2017
	Brevibacillusparabrev isMTCC 12,105	*51*	42		60	Hooda *et al.* (2018)
	Planococcus sp. TRC1	*96*	74		85	Majumdar *et al.* (2019)
	Ensifer adhaerens					Falade *et al.,*2017
	Arthrobacter sp. C2		40			Jiang *et al.,*2020
	Serratia sp	*80*	60	80	80	An *et al.,*2021
	Raoultella ornithinolytica	+	+			Falade *et al.,*2017
	Staphylococcus lentus		+			Baghel & Anandkumar, 2019
	Pseudomonas putida		+			Santos *et al.,*2014
	Thermobifida fusca		+			Rahmanpour *et al.,*2016
Enzymatic methods	***Enzymes***	**Applications**				**References**
	Cellulolytic enzymes Cellobiase Cellobio-hydrolase Exo-1,4-b-D-glucosidase Cellulase	Cellulosic sludges from pulp and paper are hydrolyzed to yield sugars and alcohol.				Pandey *et al.,*2017
	Lignin peroxidase	Degrading PAH-capable enzyme. Additionally, it catalyses the oxidation of a wide range of resistant aromatic substrates.				Yadav & Yadav, 2015
	Lacasses	Laccases oxidize many substrates like phenolic dyes, chlorophenols, phenols, and lignin-related diphenyl methanes.				Yadav & Yadav, 2015
	Manganese peroxidase (MnP)	Mn (II) oxidized to Mn(III); chelated Mn(III) oxidizes phenolic compounds to/phenoxyl radicals; other reactions in the presence of additional compounds.				Harzog *et al.,*2019

2.4. Bioreactor for the pulp and paper industry effluent: Success, problems and challenges

The fast expansion of human populations and enterprises over the past century has destroyed vital ecosystems, mainly due to insufficient industrial and municipal wastewater treatment. While industries frequently perceive compliance requirements as expensive duties, strict rules have been implemented to combat pollution. In order to find a remedy, the pulp and paper sector is looking to bioreactors. To meet regulatory standards while lessening the ecological and financial costs associated with waste management, bioreactors present a viable method for treating the effluent from this business. Implementing bioreactors is not without its triumphs, issues, and difficulties, in any case. Pollution of ocean and river quality has mainly resulted from the discharge of inadequately treated. Initially, such wastewater may contain pollutants that are readily biodegradable. However, their impact on the ecosystems can be significant, with loads in the tens of thousands of mg/L in terms of TSS, BOD and COD (Asami *et al.,*2022). To address this issue, governmental bodies are implementing increasingly stringent regulations on pollution discharge, with a primary focus on waste reduction. Nevertheless, industries frequently view the treatment systems created to adhere to these standards as a mandatory requirement, resulting in higher expenses for both initial investment and ongoing operation, and ultimately yielding unfavorable economic outcomes.

However, by conducting thorough investigation and implementing effective environmental control measures, it is possible to treat the majority of wastewater that contains biodegradable components and has a BOD/COD ratio of 0.5 or above using biological methods (Khorsandi *et al.,*2014). Biological treatment offers the advantage of reduced treatment expenses and absence of secondary contamination, distinguishing it from alternative wastewater treatment approaches (Rehman *et al.,*2017). Aerobic and anaerobic processes can be employed, with aerobic processes utilizing free or dissolved oxygen by microorganisms (aerobes) to transform organic wastes into biomass and CO2. Conversely, the latter procedure decomposes complex organic waste into methane, carbon dioxide, and water through three crucial phases without oxygenAlthough aerobic biological processes are frequently employed to achieve a high treatment efficiency in organic wastewater treatment, considerable advancements have been made in anaerobic biotechnology, focusing on resource recovery and utilization while still accomplishing pollution control objectives (Naz *et al.,*2013). Both techniques can attain a high level of effectiveness in removing organic matter. Aerobic systems are generally appropriate for treating waste of low strength and voice versa is true for aerobic system. Anaerobic treatment offers better advantages than aerobic treatment when handling influents with concentrations exceeding the crossover levels (Hamza *et al*., 2016).

In addition, anaerobic treatment typically demands lower energy consumption while offering the possibility of bioenergy and nutrient retrieval. Nevertheless, aerobic systems exhibit superior elimination, and the resultant biomass typically forms strong aggregates, leading to reduced quantities of suspended particles in the effluent (Rasool *et al*., 2017). Thus, the quality of the waste discharged from an aerobic system is typically superior to that of an anaerobic system. An anaerobic reactor is the preferred method for treating highly polluted industrial wastewater due to its ability to effectively handle high levels of COD, generate energy, and minimize surplus sludge creation. However, in real-world scenarios, anaerobic treatment encounters challenges such as slow microorganism growth and the requirement for additional treatment, which frequently contains NH^{4+} and hydrogen (Chen *et al*., 2008). Despite the great efficiency of the anaerobic process, total stabilisation of the organic matter in most applications is unattainable due to the wastewater's high organic strength. The effluent generated at the end of anaerobic treatment contains dissolved organic matter that is appropriate for aerobic treatment, suggesting the possibility of employing anaerobic-aerobic systems (Tezel *et al*., 2001). Hence, the ultimate discharge resulting from the anaerobic treatment consists of dissolved organic substances that are appropriate for aerobic treatment. This advises using anaerobic-aerobic systems and aerobic post-treatment to meet wastewater discharge requirements.

2.5.Types of integrated bioreactors for wastewater treatment

2.6.Anaerobic–aerobic bioreactors

Recently, integrated bioreactors for treating have received much attention. These bioreactors must adhere to strict criteria regarding space limitations, odor control, visual aesthetics, and the production of biosolids. To address these challenges, integrated bioreactors incorporating combined anoxic and aerobic processes within a single reactor have emerged as a promising alternative. This approach has been shown to enhance the overall degradation efficiency by combining the benefits of both aerobic and anaerobic degradation pathways. Moreover, such bioreactors are economically viable, highly effective, and occupy less space compared to conventional anaerobic-aerobic systems. Despite these benefits, integrated anaerobic-aerobic bioreactors are still in their infancy, with few research.

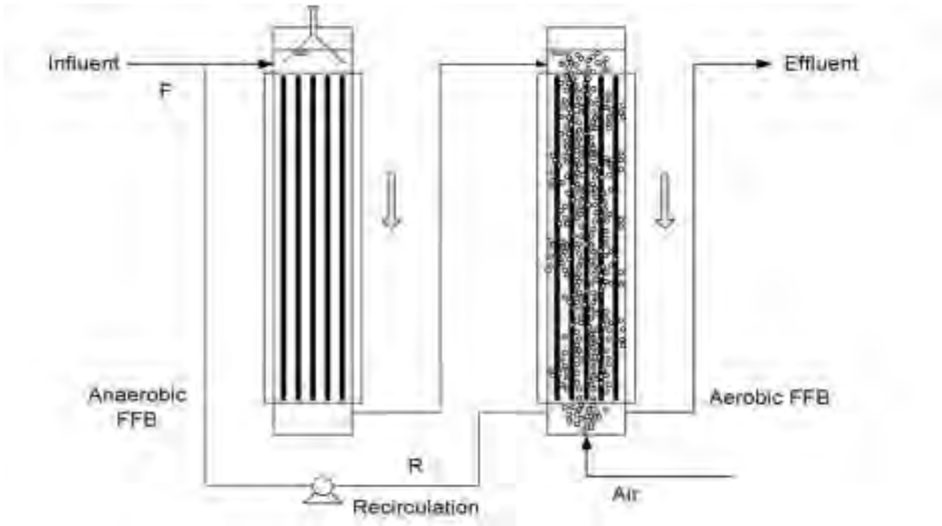


Figure 2.2 Schematic diagram of anaerobic–aerobic bioreactor (Chen *et al*., 2009)

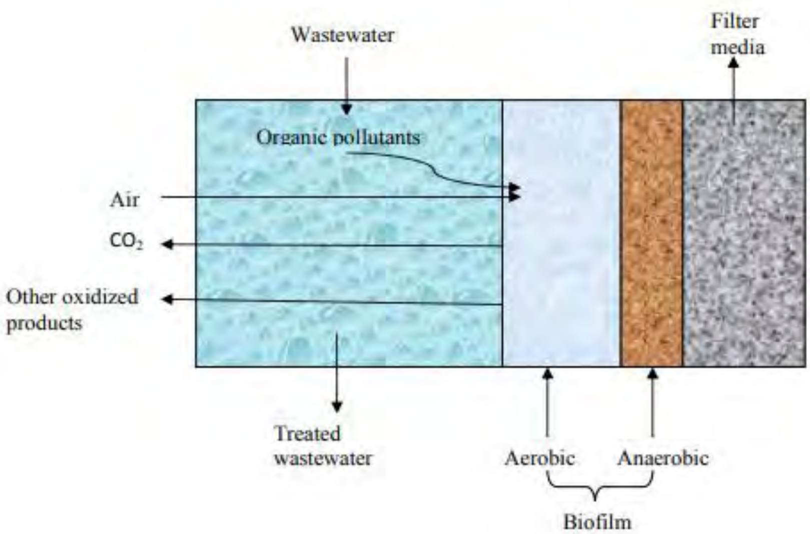


Figure 2.3 Mechanism of contaminant anaerobic–aerobic bioreactor (Naz *et al*., 2019)

2.6.1. Integrated anaerobic–aerobic fixed-film reactor (FFR)

The FFR, seen in Figure 2.4, presents a feasible solution for the treatment of BL. This innovative approach delivers exceptional efficiency in removing organic materials. It increases the decomposition of organic molecules by using membrane diffusers and corrugated tubes that are vertically oriented for aeration. Due to its strategic design and effective removal rates, the FFR is a strong option for tackling the wastewater difficulties faced by the pulp and paper sector. Del Pozo & Diez (2022) constructed a pilot-scale integrated anaerobic-aerobic fixed-film reactor (FFR) with organized medium, showcasing exceptional efficacy in the elimination of organic substances from abattoir wastewater. The reactor achieved an overall organic matter removal efficiency of 93% at an average OLR of 0.77 kg COD/m3 day and HRT of 0.94-3.8 days. The combined anaerobic-aerobic FFR exhibited superior treatment efficacy compared to the anaerobic-aerobic FFB system. The reactor is partitioned into two compartments, namely the aerobic and anaerobic zones, which are not separated by physical barriers. The wastewater is introduced into the system at the top of the non-aerated area, flows downward, and is subsequently carried through the aerated zone by the air injection's air-lift effect. Alternating diffuser activation and deactivation at the reactor bottom achieves different anaerobic-aerobic volume ratios. The percentage of anoxic removal was only 2.6%, whereas the percentage of methanogenic removal was 1.2% (Van: Vae ratio was 3:2). Upon reducing the ratio to 2:3, the number of COD eliminated by methanogenesis decreased to 0.6%. The anaerobic process exhibits low efficiency primarily because of the intense mixing pattern within the integrated reactor. This mixing pattern homogenizes both zones, inhibiting methanogenic and anoxic activities. To solve this problem, both zones must be strongly separated to generate an anaerobic environment. Two tiny barriers were added to the reactor's top and bottom. Aerated and non-aerated portions are parallel rather than series to recover methane without dilution from injected air.

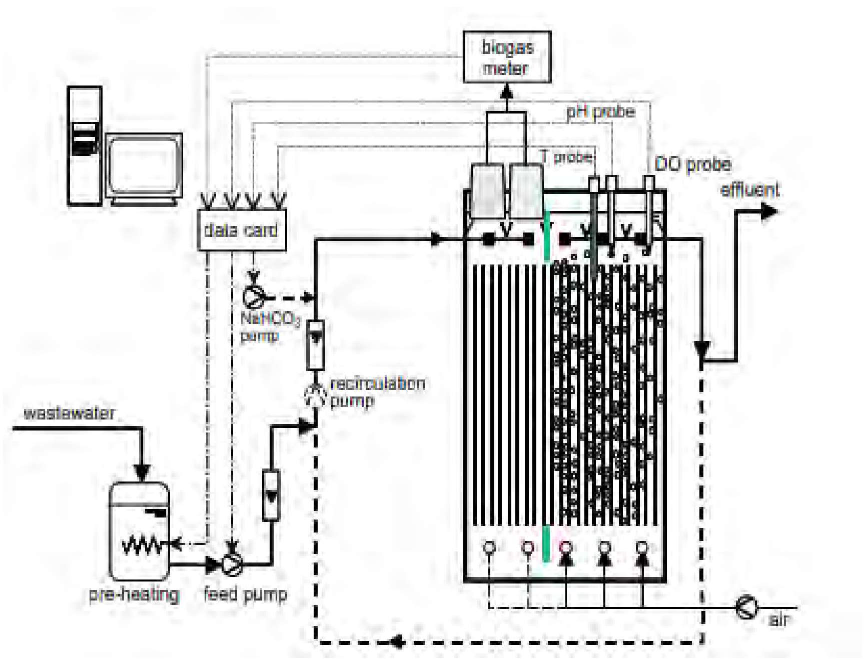


Figure 2. 4 Schematic illustration of the integrated anaerobic–aerobic FFR (Source: Del *et al*.,2005)

2.6.2. Moving-bed biofilm reactor (MBBR) technology

Biofilm-based treatment methods first appeared in the water quality restoration and remediation field in the 1980s. To integrate biofilms, these techniques use rotating biological contractors, granular media biofilters, trickling filters, and other reactor topologies. These well-established methods do, however, have some drawbacks. The MBBR technology, which provides a flexible and adaptive treatment technique to pilot-scale and small-scale standards, was created to overcome these problems. The MBBR process differentiates itself through its remarkable treatment efficiency and cost-effective operational, maintenance, and replacement expenses, with over 700 MBBR-based wastewater treatment systems running in more than 50 countries (Barwal & Chaudhary, 2014). Because it can avoid membrane surface fouling and channel blockage, MBBR technology is superior to MBR technology. However, MBBR systems' main shortcoming is the requirement for routine manual monitoring. In order to determine the presence of germs on the carriers, this monitoring method involves sample collection and several assays. Although bacteria are excellent at creating biofilms, these structures can also serve as attractants for pests like mosquitoes, sewer flies, and red worms. According to Barwal and Chaudhary (2014), the MBBR system has successfully treated wastewater from various businesses, including pulp and paper, pharmaceuticals, dairy, refineries, and slaughterhouses.

Both full-scale and pilot-scale facilities use this technique to treat industrial wastewater (Degaard, 2006). A particular MBBR is shown in Figure 2.5.

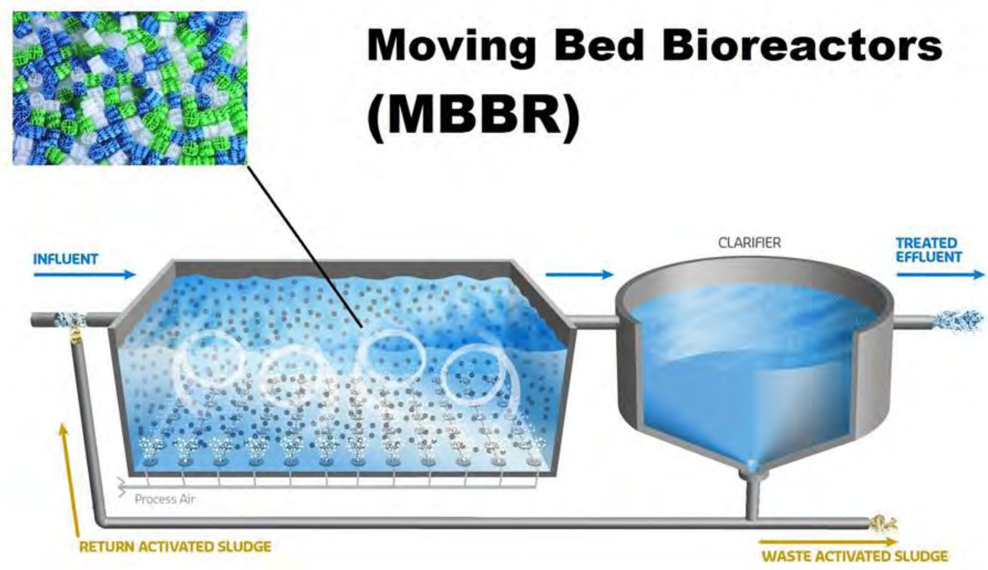


Figure 2. 5 Moving-bed biofilm reactor (Source: Liu *et al*., 2023)

Specifically, Swedish paper mills have employed the Moving Bed Biofilm Reactor (MBBR) technology for effective treatment of wastewater (Rusten *et al*.,1994). Broch-Due *et al*. (1994) observed that the MBBR system achieved an impressive 98% reduction in toxicity and a 70% decrease in COD when treating wastewater from a paper mill with an organic load of 25 kg COD/m3/day. Broch-Due *et al*. (1997) conducted a study where they used a pilot-scale MBBR system to treat integrated paper mill effluent. The system achieved a removal rate of 65-75% for COD and 85-95% for BOD within a treatment time of 4-5 hours. Embley (2001) effectively employed the MBBR technology for the treatment of effluent from kraft pulp, resulting in a significant 63% decrease in Biochemical Oxygen Demand (BOD). In addition, Jahren *et al*. (2002) showed that a laboratory-scale MBBR system can effectively treat white water from thermo-mechanical pulping by eliminating COD. Das and Naga (2011) employed a comprehensive approach to treat the combined waste streams from different sources. They utilized full-scale MBBR technology for this purpose. This system achieved significant removal rates when compared to conventional treatment methods, including 50% removal of soluble chemical oxygen demand (SCOD), 21.53% reduction of chemical oxygen demand (COD), and 33.5%

elimination of BOD. The wastewater treatment process can be enhanced and accelerated by implementing a combined aerated lagoon that consists of three interconnected Moving Bed Biofilm Reactors (MBBRs) and a Hydraulic Retention Time (HRT) system (Oliveira *et al*., 2014). Furthermore, according to Leyva-Diaz *et al*. (2013), combining MBBR with MBR technology shows remarkable COD removal efficiency of 90–91% in wastewater treatment plants. Additionally, as Chen *et al*. (2020) illustrated, recent advancements have produced a particular anaerobic MBBR-MFC system that enables bioelectricity production while also providing paper mills with effective wastewater treatment.

2.6.3. Integrated anaerobic–aerobic fluidized bed reactor.

A possible solution for treating BL is the combined anaerobic-aerobic fluidized bed reactor, shown in Figure 2.6. With the combination of aerobic and anaerobic processes within a fluidized bed structure, this cutting-edge system can increase efficiency and remove pollutants. By merging these treatment techniques, the reactor intends to improve wastewater management while reducing the adverse environmental effects of effluents produced by pulp and paper mills.

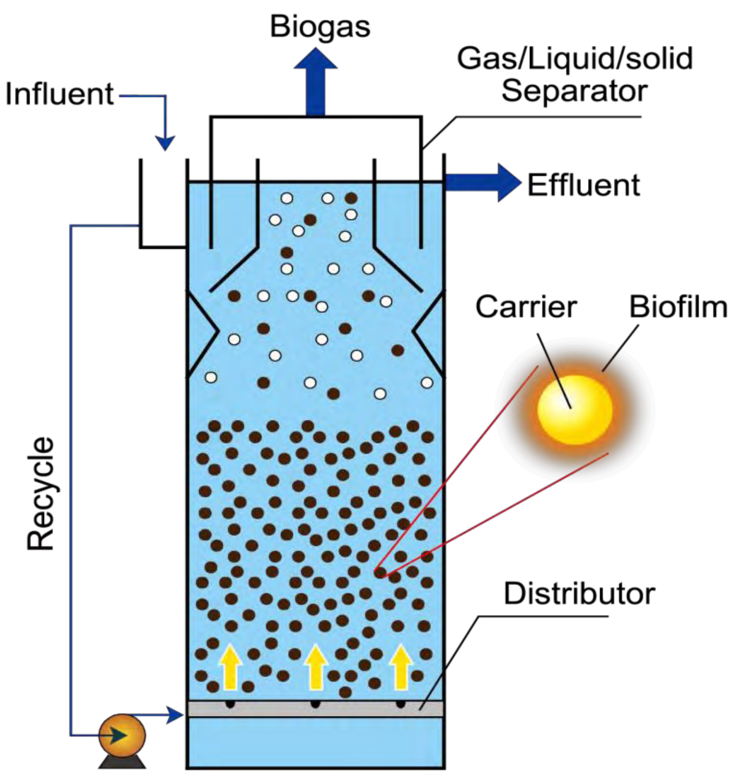


Figure 2. 6 A typical anaerobic–aerobic fluidized bed reactor (Source www.itriwater.org.tw)

Celis *et al*. (2007) used a pilot-scale anaerobic-aerobic fluidized bed reactor to treat municipal wastewater. COD removal rates above 80% were achieved with the reactor's 1.2 kg COD/m3 day OLR and 24-hour HRT. To enhance aeration, four diffusers were strategically positioned in a particular formation. Additionally, the reactor was designed with a cylindrical bed that utilized stone as a supportive medium. The system had notable potential for cost-effective automation and exhibited good stability, rapid starting, and resilience in the face of unforeseen challenges. Nevertheless, the study identified certain areas that require enhancement, such as increasing the frequency of pumping to maintain the suspension of the support material, and the potential benefits of implementing solid separation and recirculation systems to enhance the performance of the reactor.

2.6.4. Integrated bioreactor based on combined anaerobic–aerobic cultures.

Over the last twenty years, there has been a growing focus on mixed cultures, which consist of both anaerobic and aerobic bacteria a single reactor. While methanogenic and aerobic processes are often seen as different and independent, mixed cultures have shown effective application in the treatment of diverse contaminants requiring the sequential operation of anaerobic, aerobic, or anoxic reactors. In contrast to the conventional belief that anaerobic and aerobic treatment stages are mutually exclusive, this novel strategy has proven to be particularly useful for dealing with contaminants that require both of these stages of treatment. Combination cultures have shown significant performance variability in low-strength municipal wastewater USB reactors. When operated at HRT of 0.75 days, these cultures demonstrated an average BOD removal effectiveness ranging from 52% to 76% (Ergüder *et al.,*2008). The most efficient and practical aeration technique for USB reactors has been discovered to be an alternating cycle approach that alternates between anaerobic and microaerobic/aerobic conditions (Chan *et al.,*2009). Anaerobic digester sludge and aerobic mixed liquor were utilized in bench-scale batch reactors to obtain significant COD removal. A combination of methanogenic and oxygen-limited situations occurred when this outstanding performance was recorded (Shin *et al.,*2011).

Compared to traditional anaerobic and aerobic treatment systems, integrated bioreactor systems with both anaerobic and aerobic cultures have several benefits.

These benefits include the possibility for increased methane production, the capacity to fulfill mandated discharge levels reliably, improved settling, and natural aeration. However, further investigation is required to address the challenges associated these bioreactor systems, despite their promising benefits (Khin *et al.,*2004).

2.7.Constructed wetlands: Types and mechanism of contaminant removal.

Constructed wetlands are artificial systems created to remediate pollutants in a controlled setting by simulating the natural processes of wetlands. These systems mimic the complex ecological processes seen in natural wetlands by utilizing wetlands' soil, vegetation, and microbes. Built wetlands are categorized based on the kinds of macrophytes used, including floating leaves on the free-water surface, emergent macrophytes, and submerged plants. These systems can also be divided into groups based on their hydrological properties, with subsurface flow-created wetlands and water-constructed wetlands serving as the two main categories.

2.7.1. Vertical subsurface flow constructed wetland (VSSFCW).

The underlying figure (2.7) represents structure of vertical subsurface flow created wetland with its different components.

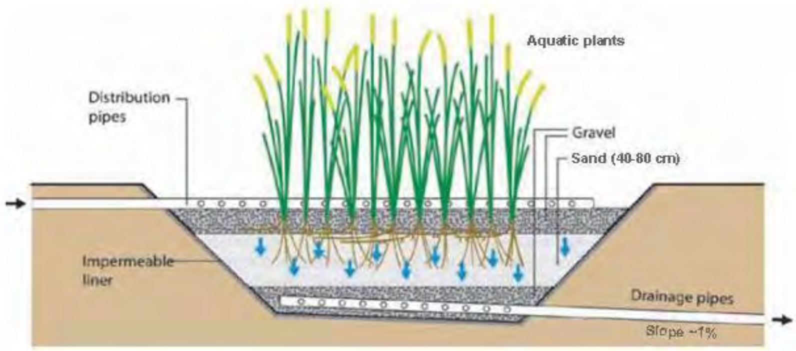


Figure 2. 7 A schematic diagram of a vertical subsurface flow constructed wetland (Chen *et al*.,2008)

The development of this particular sort of artificial wetland can be attributed to Seidel, who in 1966 applied the process of oxygenation to the effluent of an anaerobic septic tank. At first, VSSF-CWs were not as popular as HSSF created wetlands because of the expensive operation and maintenance costs involved in pumping wastewater onto the wetland surface in batches (Vymazal *et al*., 2005). The wastewater is introduced in discrete portions and left to percolate through the filter material until the subsequent portion is added. This procedure facilitates the dispersion of ambient oxygen into the treatment bed, resulting in VSSF-CWs being more oxygenated than HSSF-CWs. VSSF systems are capable of achieving nitrification, but they are not successful for denitrification. However, they are highly efficient in removing organic and suspended particles.

However, the efficiency of phosphorus removal is limited unless filter media with a high sorption capacity are used. VSSF systems typically require a land area ranging from 1 to 3 square metres of PE-1. Historically, vertical systems were built with multiple beds, where the first bed would rotate. However, modern vertical systems consist of a single bed, known as a compact bed VFCW (Vymazal *et al*., 2005). VSSF systems are frequently employed for the purification of municipal and household wastewater, especially when there are specific regulations in place regarding the allowable levels of ammonia in the discharged water. Nevertheless, multiple studies in the scientific literature have demonstrated that Vertical Flow Constructed Wetlands (VFCWs) have also been employed for the remediation of composting leachate, refinery wastewater, runoff water, and dairy wastewater. Tettleton *et al*. (1993) conducted a study on three VFCW wetlands that were parallel to each other. These wetlands had a total surface area of 1300 m^2 and were planted with Torpedo grass (*Panicum hemitomon*). The treatment of effluent from the tertiary bleach kraft pulp mill will take place in these wetlands. The removal efficiency exhibited fluctuations, with a low effectiveness observed for NH^{4-}N (25% and 18%), satisfactory outcomes for NO^{3-}N (80% in 1989, 64%), inconsistent performance for TSS (81% and 33%) and TP (53% and -32%), and minimal impact on BOD5 (7% and 6%).

2.7.2. Horizontal subsurface flow constructed wetland (HSFCW)

HSFCW consists of filter media, such as gravel and stones, along with an impermeable layer to prevent leakage. Additionally, wetland plants are included, as shown in Figure 2.8.

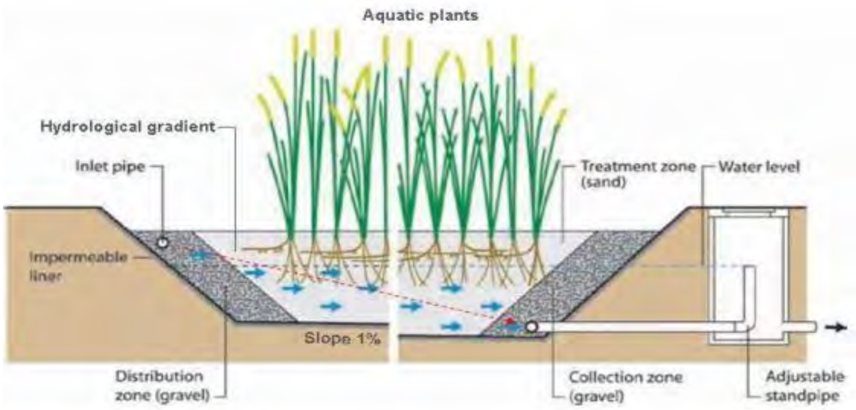


Figure 2. 8 represents a pictorial view of horizontal subsurface flow constructed wetland along with its labeled components (Bhais *et al.*,2016)

The wastewater is conveyed to the horizontal flow constructed wetlands (HF CWs) through the inlet. The fluid moves horizontally across the filter material until it reaches the exit point, where it is either released or gathered for future use. The contaminants in the wastewater were eliminated via different biochemical and physical processes under aerobic, anoxic, and anaerobic zones with the application od porous substrates (Vymazal, 2014). This system was implemented in Denmark in the 1980s, and numerous soil bed-constructed wetlands (CWs) were operated (Vymazal *et al.*, 2006). By the late 1980s, horizontal flow-constructed wetlands (HF-CWs) were adopted by various developed nations like United Kingdom and Australia (Vymazal, 2008). In the early 1990s, these systems were developed in various other countries of EU, Asia and North America with some modifications as per need. However, in the consequent decade, transformation was seen where alternative filtering material gained more attraction. It is expected to use gravel or rock stones measuring 10-20 mm (Vymazal, 2008).

The absence of oxygen in the filtration bed leads to microbial activities in anaerobic and anoxic zones, primarily facilitating the degradation of organic compounds (Vymazal & Kröpfelová, 2008). HF-CWs exhibit a high level of efficacy in removing suspended solids through filtration and sedimentation (Vymazal, 2008).

Denitrification is the primary mechanism for nitrogen removal in HSSF-CWs. Ammonia conversion does not happen in this system because the filter bed is saturated with water, and there is a low or complete absence of oxygen, as Vymazal (2007) stated. Phosphorus is mainly eliminated through ligand reaction, wherein OH ion replacement is carried out with phosphate from the surface of the aluminium and related iron oxides. The phosphorus removal efficiency in HSSF-CW is relatively low unless specific filter materials are employed (Vymazal, 2007). The plants species used in the wetlands provide an opportunity for the attachment of bacteria with plant roots and rhizomes that subsequently release oxygen in the root surrounding areas creating an oxygen-rich environment. It further support nutrients absorption and provide protected insulation in the rhizospharic area (Brix, 1994). Thut *et al*., 1990, employed eight HF mesocosms to treat tertiary pulp mill effluent. The research results showed that increasing the retention duration from 6 to 24 hours significantly enhanced the elimination of different pollutants. More precisely, it significantly increased the efficiency of removing ammonia, raising it from 31% to 88%, total suspended solids (TSS) from 55% to 68%, and phosphorus from 14% to 31%. Nevertheless, the prolonged retention duration did not impact the removal of biochemical oxygen demand (BOD_5). Significantly, there was no noticeable decrease in the color of the wastewater during the treatment process. In addition, the study examined the impact of different plant species on the removal of ammonia. The results showed a decreasing trend in the removal of ammonia in the following order: *P. australis* (82%), *Spartina cynosuroides* (63%), *T. latifolia* (53%), and the unplanted unit (16%) (Karajić *et al*., 2015). The *Spartina*, *Typha*, and *Phragmites* units achieved removal rates of 16%, 23%, and 26% for phosphorus, respectively. In contrast, the unplanted units only exhibited an efficiency of 2% for phosphorus removal. The BOD_5 and TSS elimination of both planted and unplanted units showed no significant difference compared to the *Typha* unit. *P. australis* and *S. californicus* were cultivated on a 3750 m^2 horizontal flow constructed wetland (HF CW) to treat BL. The high-quality treated wastewater provided, this system consistently achieved a removal rate of 80-90% for biochemical oxygen demand (BOD), resulting in wetland effluent levels consistently maintained within the range of 1-2 mg L^{-1} throughout the study. Removing total suspended solids (TSS) and ammonia showed inconsistent but generally satisfactory efficacy. Nevertheless, the wetland treatment had no impact on the color or AOX. As a result of hydraulic issues with the HF system, a large-scale system spanning 27 hectares featuring open water surfaces was constructed. The system consists of 33 cells and eight flow pathways, treating 60,000 m^3 of secondary wastewater daily. In a study conducted by Kadlec and Wallace in 2009, it was found that over two years, the concentrations of BOD^5 and TSS in the outflow were reduced from an average of 69 mg L^{-1} and 27 mg L^{-1} to 16 mg L^{-1} and 14 mg L^{-1} respectively (Dakua, 2015). In a study conducted by Choudhary *et al*. (2010), a wetland constructed using high-frequency (HF) techniques was employed to eliminate chlorinated resin and fatty acids (RFAs) from BL. The wetlands used for the experiment had a combined area of 5.25 square meters. They were cultivated with *Canna indica* plants and filled with gravel particles ranging from 0 to 10 millimeters. The removal efficacy ranged from 92% to 96% for 9,10,12,13-tetrachlorostearic acid when the hydraulic retention time (HRT) was 5.9 days and indicates that the primary methods of removing chlorinated RFAs are adsorption/absorption and microbial degradation within the plants' root zone.

2.7.3. Free water surface constructed wetland (FWSCW)

Free Water Surface Constructed Wetlands (FWS-CWs), as depicted in Figure 2.9, are shallow basins with a depth of 20-40 cm. These wetlands are characterized by abundant emergent macrophyte vegetation, often covering more than 50% of the surface area. Despite the construction of the first FWS-CW in the Netherlands in 1967, this technology gained little popularity in Europe, unlike subsurface flow CWs, which were prevalent in the 1980s and 1990s. Conversely, FWS-CWs were employed in North America and Australia for diverse residential and industrial wastewater treatment purposes. However, FWS-CWs eventually caught the attention of the European Union, specifically in Sweden and Denmark, where it was employed to address the issue of nitrogen removal from diffuse pollution.

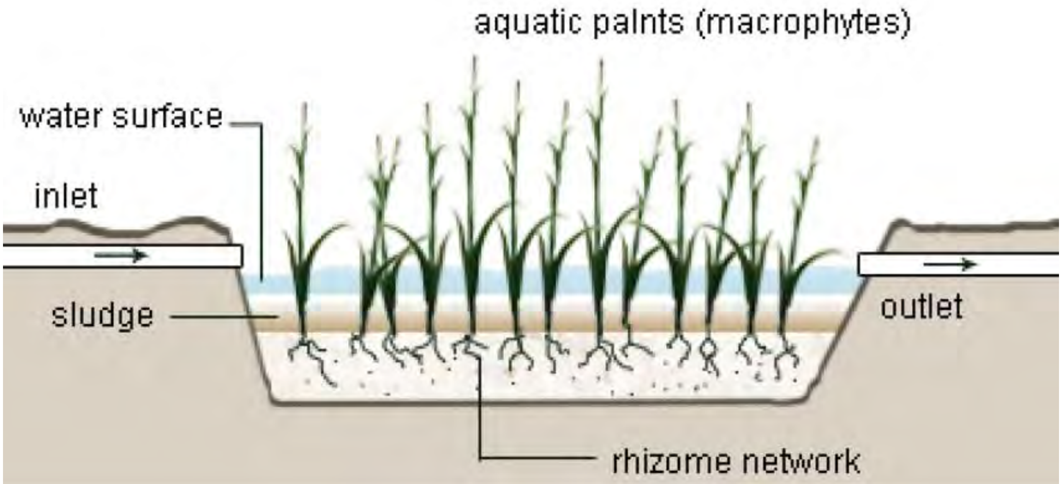


Figure 2. 9 Represents free water surface constructed wetland reported by (Vymazal, 2007)

FWS-CWs effectively remove organic matter and suspended particles through FWS-CWs efficiently eliminate organic matter and suspended particles via microbial degradation, root entrapment, settling, and filtration. Nitrogen is predominantly eliminated through nitrification in the water column, whereas denitrification occurs in the litter layer. Ammonia volatilization occurs exclusively when the pH levels are elevated due to algal photosynthesis. The limited phosphorus retention in FWS-CWs is attributed to the reduced water-soil contact, which decreases the available surface area for the adsorption and desorption of phosphates. Plants are generally left unharvested because their fallen leaves and other organic matter supply the essential organic carbon needed for denitrification. Within these systems, plants serve as temporary reservoirs for nutrients released into the water as they decompose (Baldovi *et al*., 2021). Knight *et al*. (1999) conducted a study in a bleached kraft mill facility in the USA. They examined a constructed wetland consisting of six cells that received secondary-treated effluent. Based on their research, cells with a higher length-to-width ratio (10.1) exhibited superior functionality compared to cells with lower aspect ratios (5:1 and 2.5:1). An FWS CW (Free *et al*. Constructed Wetland) was created in the Qinghe District of Beijing, China to manage the wastewater generated by a papermaking company (Xianfa & Chuncai, 1994). The wastewater underwent pretreatment through a pond and overland flow, with a wetland operating at a hydraulic loading rate (HLR) of 1.5 cm per day and a hydraulic retention time (HRT) of 5.6 days. The results indicate that BOD5, TSS, TN, and TP exhibited removal efficiencies of 38%, 84%, and 29%, respectively. Abira *et al*. (2005) researched phenol extraction from wastewater generated by pulp and paper mills. The researchers utilized an artificial wetland constructed by HF, measuring 30.7 m^2 in surface area. The wetland was populated with *Cyperus immense*, *Cyperus papyrus*, *Phragmites mauritianus*, and *Typha domingensis*. A layer of gravel measuring 0.3 metres in depth was used to fill the wetland. The phenol concentrations in the effluent ranged from 0.18 to 0.23 mg L^{-1} with a hydraulic retention time (HRT) of 5 days and from 0.1 to 0.13 mg L^{-1} with an HRT of 3 days. In contrast, the phenol content in the influent varied from 0.43 to 1.7 mg L^{-1}.

2.8.Microbiological aspects of wastewater treatment systems.

2.9.Biofilm development, structure, and function

Attached growth processes necessitate the development of biofilm on media. The biofilm's concentration and composition have a significant impact on the effectiveness of the bioreactor employed for wastewater treatment (Wang *et al*., 2000). A biofilm is a complex and stationary community of microbial cells that forms a structured network on different surfaces (Henze *et al*., 2008). Throughout the treatment process, the filter media is exposed to wastewater that passes through it by the force of gravity. This causes the formation of a stable biofilm as the microbes in the wastewater attach themselves to the surface of the media. Several environmental factors, including pH, temperature, and nutrient concentrations, influence the ability of cells within a biofilm to adhere to a substrate. Specific genetic factors, such as genes responsible for movement, water resistance, detection of the environment, electrical charge on the surface, and adhesive properties, such as the existence of particular surface proteins and appendages (Costerton *et al*., 1995; O'Toole *et al*., 2000). After the initial attachment, a single layer of small colonies forms as the cells multiply and spread out on the surface. The formation of a microcolony leads to the development of a mature biofilm, which is characterised by specific changes in the cells. These changes primarily involve the production of an exopolysaccharide matrix (EPS), which indicates a mature biofilm (Costerton *et al*., 1995; Danese *et al*., 2000). Stoodley *et al*. (2002) developed a model that elucidated the biofilm formation process on carrier material across various solid media, as depicted in Figure 2.10. The demonstration of this model can be divided into a few stages. During stage 1, bacterial cells adhere temporarily to the surface. In Stage 2, the cell attachment becomes irreversible due to the activity of exopolymer substances, leading to a loss of flagella-driven motility. Stage 3 marks the initial stage of maturation. Stage 4's second maturation phase is attained, distinguished by fully mature biofilms. The motile cells are then, finally, dispersed from the microcolonies at stage 5.

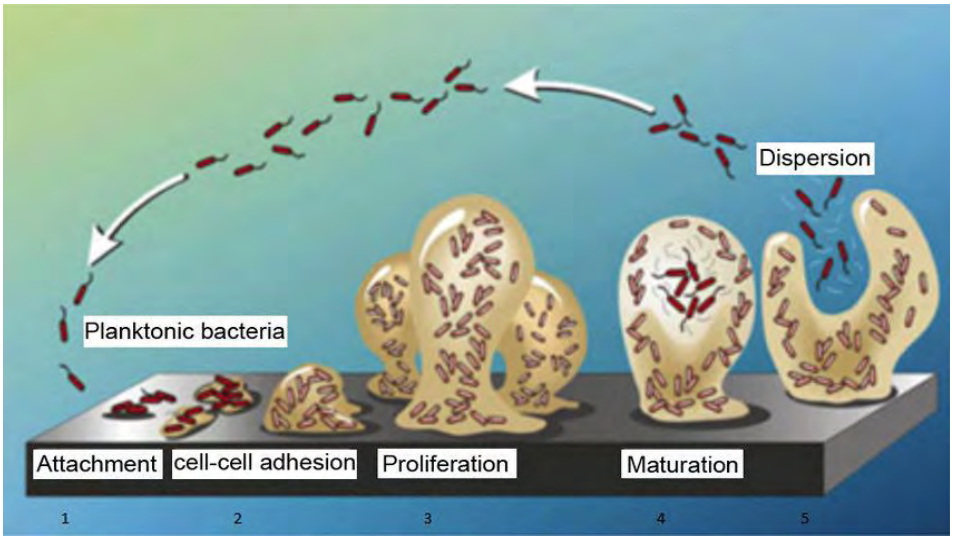


Figure 2. 10 Stages of biofilm development (Stoodley *et al*.,2002)

The developed biofilm is categorized into four components: base film, surface film, bulk liquid, and gas. Extracellular polymeric substances (EPS) produced by microorganisms adhere to the microbes and other particles, forming a complex in the underlying and surface films. The foundational film is constructed with clearly delineated boundaries. The transportation of carbon and energy source substrates, nutrients, Oxygen as an electron acceptor, and electron donors to and from the bacteria occurs through molecular diffusion. The surface film is an intermediary region connecting the case film and the liquid. The movement of materials inside is controlled by advection and turbulent diffusion (Benthack *et al*., 2001). The dispersion of wastewater onto the media provides microbes with dissolved air, which is crucial for oxidizing organic compounds (Metcalf & Eddy, 2003). The outer layer of the slime, which is approximately 0.1 to 0.2 mm thick, is where Oxygen is utilized, and the soluble and colloidal organic matter present in the wastewater is transformed into CO2 through metabolic processes (USEPA, 2000). Metcalf and Eddy (2003) also verified that when wastewater encounters a fully developed biofilm, whether aerobic, anoxic, or anaerobic, it diffuses throughout and interacts with all three zones. Recalcitrant compounds are broken down through organic mineralization, which occurs in the interior zone and leads to biomass production. To enhance the efficacy of treatment in biofilm systems, the flow of substrate to and through the liquid-biofilm interface must match the rate at which the biofilm planar area is being utilized. The distribution of microbial populations determines the flow of substances towards recalcitrant pollutants and away from mineralized organics at the biofilm interface. The distribution of microbes and their characteristics, such as porosity and density, exhibit non-uniformity. These are clusters of microorganisms with empty spaces that EPS can reach (open structures containing relatively low amounts of polymers). Hence, the biofilm's depth and its structure alterations impact the effective diffusion coefficients (Kreft & Wimpenny, 2001; Sutherland, 2001). Controlling the hydraulic application rate is crucial to preserve the metabolically active biofilms on media surfaces, especially when dealing with high organic loads. The thickness of the biofilm determines the active surface area in a trickling filter. According to Albertson and Eckenfelder (1984), an increase in biofilm thickness results in a decrease in available active surface area. A high rate of hydraulic flow typically causes a high level of shear stress, which in turn causes the release of EPS and leads to forming a thicker and denser biofilm. This is a result of increased detachment forces (Rochex *et al*.,2008).

2.9.1. Biofilm development and arrangement

The biofilm formation process on a suitable and conducive substrate is intricate and captivating. A cohesive slime layer or biofilm can develop on virtually any surface under unfavorable and humid circumstances. This habitation employs specific methodologies, such as activating genes that control the release of extracellular polymeric substances (EPS) and communication through quorum sensing. The process of maturation in a biofilm is illustrated in Figure 2.10. Initially, a deposition of adsorbed inorganic and organic molecules occurred on the surface of the support matrix. Subsequently, bacteria exhibit chemotaxis, directing their movement towards the damp surface, resulting in temporary interactions between bacteria and the surface (Gottenbos *et al*., 1999). Subsequently, bacterial excretion of extracellular polymeric substances (EPS) occurs, leading to the irreversible adhesion of cells to the surface. Dunne (2002) explained that permanent bonding occurs due to EPS, a combination of polypeptide complexes and exopolysaccharides released in moist environments. The initial attachment is facilitated by various forces, such as hydrophobic interactions, covalent and ionic bonds, and hydrogen bonding (Kumar & Anand, 1998). As per the findings of Palmer *et al*. (2007), this attachment is temporary and typically lasts for a brief duration. Following this, bacteria undergo division and establish mature colonies on the surface. The deposition of organic and inorganic molecules occurs at the beginning of biofilm synthesis. These molecules serve as a source of nutrients for bacteria, allowing them to form microcolonies that gradually grow and develop a slimy layer on the surface (Kumar & Anand, 1998). The study conducted by Garrett *et al*. (2008) examined the genetic aspects of bacterial cells in biofilm development. An increase in the expression of genes involved in the synthesis of extracellular polymeric substances (EPS) and other proteins involved in transport was observed. Conversely, the genes responsible for motility showed a decrease in expression. In addition, Dunne (2002) stated that bacterial gene expression and regulation primarily rely on the collective size of the population and communication facilitated by a signaling molecule. The structure of mature biofilms is intricate and mainly influenced by nutritional and environmental factors, including pH, temperature, nutrient composition, availability, and microbial diversity and composition (Pulcini, 2001). Researchers have observed that oxygen and nutrients are transported to the inner regions of a complex biofilm structure with a mushroom-like shape. They have also discovered that water surrounding the biofilm helps facilitate the diffusion processes. These findings have been documented by Pulcini (2001), Stoodley *et al*. (2002), and Kolter and Greenberg (2006). The biofilm development process is relatively slow and lasts approximately two weeks (Stoodley *et al*., 2002). Microorganisms can endure in a biofilm for long periods and adapt to challenging circumstances through genetic exchange. They also actively seek out a suitable environment for colonization. This occurrence is referred to as detachment and can arise from various factors. The factors contributing to this issue include unclean extracellular polymeric substances, hindered signal-induced communication, operational instability characterized by fluctuating hydraulic loading rates, and a lack of nutrients (Chambless & Stewart, 2007). The limited evidence regarding the arrangement of bacterial cells in biofilm is primarily attributed to the need for more accurate and precise analytical techniques. The empirical models offered a valuable understanding of the structure of biofilms, predicated on the hypothesis that they adopt a consistent monolayer arrangement (Watanabe *et al*., 2016). Over the years, confocal scanning laser and light microscopy techniques have been developed and are widely employed for examining and analyzing biofilm structure (Lazarova *et al*., 1994). These methods are susceptible and can produce precise images of the microbial slime layer by staining the binocular magnifying glass with INT [2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride].

Nevertheless, light microscopy is limited by its resolution, which falls just below or nearly equivalent to bacterial cell size. A high-resolution confocal scanning laser microscope was developed to examine the innermost regions of a biofilm, which also served as the foundation for electron microscopy (Caldwell *et al*., 1992). Researchers frequently employ these techniques to illustrate intricate biofilm structures and visualise their dynamic and dormant components (Watanabe *et al*., 2016). Scanning electron microscopy is frequently employed to achieve detailed visualisation of biofilm structures with high resolution. In addition, SEM also offers X-ray dispersive microscopy, which can be utilised to infer the composition of the support matrix and biofilm (Robinson *et al*., 1984). The application of these technologies allows for the acquisition of a distinct visual representation of biofilms, revealing that they are not simply modest collections of microorganisms but composed of bacterial cells enclosed within a polymeric environment (Lazarova *et al*., 1992). Transmission Electron Microscopy (TEM), a more advanced iteration of SEM, can generate comprehensive images of the internal structural arrangements of biofilms (Kinner *et al*., 1983). In a study conducted by Eighmy *et al*. (1983), the structure of aerobic biofilm was examined using the TEM technique. The researchers found that the biofilm consisted of three different types of matrix polymers distributed three-dimensionally and possessed various physical properties.

2.9.2. Application of biofilms in wastewater treatment processes

For more than ten years, microbial biofilm or aggregates have been significantly used in wastewater and water management, as reported by Miranda *et al.* in 2017. In the early 1980s, numerous researchers regarded biofilm as a captivating subject, encompassing environmental scientists and biotechnologists. Biofilm has emerged as a central focus in various research endeavors to improve groundwater quality, produce bioactive compounds using attached growth bioreactors, and treat sewage water. Klein & Ziehr (1990) initially presented reports on the significance of biofilms in wastewater treatment, which were subsequently validated by other researchers (Blenkinsopp & Costerton, 1991). Certain researchers exploited the genetic exchange occurring within biofilms among microorganisms to treat wastewater in a biofilm reactor (Fletcher, 1986). In contrast, other studies focused on measuring the activity of biofilm reactors by assessing nutrient concentration, enzyme production under different environmental conditions, and the presence of toxins or inhibitors (Rittmann *et al.*, 1986; Manem, 1988). According to reports, attached growth bioreactors are less susceptible to the influence of various environmental factors such as pH, temperature, growth limiting elements, secondary metabolites production, and carbonaceous compounds, compared to suspended growth bioreactors (Pedersen, 1990; Das *et al.*, 2017). Biofilm activity does not depend on a fixed amount of biomass. Instead, there is an observed increase in activity up to a specific limit of biofilm thickness, referred to as active biomass thickness. If the thickness exceeds this threshold, the supply of nutrients to the biofilm becomes restricted, leading to a distinction between inactive and active biomass (Kornegay & Andrews, 1968; LaMotta, 1976). Hence, it is imperative to maintain a consistent and dynamic biofilm in wastewater treatment facilities to optimize the neutralization of waste materials. An expedient and meticulously crafted approach is necessary to ascertain the boundaries of active biomass. The attached growth system in wastewater treatment offers several advantages compared to the suspended growth system. The advantages of this system include its cost-effectiveness, resistance to toxins and shock loads, low operational and space requirements, extended retention time, environmental friendliness, compliance with operational standards, increased rate of biodegradation, and reduced sludge production due to slow microbial growth (Chen & Chen, 2000; Lazarova & Manem, 2000; Wilderer & McSwain, 2004). The attached growth system can control the rates of reactions according to the requirements (Lazarova & Manem, 2000).

Rock-media systems, rotating biological contactors, fluidized bed bioreactors, high-rate plastic media trickling filters, granular filters, immobilized cell membrane bioreactors, and constructed wetlands are various technologies that employ biofilm or microbes adhered to appropriate support media for wastewater treatment (Seeger, 1999; Lazarova & Manem, 2000). The distinction between fixed and moving bed systems lies in the filter media's type, shape, and nature. The fixed bed system employs inert media such as stone, ping pong balls, plastic sheets, and polystyrene sponges to facilitate biofilm formation (Lazarova & Manem, 2000). The transportation of wastewater through filter media facilitates the provision of nutrients and dissolved oxygen to microbe communities residing in the biofilm. Conversely, moving or floating media is selected to promote biofilm formation in the moving bed or fluidized bed system (Rodgers & Zhan, 2003). The selection of filter media for both fixed and moving bed systems is determined by the specific surface area of the carrier materials and their size, shape, and density. Consequently, the materials used are of exceptional quality and are resistant to toxins and shock loads (Christensson & Welander, 2004). The fungal-fixed biofilm reactor demonstrated an efficiency of over 85% in degrading mano-azo and complex dyes found in textile wastewater. The cumulative impact of both reactors was analyzed, revealing a treatment efficiency exceeding 95% within 14 days. Almstrand *et al*. (2011) found that biological processes are more effective than physical and chemical wastewater treatment methods in removing ammonia and other nitrogenous compounds. The researchers employed a biofilm of ammonia and nitrite-oxidizing bacteria with a slow growth rate to create a nitrifying trickling filter. They then tested the filter under various organic and hydraulic loading rates. The experiments concluded that there is a direct correlation between the ability of these microbes to eliminate ammonia and nitrogenous compounds from wastewater and the rates of organic and hydraulic loading.

CHAPTER 3

MATERIALS

&

METHODS

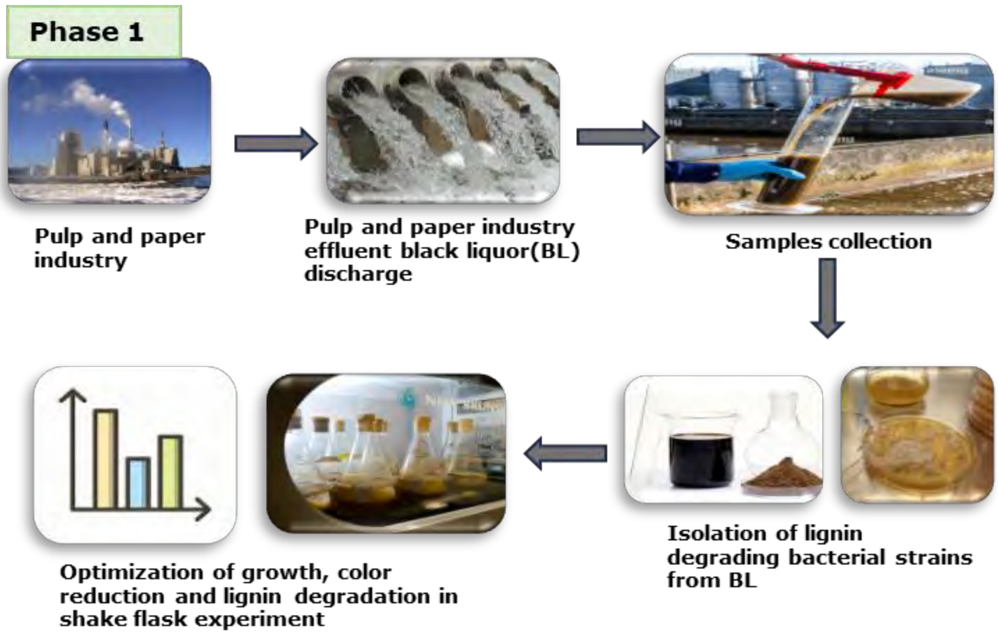
3. Materials & Methods

A comprehensive and integrated approach is required to treat black liquor (BL). In pursuing this aim, the current study is designed with four distinct objectives. First, the physicochemical characterization of BL is conducted, along with the isolation and characterization of lignin-degrading microbes. Isolation and characterization of lignin-degrading fungal strains were part of the project and already reported by Parveen *et al*., 2022. The present study exclusively focused on isolating and characterizing lignin-degrading bacterial strains. In the first phase, physicochemical analysis of PPI effluent isolation of lignin-degrading bacterial strains established the foundation for understanding the effluent's composition. This fundamental understanding is critical in identifying potential biological agents capable of degrading lignin and reducing the color of the BL. The presence of indigenous lignin-degrading bacterial populations in the BL affirms their potential as the key players in an integrated biological reactor designed to treat pulp and paper industry wastewater effectively. These bacterial communities, specifically when provided with filter media such as small stones or pebbles, form an efficient and robust biofilm in the bioreactor and play an essential role in containment removal. This correlation highlights the strategic utilization of indigenous bacterial flora's natural capabilities to enhance the biological reactor's effectiveness and self-sustainability in addressing the challenges posed by PPI effluent. Subsequently, the research project transitions into designing and constructing two distinct bioreactors: a gravity-driven biological reactor (GDB) and an integrated vertical flow constructed wetland (IVFCW). In the GDB, the natural bacterial flora of the GDB was provided with stone media as support to form biofilm in the bioreactor, while in IVFCW, in addition to the biofilm, rhizosphere microbes of *Typha latofolia* played a significant role in the contaminant removal. These engineered systems are tailored to facilitate the treatment of PPI effluent by harnessing the synergistic capabilities of biofilm. The efficiency of these treatment systems is rigorously monitored for different physiochemical parameters, and significant results were observed. However, the treated effluent still contains a considerable contaminant and does not fall under the permissible limits of environmental protection agencies. The hypothesis "domestic wastewater forms a biofilm with greater microbial diversity, particularly owing to the substantial presence of bacterial species originating from the human gut" was developed and tested in both bioreactors. BL was blended with equal volumes of domestic wastewater and fed as an influent. The strategic amalgamation enhanced the biofilm's capabilities for contaminant removal and synergistic microbial interactions in mixed-effluent treatment scenarios. In the final stages, a focused investigation of biofilm developed on stone media within the GDB and in the rhizosphere of IVFCW was conducted. The study provides a deep insight into microbial communities involved in the treatment process in each system. The subsequent sections provide a specific methodology employed in each objective, depicting a systematic approach to treating BL efficiently.

Phase 1

Objective 1: Physicochemical analysis of wastewater and characterization of lignin degrading bacterial strains isolated from black liquor.

<u>Graphical Methodology</u>



3.1. Sampling site and collection

The samples of BL were collected from Premier Paper Mills Ltd. Lahore, Pakistan. Sample was collected from wastewater treatment plant prior to any treatment in a sterile container and maintained its temperature at 4°C and transported to lab for further analysis.

3.2.Enrichment of samples

Nutrient broth (Yeast extract 2g/L, Peptone, beef extract 1g/L, and NaCl 5g/L) media were prepared and autoclaved. After the autoclave cooled the media and taken to the laminar flow hood where the collected samples were inoculated into the autoclaved media. The inoculum (nutrient broth + sample) was placed in a shaking for 10days to increase the biomass. The temperature of the shaker was maintained at 37°C, 120 rpm under aerobic conditions.

3.3.Isolation of lignin degrading bacteria

After ten days the enriched samples were serially diluted. The process involves the dilution of the cultures in a series of test tubes having 9ml normal saline mixed with 1ml of 10 days old inoculum to maximize the chances of isolated colonies. For spread plate, agar plate (Yeast extract 2g/L, Peptone beef extract 1g/L, and NaCl 5g/L, agar 4%) method was used. After 24 hours of incubation at 37°C, the general form of the colony, shape of the edge or margin were observed. Pure cultures were obtained using the streak plate method.

3.3.1.Primary screening of bacterial strains

The isolation of the bacteria yielded thirteen pure bacterial cultures which were initially named as strain TR-1 to TR-13. These bacterial strains were checked for their ability to utilize lignin as an energy source. For this purpose, mineral salt agar media (MSM) having following composition was used; Yeast extract, Peptone, $MgSO_4$, KH_2PO_4, NaH_2PO_4, Black Liquor 100 mg/L and 2% agar. The plates were prepared and after that each bacterial strain was point inoculated in the center of the plate and incubated at 37 °C for 8 days. The plates were monitored at a regular interval throughout the five days to check the zone of visible bacterial colony. Out of 13 pure cultures, three bacterial strains showed comparatively larger zones and were selected for further screening.

3.3.2.Secondary screening of lignin degrading bacteria

3.3.2.1.Lignin degradation and color reduction assay in shake flask experiments

To assess the effectiveness of the bacterial isolates to degrade the lignin, shake flask experiments were designed. Submerged fermentation was carried out using the MSM media previously described. After preparation of the media in 250 mL flasks, each selected bacterial strain was inoculated. The flasks were incubated for 8 days and lignin degradation and color reduction were monitored after every 24 hours interval.

3.3.2.1.1. Color reduction assay

For the measurement of the color reduction, samples were centrifuged. Change in the color of the media was determined by using spectrophotometry at Pt Cob. 0.1214 standard solutions at 465 nm.

3.3.2.1.2. Lignin degradation assay

The lignin degradation was determined by using USA standard Pt. Cob01214 standard solution. For this purpose, sample from the same flasks were centrifuge at 8000 rpm for 30 minutes, followed by discarding the pellet and mixing the supernatant with 3ml of phosphate buffer (pH7.6) to measure the absorbance at 280 nm with UV-visible spectrophotometer.

3.3.3.Preservation of bacterial culture

Bacterial strains showed better results and were preserved in the glycerol medium at -20 °C for future use.

3.4.Physicochemical characterization of pulp and paper industry effluent

Standard sampling method (APHA, 1998) was used to collect the wastewater and treated water for analysis. Pre autoclaved, plastic bottles were used to take the sample from the inlet and outlet of the TBF. Samples were immediately transported to the Environmental Microbiology Lab for the microbial characterization. Dissolved oxygen (DO) was calculated on the site just after the collection of the sample however sample was stored at 4°C for other physicochemical analysis.

3.4.1.pH Analysis

pH was measured by PCS Multi test meter Electrode was cleaned with deionized water before and after using the pH meter.

3.4.2.Electrical Conductivity and Turbidity

Electrical conductivity was measured in micro-Siemens per centimeter. PCS Multi test meter was used to measure the conductivity of wastewater before and after the treatment. Turbidity was measured using pHotoFlex WTW - Model 251110 - Turb Portable Multiparameter Colorimeter.

3.4.3.Dissolved oxygen (DO)

DO is directly related to water quality. For the measurement of dissolved oxygen Digital DO meter (MM60R, TOA-DKK, Tokyo, Japan) was used. DO of each sample was taken immediately after taking the sample and was calculated very carefully.

3.4.4.Biological oxygen demand (BOD)

Standard method 5210B (APHA, 21st Edition) was employed to measure the BOD of the water samples both influent and effluent.

3.4.4.1.Reagents

- **Calcium Chloride Solution ($CaCl_2$):** 27.5g of $CaCl_2$ was dissolved in distilled water and then solution was diluted up to 1L.
- **Ferric Chloride Solution FeCl3:** 0.25 grams of Hexa-hydrated Ferric Chloride ($FeCl_3$.6H2O) were dissolved in 1 liter of deionized water.
- **Magnesium Sulphate ($MgSO_4$) Solution:** 22.5 grams of Hepta-hydrated Magnesium Sulphate ($MgSO_4$.7H20) were dissolved in 1 liter of deionized water in a round bottom flask.
- **Phosphate Buffer Solution:** 8.5 g of KH2PO4, 33.4 g of Na_2HPO4•7H2O, 21.8 g of $KHPO_4$ and 1.7 g of NH_4Cl were dissolved in deionized water in round bottom flask with final working of 1 litter.

3.4.4.2.Procedure

Preparation of Dilutions: Dilutions of samples were made in such a way that each 1 ml of each chemical in 1 liter of solution. In the aspirator bottle 5 ml of sample and 295 ml of dilution are mixed in such a way that aspirator bottle should not trap the air.

Initial BOD was measured in terms of dissolved oxygen and the second bottle was incubated for 5 days at 20°C. After 5 days final dissolved oxygen was measured. BOD_5 was calculated by using the following formula,

$$BOD_5\ mg/L = (DO_i - DO_f) \times 1000 / \text{used dilution sample volume}$$

Where,

DO_i= *Initial Dissolved Oxygen* DO_f=*Final Dissolved Oxygen*

3.4.5.Chemical oxygen demand (COD)

The COD was determined using the Kit method. Kits with different range are available by different multinational companies. For COD measurement, 3 ml of pre filtered were sample was transferred into vial, shacked for 2-3 minutes and were put in the digester at temperature 150°C for 120 minutes. Kits removed from digester were allowed to cool for 25-30 minutes and reading were taken by using Spectro quant Pharo 300.

3.4.6.Total dissolved & total suspended solids (TDS & TSS)

TDS and TSS of influent and effluent samples were measured using digital PCS multi test meter.

3.4.7.Lignin content

The lignin content was measured using the standard Biorefreniary Test Method L2:2016(Costa *et al.,*2017). The procedure relies on sulphric acid hydrolysis of the samples, enabling the determination of total lignin content by summing up the acid-insoluble matter (AIM) and acid-soluble matter (ASM) concentrations after the hydrolysis.

3.4.8.Sulfates

Standard EPA method, 0375 Barium Chrometry (APHA, 21st Edition) was used to measure the sulfates in the samples.

3.4.8.1.Reagents

Following regents were used in the determination of sulfates,

- **Buffer Solution A:** 30 g of $MgCl_2.6H_2O$, 1g of sodium acetate and 20 ml of 99% acetic acid were added in distilled water and diluted up to 1 liter.
- **Buffer Solution B:** 30 g magnesium chloride, 20 ml 99% pure acetic acid, 5g sodium acetate, 0.111g sodium sulphate, and 1g potassium nitrate were mixed in 500ml water and diluted to 1 litre.
- **Dehydrated Barium Chloride**

3.4.8.2.Procedure

A 250-ml flask contained 25ml of pre-filtered sample. 2.5 ml Buffer A and B and a few Dehydrated Barium Chloride crystals were added. The sample was gently mixed. Readings were taken with Spectroquant Pharo 300.

3.4.9.Orthophosphates

Standard method, 45000-P (APHA, 21st Edition) was used to determine the orthophosphates.

3.4.9.1.Reagents

- **$SnCl_2$ Solution:** At high temperature, 2.5 g SnCl2 was dissolved in 100 ml glycerol.
- **Ammonium Molybedate Solution:** 25 g of Ammonium Molybedate was added to 175 ml of distilled water. In 400ml of distilled water, 80ml of Con.H_2SO_4 was mixed, as reaction is highly exothermic allowed to cool for some time. Both solutions were mixed, and volume made up to 1 Liter.
- **Phenolphthalein Indicator:** 0.5 g powdered Phenolphthalein was added to 100ml of 60% ethanol.
- **Strong Acid Solution:** 300 ml of concentrated sulphric acid was to 600 ml of distilled water. 4ml of concentrated HNO_3 was added and solution was diluted to make 1 liter working volume.

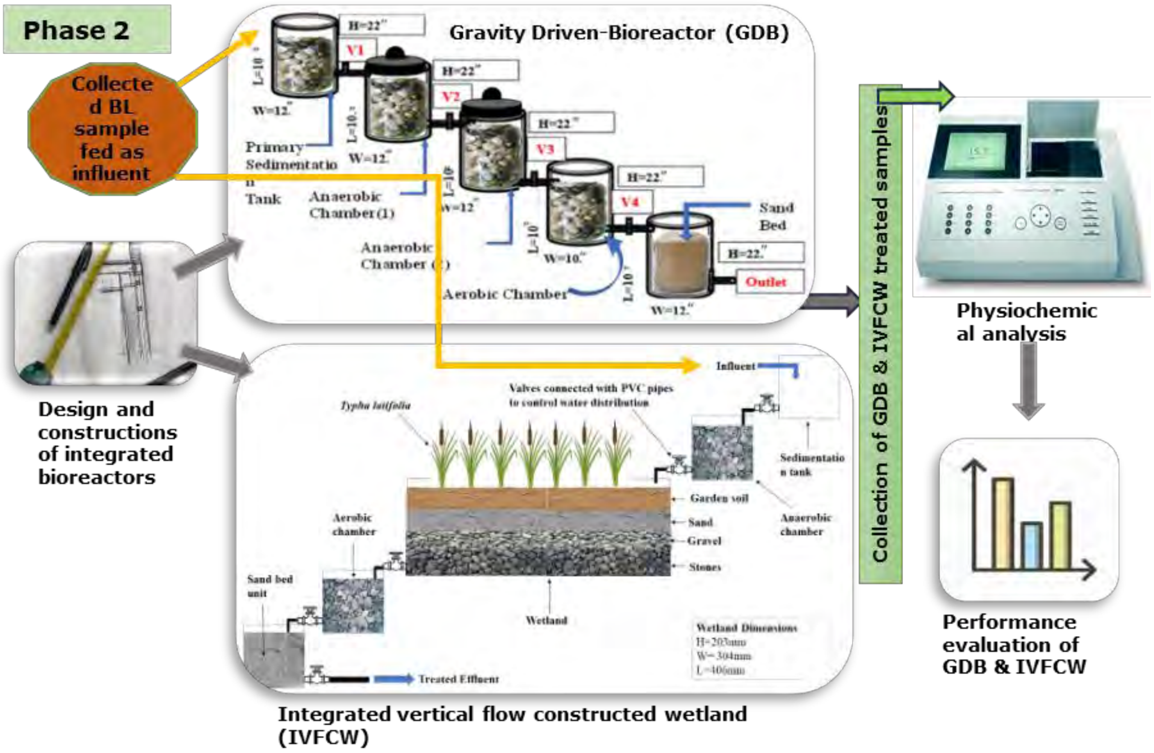
3.4.9.2.Procedure:

Flask contained 25 ml pre-filtered simple. A few drops of Phenolphthalein indicator and sting acid solutions were added, followed by 1 ml of ammonium molybedate and 25 microliters of stannous chloride. Sample settled for 10 mints under static conditions. The Pharo 300 spectroquant was used.

Phase 2

Objective 2: Design, construction, and performance evaluation of lab-scale gravity-driven biological reactor (GDB) and integrated vertical flow constructed wetland (IVFCW) for the treatment of paper and pulp industry effluent (black liquor)

<u>Graphical Methodology</u>



3.5. Designing and construction of gravity driven bioreactor (GDB) for the treatment of pulp and paper industry effluent

A lab-scale gravity-driven biological reactor (GDB) (having both aerobic and anaerobic chambers) was constructed in the Applied and Environmental Microbiology Lab of Quaid-i-Azam University, Islamabad, Pakistan. GDB consisted of five plexiglass chambers (dimension: length=10″, width =12″ and height= 22″) arranged on a tabletop at different heights to maintain water flow under the force of gravity. The first chamber was used as a primary sedimentation tank (PST), the subsequent two chambers were used as anaerobic chambers (ANC), the next chamber was used as aerobic chambers (AC), and the last chamber was a sand bed for the final treatment. Stones or pebbles having a rough surface area and volume of about $2mm^3$ were used as packing materials in all the chambers. In order to create an anaerobic environment, chambers were sealed with paraffin wax, while in aerobic chambers, oxygenation was carried out using aquarium pumps. All these chambers were interconnected with each other through a polyvinyl chloride (PVC) pipe system fitted with plastic valves (V1, V2, V3 and V4 having internal diameter of 0.5″and length of 10″) and all these units were placed in such manner that the flow of wastewater from one chamber to another was maintained under natural gravitational force. Before being subjected to working conditions, the stones were kept soaked with black liquor for 10 days as an incubation period to establish biofilm over the surface of stones suitable for wastewater treatment. The schematic illustration of GDB is schematically represented in Figure 3.1.

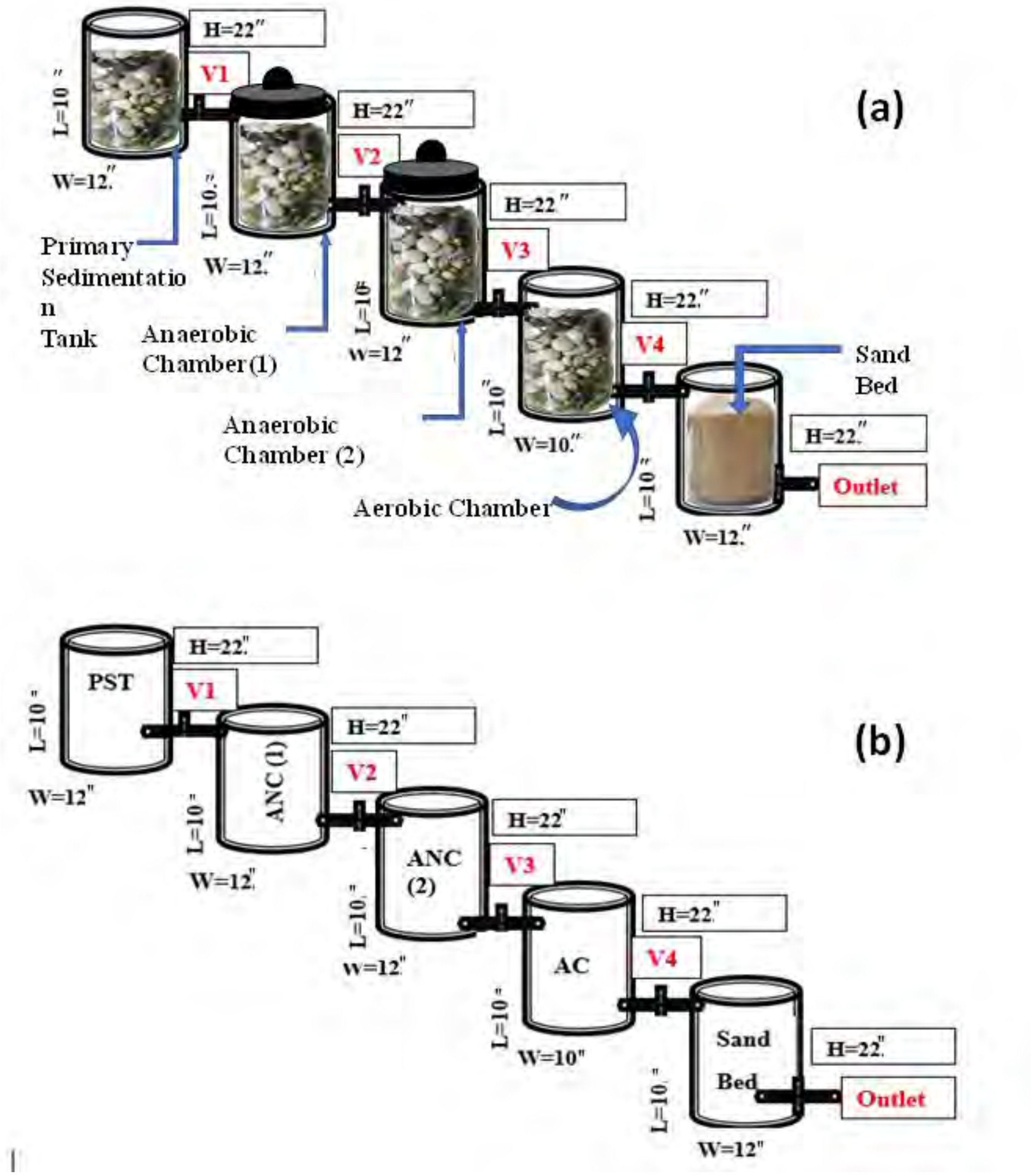


Figure 3.1The schematic illustration of GDB, (a) with filter media (b) without filter media

3.6.Operational set up of GDB.

Wastewater (black liquor, BL) was collected in pre-washed (with detergent dilute nitric acid and doubly deionized water, respectively) polyethene bottles from Premier Paper Mills Ltd. Lahore, Pakistan. The lab scale GDB (working volume 2.6 liters) was fed with 2 liters of black liquor (BL) daily in a continuous mode with an organic loading rate (OLR) of 1750 mg-COD/L-Day. The wastewater (2L) was fed to the first chamber, the primary sedimentation tank (PST), at the flow rate of approximately 12mL/minute till the PST filled; then, wastewater retained in the PST and was fed to the remaining chambers in such a way that retention time of almost 12 hours in PST to ensure settling of suspended particles and particulate material. After primary sedimentation, valve V1 was turned ON. Water flows to anaerobic chamber 1 (ANC-1) under the gravitational flow force and subsequently to anaerobic chamber 2 (ANC-2) by opening valve V2. A retention time of about 24 hours was given for anaerobic treatment. This anaerobic semi-treated black liquor flows to the aerobic chamber (AC-1) by opening valve V3. Black liquor was subjected to aerobic treatment for 24 hours and then passed to the sand bed for final filtration by opening valve V4. The hydraulic retention time of sand bed filtration was about 12 hours for each sample. Hydraulic retention time (HRT) and flow rate in each chamber were controlled using valves (V1-V4). Temperature was continuously monitored throughout the functional phase of GDB. This lab scale GDB was run from June 2021 to August 2021 under the natural temperature of workstation (35-40°C). Inlet (influent) and outlet (effluent) samples were taken after 72 hours in sterilized disposable bottles and analyzed for different physicochemical parameters.

3.7. Physicochemical characterization of wastewater before and after treatment through GDB

Various physicochemical characteristics like odor, BOD, COD, TDS, TSS, color, pH, phosphates, sulfates, total nitrogen, dissolved oxygen content, and lignin content were determined according to the standard described in section 3.4. All the readings were taken thrice, experiments were performed in triplicates, and results were recorded as the mean ± standard deviation (S.D.). $p < 0.05$ was considered as the minimum value for statistical significance.

3.8. Designing and construction of IVFCW for the treatment of black liquor

A mesocosm IVFCW integrated with anaerobic and aerobic chambers fitted with a sand bed for treating wastewater was constructed in the Applied Environmental Microbiology Lab at Quaid-i-Azam University Islamabad, Pakistan. This mesocosm IVFCW consisted of five plexiglass chambers (W=240 & H=220mm) arranged on a tabletop at decreasing heights (1ft) to maintain water flow under natural gravitational flow. These chambers were designated as one primary sedimentation tank (PST) with a loading capacity of 4 liters, one anaerobic chamber (ANC), one VFCW, one aerobic chamber (AC), and one sand bed chamber. Anaerobic conditions were maintained using paraffin wax, while aquarium pumps were used for oxygenation. In mesocosm, IVFCW, four layers were placed from top to bottom (8 cm soil, 4 cm sand,4 cm gravel, and 4 cm stones or pebbles), and the *Typha latifolia* was planted in the uppermost layer (soil). All these chambers were connected through polyvinyl chloride (PVC) pipes (having ½ inch diameter), and the flow rate was maintained using peristaltic valves. Before being exposed to working conditions, the middle two units underwent a soaking process with BL obtained from the pulp and paper industry in Lahore, Punjab-Pakistan. This soaking process lasted for 3 to 4 weeks, intending to facilitate the growth of plants and the development of biofilm in both aerobic and anaerobic chambers on filter/packing media. The IVFCW system was implemented and supervised for twelve samples (W1-W12), with a consistent HRT of 3 days for each sample. The temperature of the workstation was consistently monitored and was observed in the range of 35-40°C. Figure 3.2 illustrates a schematic depiction of the IVFCW system's various components.

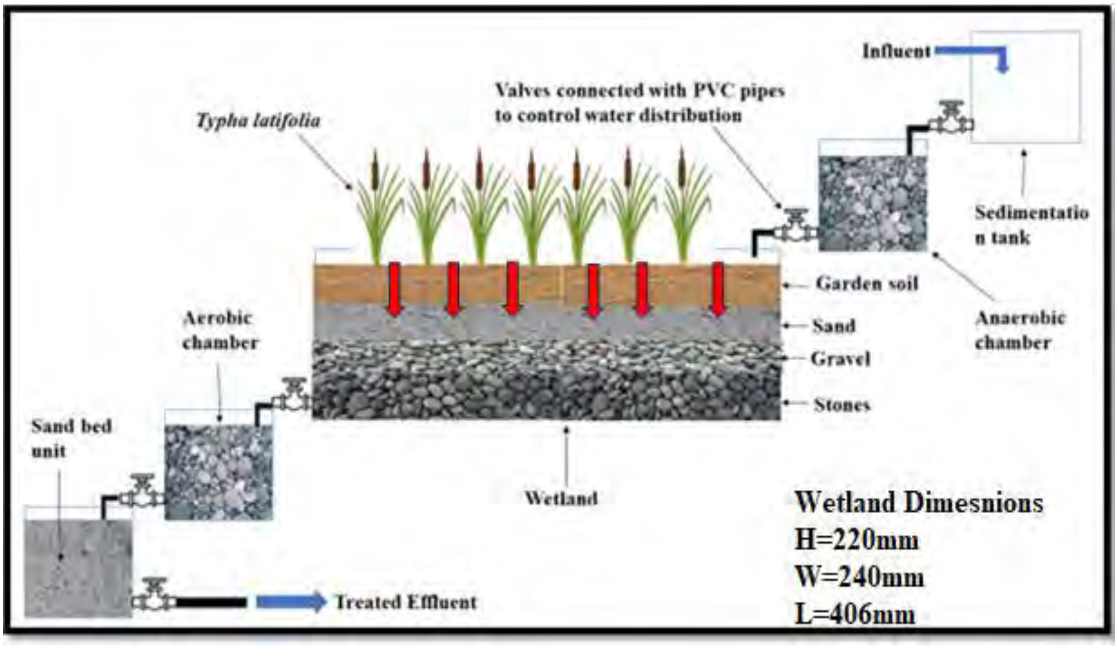


Figure 3.2 A schematic depiction of the IVFCW for the treatment of black liquor

3.9.Operational setup of IVFCW for wastewater treatment

Samples of BL were collected from the pulp and paper industry, Lahore, Punjab-Pakistan, in bulk quantity and stored at 4°C in the laboratory. This lab-scale vertical flow constructed wetland (working volume of 4liters) was fed with 4 liters of BL in a continuous mode with an organic loading rate (OLR) of 1750 mg-COD/L-Day.

Valves controlled each chamber's hydraulic retention time (HRT) and flow rate of 12mL/minute. The BL was fed to the first chamber, the PST. Then, wastewater was retained in the PST for 12 hours to ensure the settling of suspended particles and particulate materials and then fed to the remaining chambers so that wastewater remained in contact with anaerobic, aerobic, and wetland for the rest of the time (60 hours) for the efficient treatment. Each sample of wastewater completed 60 hours in the following sequence: anaerobic treatment for 24 hours and then passed sequentially to VFCW for 24 hours, aerobic treatment of 12 hours, and finally, the sand bed for filtration. A retention time of 72 hours was completed from PST to the sand bed for each sample. This IVFCW was monitored from June 2021 to August 2021 under the natural temperature of workstation (35-40°C).

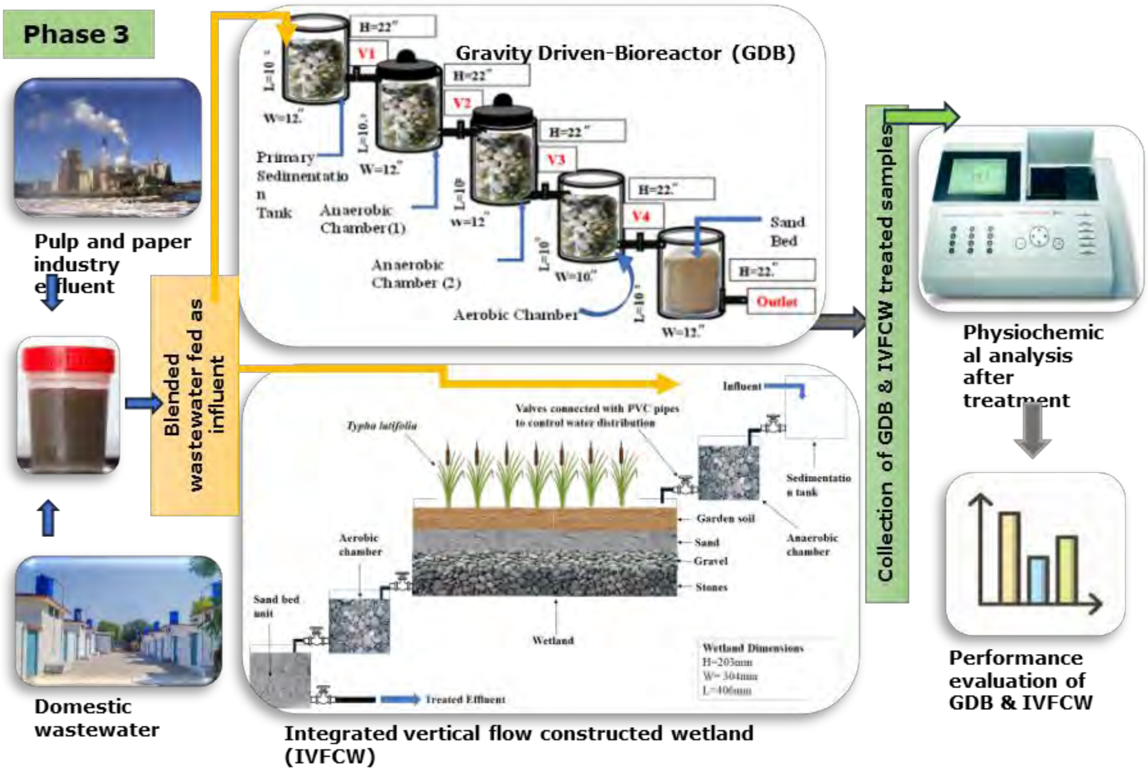
3.10. Physicochemical characterization of wastewater before and after treatment through IVFCW

The inlet (influent) and outlet(effluent) samples were taken in sterilized disposable polyvinyl bottles before and after treatment and subjected to different physicochemical parameter (odor, BOD, COD, TDS, TSS, color, pH, phosphates, sulfates, total nitrogen, dissolved oxygen content, and lignin content etc.) analyses according to standard procedure described in section 3.4. All these analyses were conducted in triplicates, and mean values were recorded (±SD).

Phase 3

Objective 3: Efficiency monitoring for GDB and IVFCW for the treatment of black liquor blended with domestic wastewater.

Graphical Methodology



3.11. Operational setup of GDB and IVFCW for blended wastewater treatment

GDB and IVFCW design construction are described in section 3.5 and 3.8 respectively. In order to enhance the efficacy of GDB and IVFCW, black liquor was mixed with equal proportion of domestic wastewater collected from residential colony of Quaid-i-Azam University, Islamabad Pakistan, both GDB and IVFCW were operated with a blend of black liquor and domestic wastewater at a 1:1 and maintained at an average organic loading rate of 1235 mg-COD/L-Day, at a flow rate of approximately 12 mL/minute for twelve samples (W1-W12) at a weekly frequency. The entire process was conducted from June 2022 to September 2022 at the workstation's natural temperature range of 35-45°C.Same operation conditions were employed as mentioned in section 3.6 and 3.9 for both bioreactors.

3.12. Physicochemical characterization of wastewater before and after treatment through GDB and IVFCW

Both GDB and IVFCW were operated for a period of four months and 12 samples (W1-W12) were of the inlet (influent) and outlet(effluent) were taken in sterilized disposable polyvinyl bottles before and after treatment and subjected to different physicochemical parameter (odor, BOD, COD, TDS, TSS, color, pH, phosphates, sulfates, total nitrogen, dissolved oxygen content, and lignin content etc.) analyses according to standard procedure described in section 3.4. All these analyses were conducted in triplicates, and mean values were recorded (±SD).

3.13. Microbiological Characterization of the wastewater before and after treatment through GDB and IVFCW

Two microbiological tests were performed routinely i.e. total count of bacterial population by plate count method and Most Probable Number (MPN) for the estimation of *Fecal coliforms.*

In this study culture media of different multinational companies like BioLife Chemical Company Italy, DIFCO Laboratories USA, Oxoid Company UK, Sigma Chemicals, ICI America 9211Portland and Merck Germany. All culture media were made according to the directions and standards of Manufacturer Company. In order to avoid contamination in microbiological work media was autoclaved at 121°C for 15minutes and for sterility test it was incubated at 37°C for 24 hours.

3.13.1. Total count of bacteria by plate count method (CFU)

In order to determine the total number of different bacterial communities such as *E.coli*, *Salmonella*, *Enterobacterace*, *Klebsiella* etc, a plate count method was used. As there is a high load of bacterial communities in the wastewater, it was serially diluted. For the serial dilution of each sample 6 test tubes, each containing about 9ml of distilled water, was autoclaved. In the first test tube 1ml from the sample was added and mixed thoroughly. After that 1ml from the first test tube was taken and transferred to the next test tube. In the same way different dilutions were made up to 10^{-5}. After making the different dilutions each was spread on the nutrient agar plate. For the spreading 0.1ml of sample was taken and spread over the plate by using sterile spreader. Plates were incubated at 37°C for 24 hours and the colonies were counted by using digital colony counter. All work was done in the laminar flow hood to avoid any sort of contamination. CFU was counted by using formula,

CFU / ml = Number of colonies × Dilution factor / Volume inoculated

3.13.2. Most Probable Number (MPN) Test

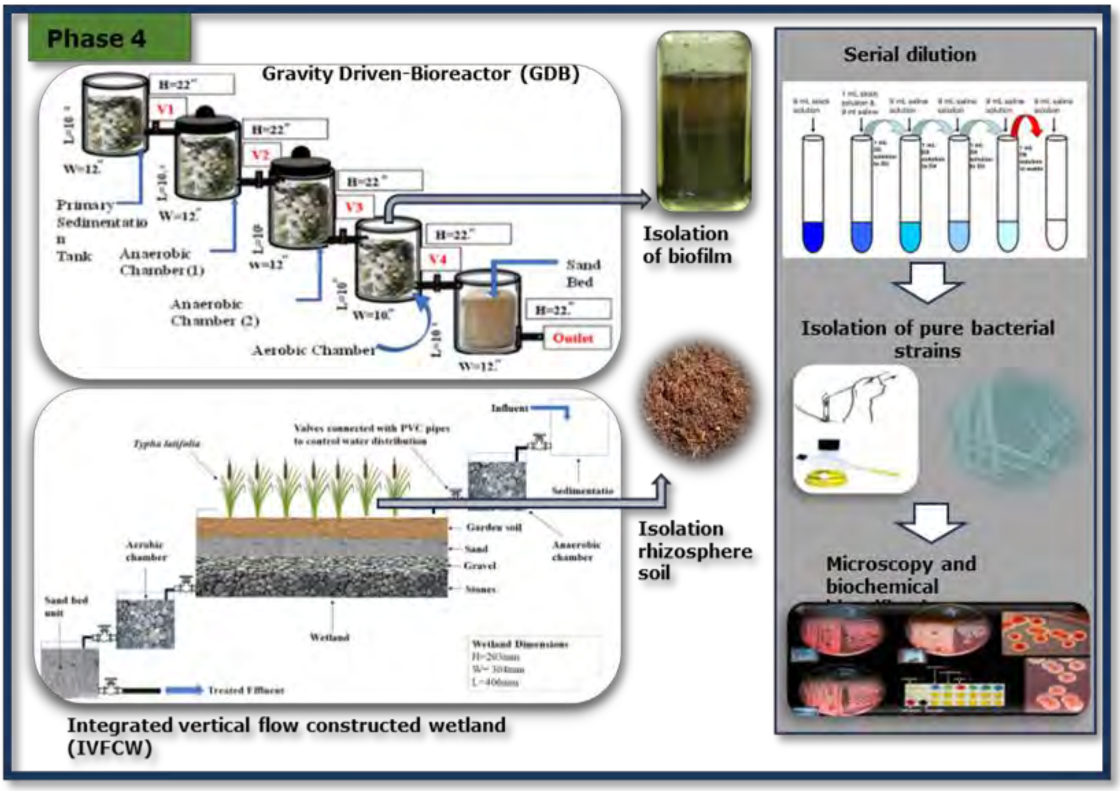
MPN is qualitative as well as quantitative test for water quality. Qualitatively it tells about the faecal contamination of water and quantitatively about the number of *faecal coliforms* and coliforms. Two different concentrations of Lactose Broth, LB1x and LB 2x were used under sterile conditions. Production of gas bubbles in the Durham tubes is an indication for presence of *faecal coliforms.*

3.13.2.1. Procedure:

To carry out the MPN, for each sample, 9 test tubes were arranged in 3 sets and labelled as 0.1 x, 1.0x and 2x. Each tube contains an inverted Durham tube for the determination of gas bubbles. 10ml media of single strength (LB1x) was added in the first two sets (0.1 x and 1.0x) and 10 ml of double strength media (LB2x) was added in the third set (2x). All sets were autoclaved for 15 minutes at 121°C. After autoclaving bubbles were removed from the Durham tubes. After 30 minutes 0.1ml of sample was added in the first set, 1ml in the second and 2 ml in the third set under sterile conditions. Caps were closed tightly, and tubes were incubated for 24 hours at 37°C. Results were checked as gas producers (+) and non-gas producers (-) and results were recorded according to MPN index.

Phase 4

Objective 4: Study of biofilm: Developed on stone media in GDB and from the rhizosphere of IVFCW.



3.14. Biofilm characterization

Stones having a dense biofilm were picked from the aerobic chamber of GDB and rhizosphere of IVFCW with sterile handler and biofilm was removed with the help of sterile spatula in pre autoclaved glass container and mixed with 25mL of autoclaved distilled water. Sample was vortex form 10 minutes. Different dilutions up to 10^{-5} were made according to standard protocols and from each dilution 0.1mL was spread on the nutrient agar plate and incubated for 24 hrs. at 37°C. Different colonies were picked from the nutrient agar plates and spread over various differential Medias like SSA (*Salmonella sheigella agar*), *E. coli/coliform* agar, MacConkey agar, Blood agar plates and eosin methylene blue agar. Plates were incubated overnight and were checked for growth.

3.15. Characterization and identification of aerobic bacterial colonies

3.15.1. Morphological characterization

Each microbe has a specific shape and morphology. Based on different characteristics like form, size, opacity, pigmentation, and colony morphology like elevated colonies, spread colonies and margins of the colonies bacterial communities were differentiated.

3.15.2. Gram Staining and microscopic characterization

Hans Christian Gram devised a technique to differentiate Gram Positive and Gram-negative bacteria. Each bacterial colony was subject to differential technique. Gram positive bacteria give purple color while Gram negative bacteria give pink color under microscope.

3.15.3. Biochemical Identification

The identification of isolated bacterial colonies was conducted in accordance with the guidelines outlined in the 9th Edition of Bergey's Manual of Determinative Bacteriology. Following tests were carried out,

3.15.3.1. Catalase Test:

With the help of sterile loop bacterial colony was transferred to aseptic glass slide and then a drop of hydrogen peroxide was added on it. Catalase positive colonies produce bubbles while others do not.

3.15.3.2. Nitrate Reduction Test:

Nitrate reduction broth was used as a medium.

Solution A		**Solution B**	
Reagent	Quantity	Reagent	Quantity
30% acetic acid (5M)	1000 mL	Acetic Acid (5M)	1000 mL
Sulphuric Acid	8 mL	Alpha-napythylamine	5 g

The nitrate reduction test tubes were inoculated aseptically and incubated for 24 hours at a temperature of 37°C. The appearance of a cherry red color indicates positive results when solutions A and B are added. At the same time, the presence of a cherry red color upon adding zinc dust indicates negative results. This test provides information on converting nitrate (NO^{-3}) to nitrite (NO^{-2}).

3.15.3.3. Citrate Utilization Test (CUT)

Citrate Utilization Test is usually carried out to check the ability of the microbes to utilize citrate as a sole source carbon source. Slants of Simon citrate agar was made and incubated for overnight at 37°C and results were checked for growth of microbes. Color change from green to blue along with growth showed a positive result.

3.15.3.4. TSI (triple sugar iron) Test

The triple sugar iron slants, containing 5% NaCl, were inoculated with sterile technique using an inoculating needle. The inoculation process involved initially puncturing the Centre of the bottom and subsequently streaking the surface of the slant. Tubes were incubated at 37°C for 24 hours. The appearance of pink color showed alkaline reaction while yellow color of the butt showed acid production and fermentation. The color change in the butt was due to glucose fermentation while in the slants was due to lactose fermentation.

3.15.3.5. Oxidase Test

Oxidase reagent was prepared by addition of 1g of Tetramethyl-pphenylenediamine·2HCl in 1000 ml of distilled water. 2-3 drops of oxidase reagent were placed on the filter paper and bacterial colony was transferred over it. The appearance of dark blue color showed a positive oxidase test.

3.15.3.6. Methyl Red Vogas-Proskaeur (MRVP) Test

5ml of autoclaved MR-VP broth was transferred to test tubes and then inoculated under sterile conditions and then incubated for a day or two at a suitable temperature. On growth appearance tubes were divided into two groups for Methyl Red and Vogas Proskaeur test.

- **Barritt's Reagent:**

Barritt's Reagent comprises of solution A & B.

Solution A		**Solution B**	
Reagent	**Quantity**	**Reagent**	**Quantity**
Alpha-naphthol	5gm	Potassium hydroxide	40g
Ethyl alcohol	100ml	Deionozed Water	100ml

- **MR-VP Broth: Methyl Red Indicator:**

Reagent	**Quantity**
Methyl Red	0.1g
95% Ethanol	300ml
Dis.Water	2000ml

For methyl red test 2 drops of indicator were added to one set of test tubes and appearance of red color was taken as positive result. Similarly for VP test, appearance of red color on addition of 0.6ml of a-naphthol and 0.8ml of KOH and shaken for 2-3 minutes show a positive result and no change in color was taken as negative result.

3.15.3.7. Urease Test:

Urea broth (0.9/95 g/ml) was needed for urease testing. Syringe-filtered 5ml of urea was added to 0.2/5 g/ml distilled water after autoclaving media. Inoculation was followed by a day or two of incubation and recording. The appearance of light pink color in the media showed a positive result while no color change was taken as negative result.

CHAPTER 4

RESULTS

Phase 1

Results -Objective 1

4. Results

4.1. Physicochemical characterization of PPI effluent, isolation, and characterization of lignin degrading bacterial strains

4.1.1. Isolation and screening of lignin degrading bacteria

4.1.1.1. Primary Screening of bacterial strains

The BL samples were collected from Premier pulp and paper industry, Lahore, Punjab-Pakistan, were used for the isolation of the lignin degrading microorganisms. BL has sufficient carbon source in the form of lignin, therefore, support growth of diverse microbial communities. After serial dilution of the samples, 13 pure bacterial strains were obtained. These bacteria were then analyzed for their metabolic potential to use and degrade lignin on agar plate containing lignin. Based on primary screening three bacteria namely TR-1, TR-5 and TR-8 were found to be comparatively better growth than the other isolates. The lignin degradation zones for the bacterial strains were TR-1, 14 mm, TR-5, 15 mm, and TR-8 12 mm. The zones for other strains were comparatively lesser. Based on these results, strains were subjected to secondary screening assay.

Table 4. 1 Zones of lignin degradation (mm) and growth of bacterial strains

Bacterial Strain	Zone of lignin degradation (mm)	Growth
TR-1	14	+++
TR-2	9	++
TR-3	10	++
TR-4	9	++
TR-5	15	+++
TR-6	6	++
TR-7	9	+
TR-8	12	+++
TR-9	3	++
TR-10	11	++
TR-11	9	++
TR-12	7	++
TR-13	8	++

(Key: +++ rich growth, ++, medium growth, + less growth)

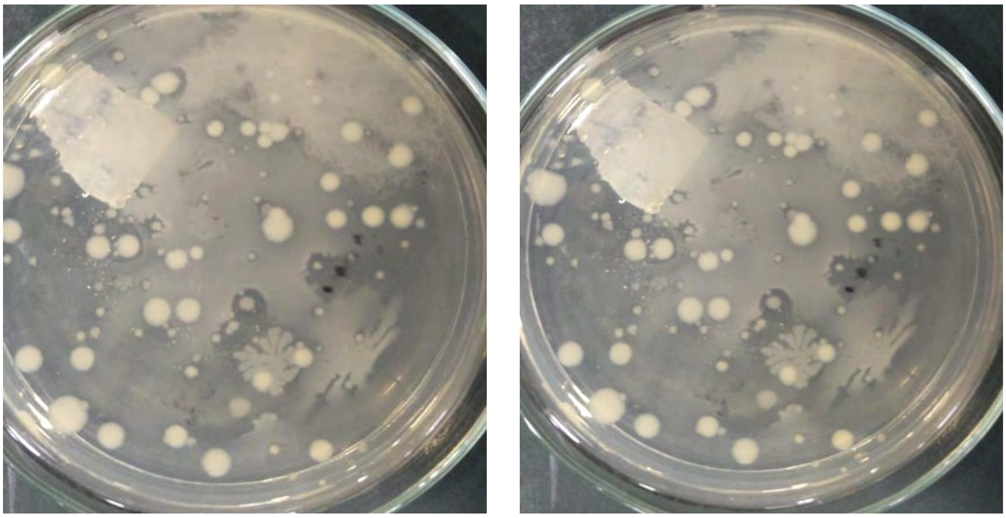


Figure 4.1 Bacterial colonies on nutrient agar plate after serial dilution

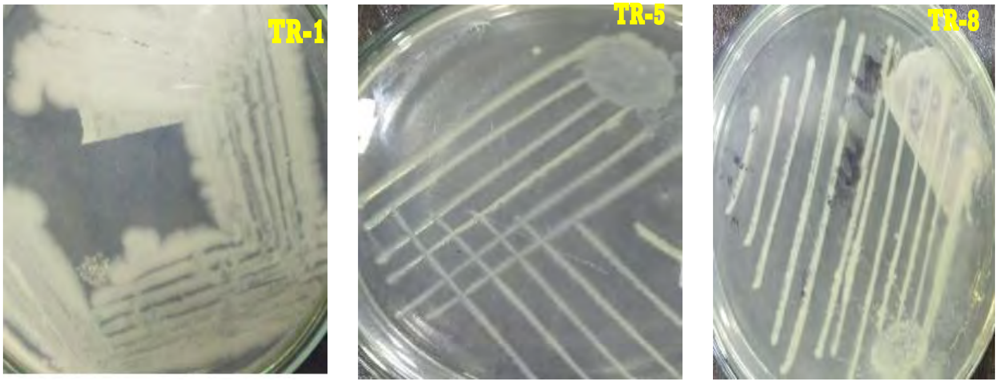


Figure 4.2 Purification of selected bacterial strains by streak plate method

4.1.1.2. Secondary screening based on growth, lignin degradation and color reduction assay in shake flask fermentation.

The three bacterial strains (TR-1, TR-5, and TR-8) showed significant growth and lignin degradation results on the agar plate assay (as shown in figure 4.3) were subjected to the secondary screening using MSM broth amended with 2% black liquor containing lignin at, 150 rpm for 96 hours at different pH and temperature. Bacterial strains were observed for their growth, ability to degrade lignin and decolorize the black liquor in shake flask experiment under varying pH and temperatures. Our results indicated that the bacterial strains TR-1, TR-5 and TR-8 showed positive results in terms of growth, lignin degradation and color reduction.

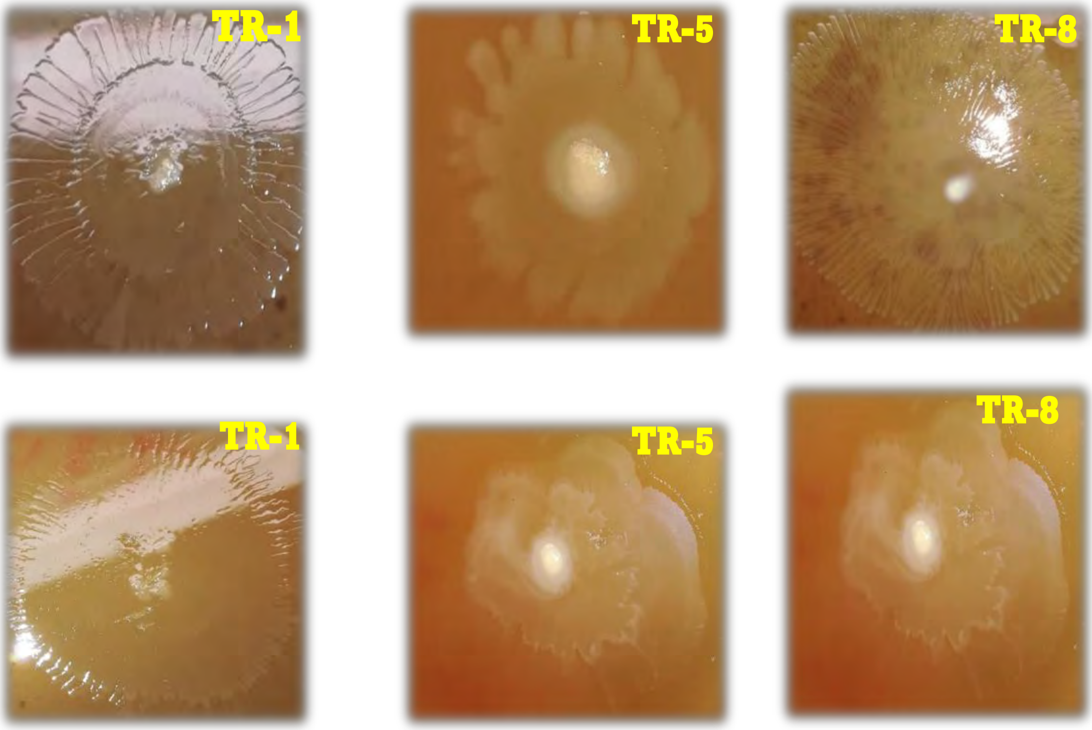


Figure 4.3 Growth of selected bacterial strains TR-1, TR-2, & TR-3 on MSM amended with 2% lignin.

In the figure 4.4, bacterial strain TR-1 showed a maximum growth of 3.12g/l at 41°C and pH 8. The highest lignin degradation was recorded for the strain TR-1 showed a maximum 77% lignin degradation and color reduction of 65% was observed (Figure.4.5). The fermentation conditions where maximum lignin degradation and color reduction achieved were 37 °C, and pH 8.

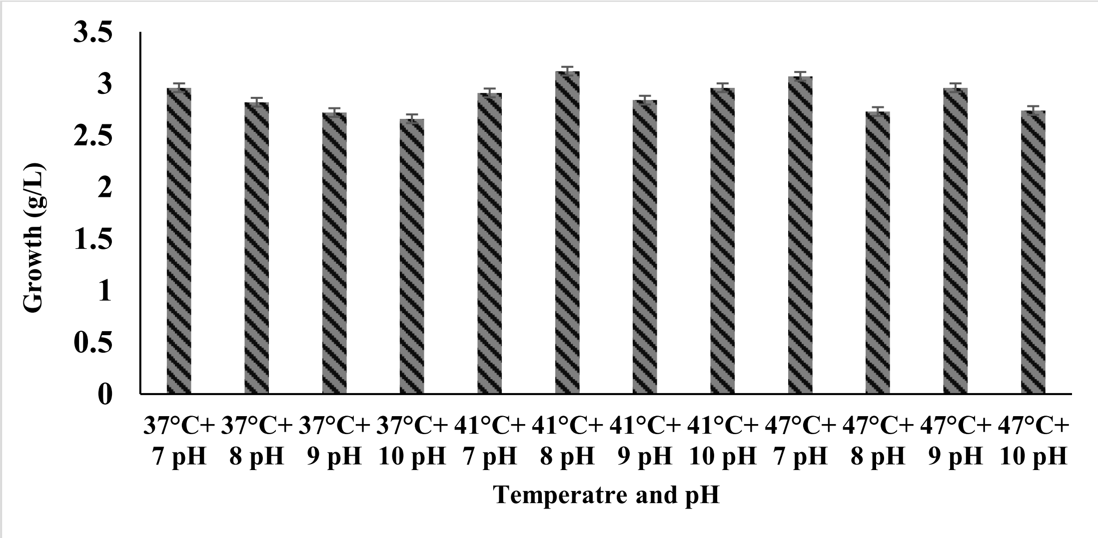


Figure 4.4 Growth pattern of bacterial strain TR-1 under varying pH and temperatures

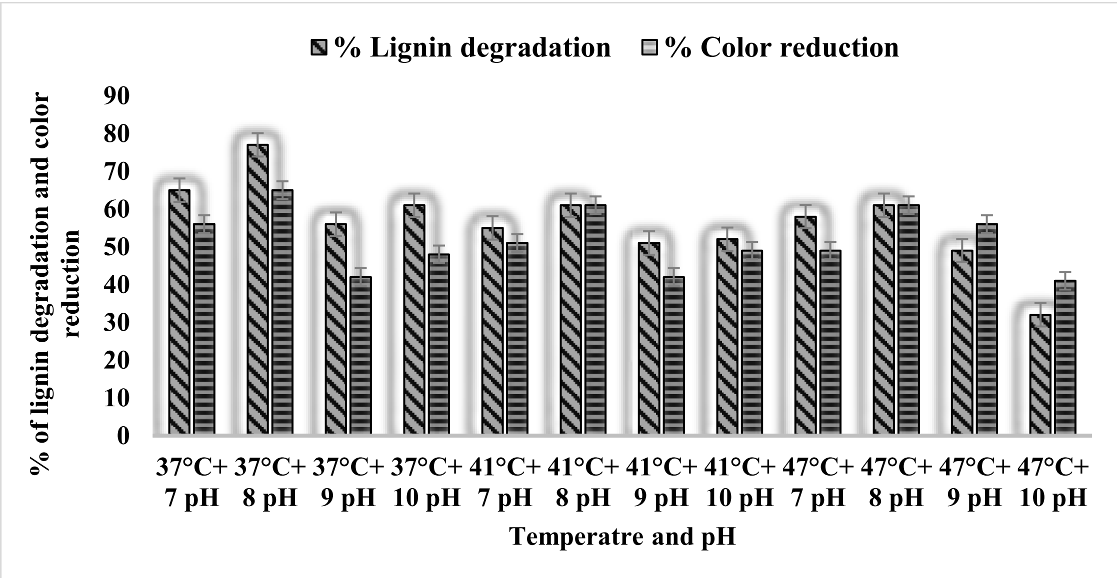


Figure 4.5 Lignin degradation and color by bacterial strain TR-1 under varying pH and temperatures

In case of the bacterial strains TR-5 a maximum growth of 2.9g/L was recorded at 37°C and the pH was 8 after 96 hours of incubation period (figure 4.6). The color reduction and lignin degradation were maximum 65% (pH 8) and 65% (pH 7) respectively at temperature 37°C as shown in the figure 4.7.

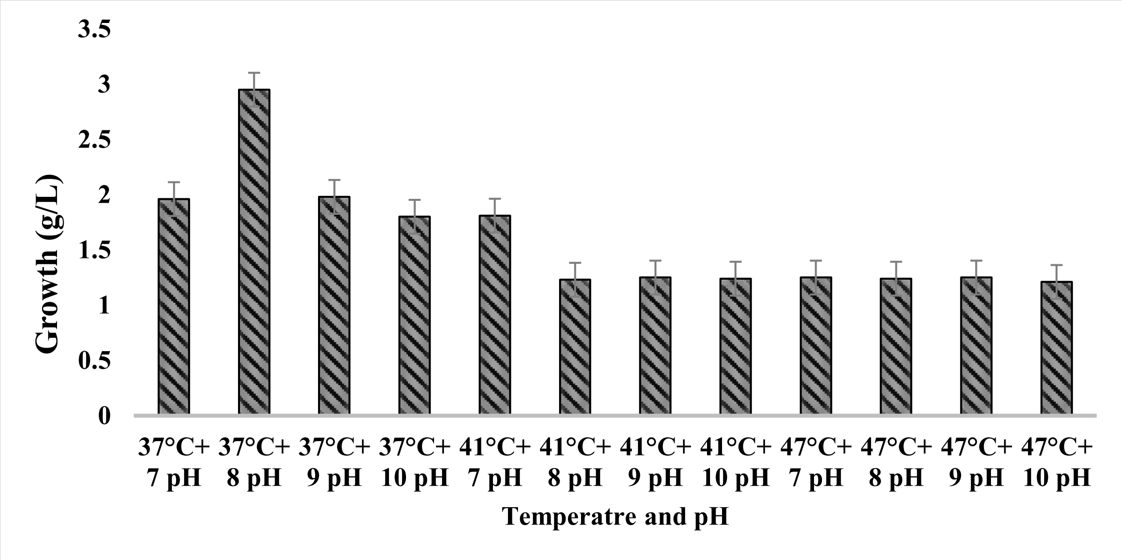


Figure 4.6 Growth pattern of bacterial strain TR-5 under varying pH and temperatures

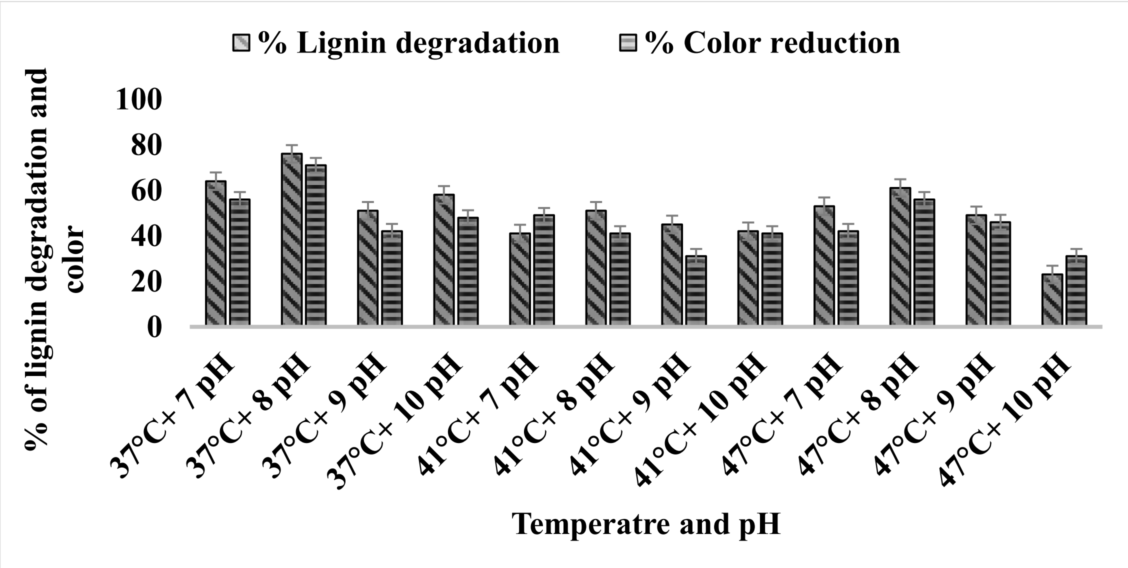


Figure 4.7 Lignin degradation and decolorization using 2% black liquor by the bacteria strain TR-5

The results of the growth, color reduction and lignin degradation for the bacterial strain TR-8 are presented in figures 4.8 and 4.9 respectively. Results indicated that TR-8 showed maximum growth (2.34g/L) at 37 °C and pH 8. Similarly, maximum lignin degradation (66%) and color reduction (65%) were observed at 37 °C. However, the pH for maximum lignin degradation was pH 7 but for color reduction was 8.

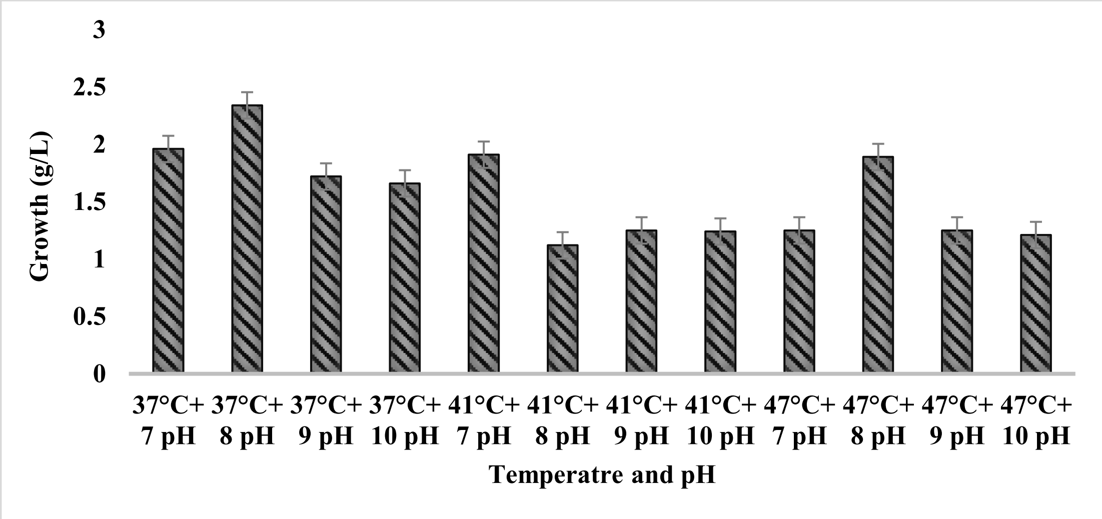


Figure 4.8 Growth pattern of bacterial strain TR-8 under varying pH and temperatures

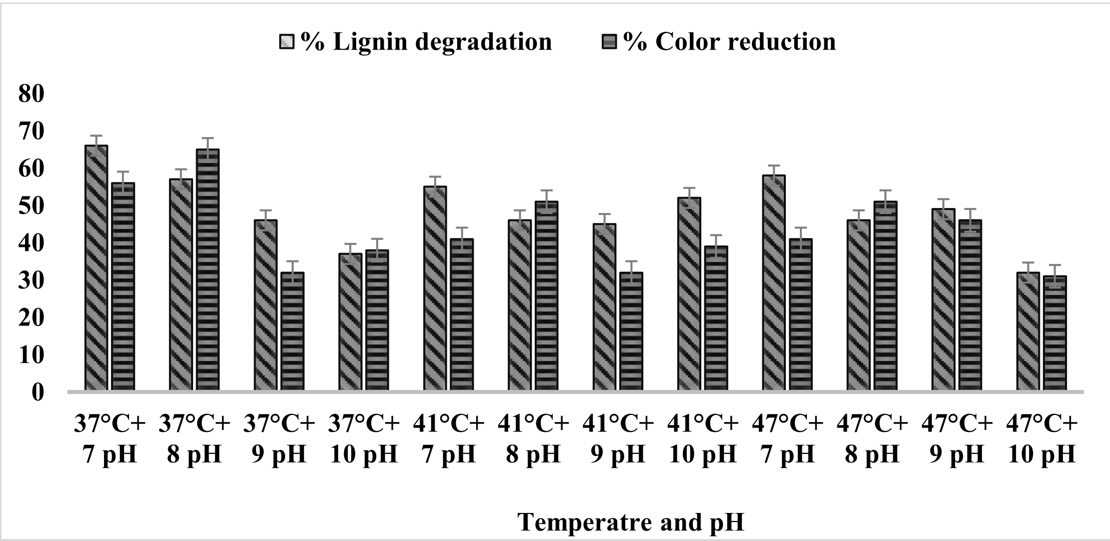


Figure 4.9 Lignin degradation and color reduction using 2% black liquor by the bacterial strain TR-8

4.1.2. Biochemical identification of selected bacterial strains

Table 4. 2 Biochemical identification of selected bacterial strains

Strain Code	Gram's Staining	Morphology	Oxidase	Catalase	Urease	MR	VP	Indole	Nitrate	Citrate	TSI	H_2S	Identified Genera
TR-1	G^+	Rods	+	+	-	-	+	-	+	+	A	+	***Bacillus subtilis***
TR-5	G^-	Chain	+	+	-	-	+	-	+	+	A	-	***Bacillus cereus***
TR-8	G^-	Rods	-	+	-	+	-	-	+	+	-	-	***Pseudomonas***
(Key: + = positive test; - = negative test; G^+ =Gram's Positive; G^-= Gram's Negative; A = Acidic)													

4.1.3. Physiochemical characterization of wastewater

The wastewater samples were collected, and physicochemical characterization was done. It was noted that the BL samples were high strength as compared to domestic wastewater, as indicted in Table 4.3. BL was alkaline with high lignin contents. Furthermore, total dissolved and suspended solids were found to be 3571.455±48.23 and 1890.411±57.28 mg/L respectively, with 1750.56±35.50 chemical oxygen demand (COD). The nitrates (as TN) and sulphates in the BL sample were 160.911± 4.48 and 2570.98± 62.80 mg/L.

Table 4. 3 Physicochemical characterization of wastewater

Physicochemical characterization of wastewater			**Permissible limits**	
Parameters	**Pulp and paper industry effluent, Black liquor (BL)**	**Domestic wastewater**	**NEQ's Pakistan**	**USEPA**
pH	**8.2±0.15**	**7.3±0.14**	**6–9**	**5–9**
COD (mg/L)	**1750.56±35.50**	**410.55±22.54**	**150**	**120**
BOD_5 (mg/L)	**1513.53± 32.35**	**365.11±11.22**	**50-75**	**5<**
Sulfates (mg/L)	**2570.98± 62.80**	**174.54±3.44**	**400**	**252**
Phosphates (mg/L)	**4.2.21±0.117**	**2.5±0.67**	**NGV**	**0.05**
TDS (mg/L)	**3571.455±48.23**	**1255.49±23.11**	**3500**	**500-1000**
TSS (mg/L)	**1890.411±57.28**	**350.77±2.91**	**200**	**25-80**
Total Nitrogen (mg/L)	**160.911± 4.48**	**33.02±2.91**	**NGV**	**10**
Lignin (mg/L)	**189.33±6.53**	**0.56±0.018**	**NGV**	**0.05**
Values are given as mean with S.D(n=3)			**Key: NGV=Not given value, NEQ's national environmental quality standard**	

Phase 2

Results-Objective 2

4.2. Performance evaluation of lab-scale gravity-driven biological reactor (GDB) and integrated vertical flow constructed wetland (IVFCW) for the treatment of paper and pulp industry effluent (black liquor)

4.2.1. Performance evaluation of lab-scale gravity-driven biological reactor (GDB) for the treatment of paper and pulp industry effluent (black liquor)

Pulp and paper industry effluents contain multiple pollutants of complex nature and environmentally harmful, thus requires creative, cost-effective, and sustainable methods. A unique design incorporating multiple treatment modalities, the gravity-driven bioreactor (GDB), presents a suitable novel approach. This objective evaluates the lab-scale gravity-driven bioreactor's performance for treating black liquor. The evaluation focuses on a variety of physicochemical factors, highlighting the effectiveness of the bioreactor in treating the effluent. Various parameters, including biological oxygen demand $(BOD)_5$, chemical oxygen demand (COD), phosphates, sulphates, total nitrogen (TN), total dissolved solids (TDS), total suspended solids (TSS), electrical conductivity (EC), turbidity, dissolved oxygen (DO), pH variation, and the degradation of recalcitrant compounds such as lignin, were subjected to meticulous analysis. The results demonstrate that the GDB has the ability to effectively decrease pollutant levels, indicating its potential to bring about transformative changes in the treatment of effluents from the pulp and paper industry. This chapter provides a comprehensive analysis of the experimental results, presenting significant findings about the performance of the GDB and its potential implications for sustainable wastewater management in the sector.

Table 4. 4 Efficacy of GDB for the treatment of pulp and paper industry effluent

Sample Code	COD (%Efficiency)	BOD (%Efficiency)	DO (%Increase)	pH (%Variation)	EC (%Efficiency)	TDS (% Efficiency)	TSS (% Efficiency)	PO_4^{3-} (%Efficiency)	SO_4^{2-} (% Efficiency)	TN (%Efficiency)	Lignin (%Efficiency)	Turbidity (%Efficiency)
S1	73.8403	63.97908	151.9793	27.57837	52.0431	50.11783	92.51701	54.08745	51.19942	58.62069	48.95397	87.59152
S2	74.36625	67.03155	144.5578	27.52294	54.59288	51.14173	92.52276	59.82684	53.34755	54.96432	46.97529	87.8363
S3	69.84492	66.6226	161.6725	40.12784	47.4802	56.47608	92.39964	47.8489	52.87904	55.95	44.69226	87.79327
S4	68.92021	68.78967	228.0357	33.45242	56.89421	54.50949	93.6296	46.40434	54.16114	54.86111	46.33464	88.53413
S5	74.30838	67.64203	328.5714	31.75101	65.6568	50.6132	91.86903	58.2199	54.4494	57.27153	48.20883	88.13585
S6	74.79914	66.65927	149.7364	37.16195	52.68293	54.40615	92.47552	46.79696	54.07693	53.59504	52.30898	88.67051
S7	73.88951	69.65443	124.0283	30.0885	53.16654	55.31872	92.14872	61.00917	54.02333	54.96432	55.50472	88.38455
S8	71.27103	67.80269	182.9314	32.54148	56.04912	55.68445	92.93249	57.14286	55.82913	58.51887	55.46456	88.76961
SS9	77.5794	65.14323	218.9687	27.02703	59.90465	54.6518	91.58811	64.4485	54.72173	57.46897	56.58231	87.74451
S10	77.66507	68.89376	231.6888	30.80169	62.77659	57.16118	93.17796	48.87586	55.56374	54.75743	58.05073	88.14137

4.2.1.1. Biological oxygen demand (BOD)

The assessment of ten samples (S1-S10) of untreated and black liquor treated with the GDB reveals a significant reduction in BOD_5 levels. The untreated samples had an average BOD of around 1530.53 mg/L. In contrast, the samples subjected to treatment with GDB revealed a much lower average BOD of 498.70 mg/L, showing an average reduction of approximately 67.46% as shown in figure 4.10. The results of this study provide empirical support for the effectiveness of the GDB in decreasing BOD_5 concentrations in BL. This underscores the efficacy of the GDB as a very promising strategy for mitigating BOD-related challenges in the treatment of effluent originating from the paper and pulp industry.

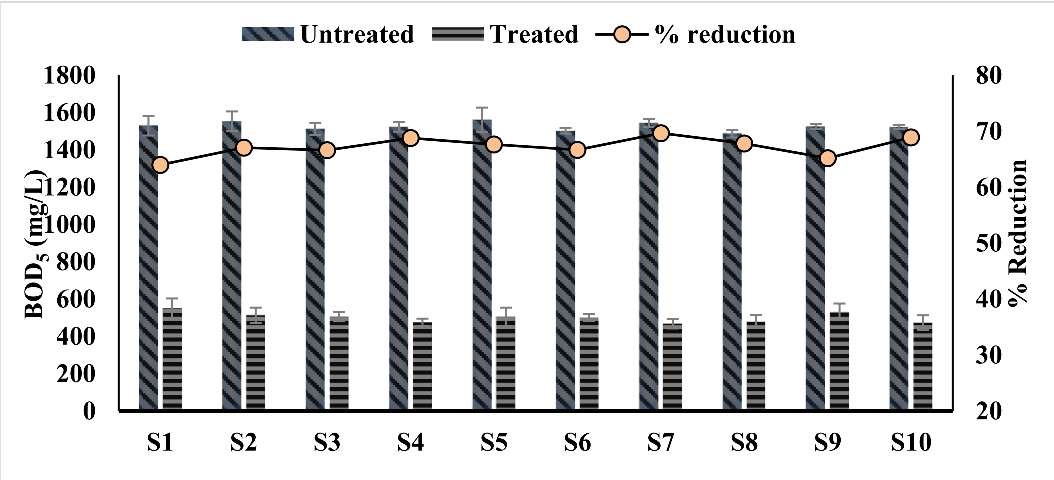


Figure 4.10 BOD of untreated and GDB-treated pulp and paper industry effluent.

4.2.1.2. Chemical oxygen demand (COD)

The present study examines the impact of the GDB treatment on the COD of black liquor. Ten samples, labelled S1-S10, were analyzed to compare the untreated black liquor with the treated samples. The results indicate a significant decrease in COD after implementing the GDB treatment. The untreated samples had an average COD of roughly 1763.93 mg/L, whereas the samples treated with GDB showed a much lower average COD of around 464.66 mg/L, indicating an average reduction of approximately 73.50% % as shown in figure 4. 11. The results, as mentioned earlier, emphasize the effectiveness of the GDB in effectively reducing COD levels in BL, thereby demonstrating its potential as a viable approach for tackling difficulties connected to COD in the treatment of effluent from the pulp and paper industry.

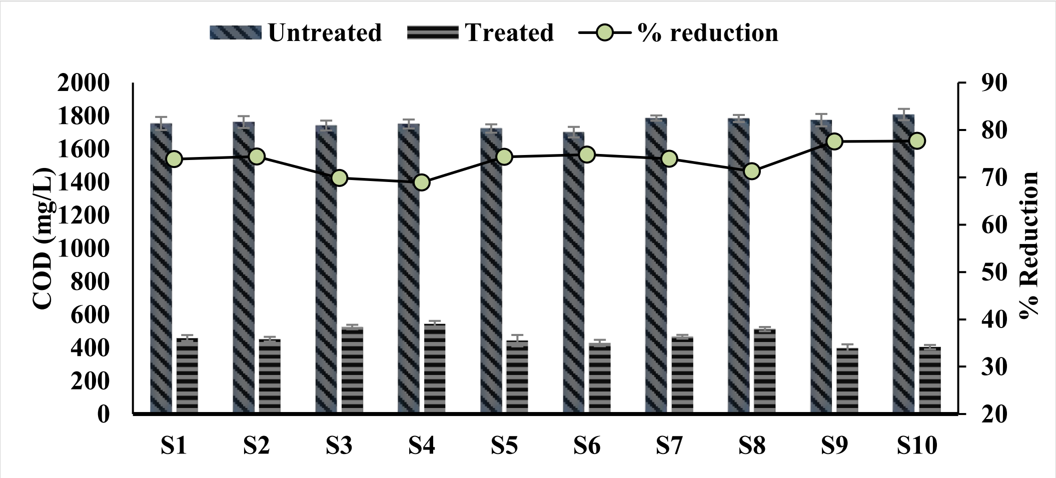


Figure 4.11 COD of untreated and GDB-treated pulp and paper industry effluent.

4.2.1.3. Dissolved oxygen (DO)

The analysis of ten samples (S1-S10) of untreated black liquor compared to black liquor subjected to treatment utilizing the GDB demonstrates a noteworthy augmentation in levels of DO. The untreated samples had an average DO concentration of around 1.81333 mg/L. In contrast, the samples treated with GDB showed a much higher average DO concentration of about 5.022 mg/L, showing an average increase of approximately 177.91%. The results of this study highlight the effectiveness of the GDB in increasing DO levels in black liquor as shown in figure 4.12.

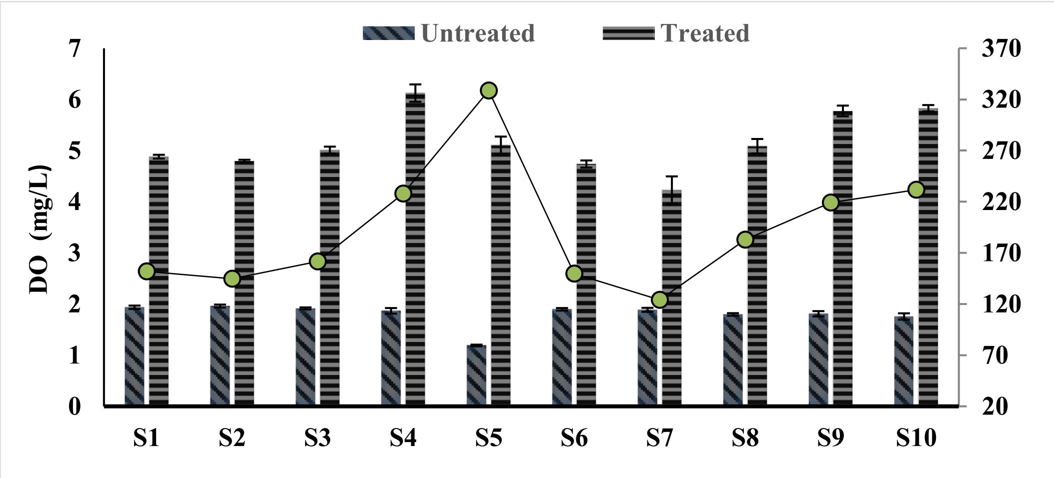


Figure 4.12 Dissolve oxygen of untreated and GDB-treated pulp and paper industry effluent.

4.2.1.4. Electrical conductivity (EC)

Assessing ten samples (S1-S10) of untreated and treated black liquor subjected to treatment using the GDB highlights a significant decrease in EC as depicted in figure 4.13. The untreated samples had an average EC of around 932.07 µS/cm. In contrast, the samples treated with GDB showed a much lower average EC of roughly 400.20 µS/cm, indicating an average decrease of approximately 57.11%. The findings of this study demonstrate the efficacy of the GDB in effectively reducing EC levels in black liquor. This suggests that the GDB has the potential to be a very successful method for resolving problems related to conductivity in the treatment of effluent from the pulp and paper industry.

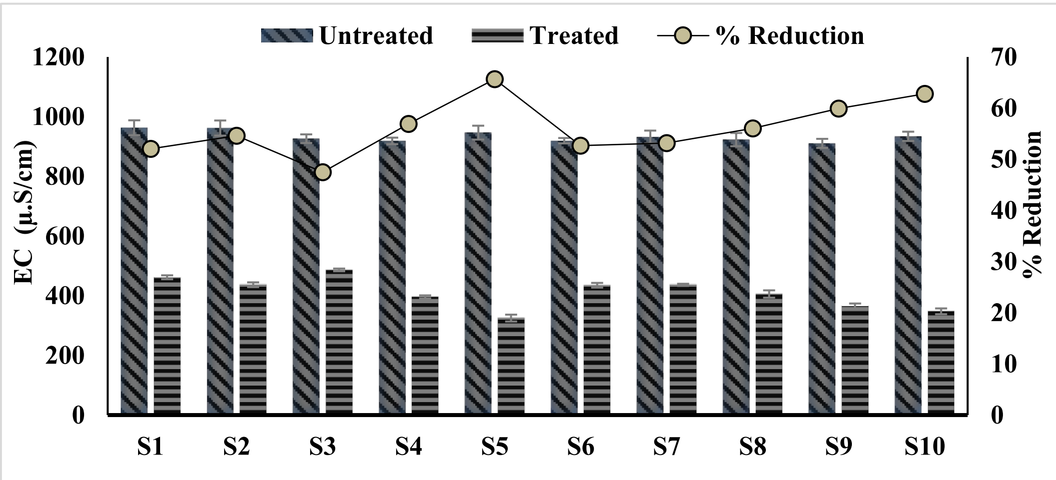


Figure 4.13 Electrical Conductivity of untreated and GDB-treated pulp and paper industry effluent.

4.2.1.5. Lignin content

The study of ten samples (S1-S10) of untreated and treated black liquor using GDB reveals a significant decrease in the amount of lignin present. The untreated samples had an average lignin content of about 1184.70 mg/L, while the samples treated with GDB displayed a drastically reduced average lignin content of around 576.15 mg/L, indicating an average decrease of roughly 51.35% as shown in figure 4.14. The results of this study highlight the effectiveness of the GDB in effectively reducing lignin concentrations in black liquor.

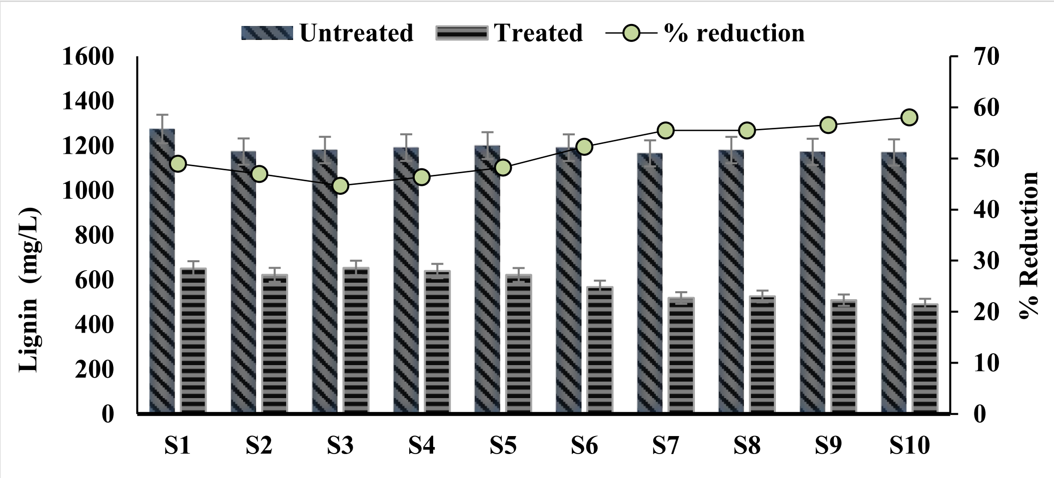


Figure 4.14 Lignin content of untreated and GDB-treated pulp and paper industry effluent.

4.2.1.6. pH analysis

Investigating ten samples (S1-S10) of raw and GDB treated black liquor reveals a notable difference in pH values, the average pH of untreated samples was 7.777, the treated samples exhibited an average pH of 7.65, resulting in an average pH change of 0.127. The maximum pH change observed in any individual sample was 11.66%, indicating variations in the pH modification efficiency of the Gravity-Driven Bioreactor (GDB) across the samples. and the results are indicated in figure 4.15.

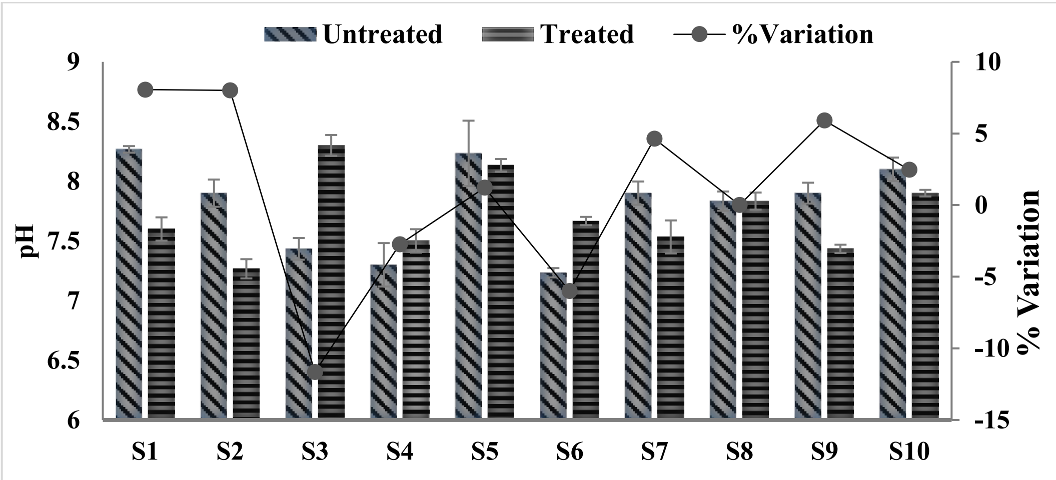


Figure 4.15 pH of untreated and GDB-treated pulp and paper industry effluent.

4.2.1.7. Phosphates-orthophosphates

The current investigation involves the analysis of ten samples (S1-S10) of black liquor in its untreated state, as well as black liquor that has undergone treatment utilizing the GDB. The results of this analysis highlight a significant decrease in phosphate concentrations in the treated black liquor compared to the untreated samples. The untreated samples had an average phosphate concentration of around 3.29467 mg/L. In contrast, the samples treated with GDB showed a much lower average phosphate content of roughly 1.503 mg/L, indicating an average reduction of approximately 54.39% as depicted in Figure 4.16. The results highlight the efficacy of the GDB in substantially reducing phosphate levels in black liquor.

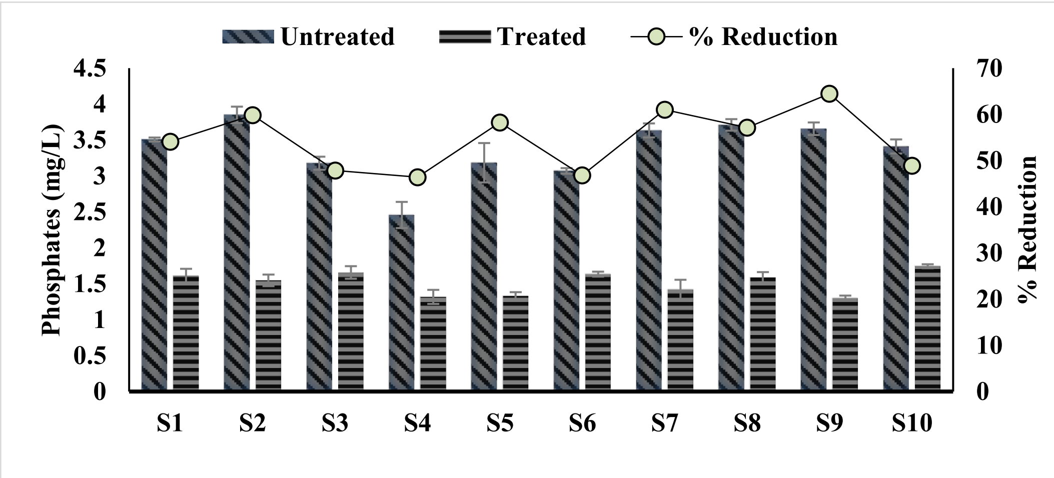


Figure 4.16 Phosphates of untreated and GDB-treated pulp and paper industry effluent.

4.2.1.8. Sulfates

The analysis of ten samples (S1-S10) of untreated and GDB-treated black liquor demonstrates a notable reduction in sulfate levels. The untreated samples showed an average sulfate concentration of around 2266.93 mg/L, while the GDB-treated samples exhibited a substantially decreased average sulfate concentration of approximately 1045.33 mg/L, signifying an average reduction of about 53.94%, as presented in Figure 4.17. These findings underscore the GDB's effectiveness in efficiently lowering sulfate levels within black liquor.

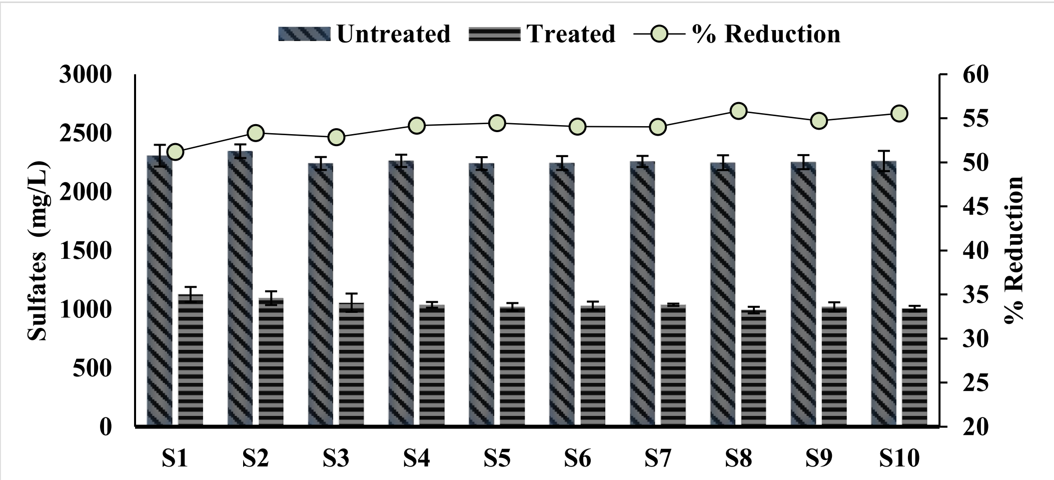


Figure 4.17 Sulfates of untreated and GDB-treated pulp and paper industry effluent.

4.2.1.9. Total dissolved solids (TDS)

The evaluation of ten samples of untreated and black liquor treated with the GDB showed a significant decrease in TDS. The untreated samples had an average TDS concentration of around 2548.5 mg/L. In contrast, the samples treated with GDB showed a much lower average TDS concentration of roughly 1163.15 mg/L, indicating an average reduction of approximately 54.31%. This information is visually shown in Figure 4.18. The obtained findings demonstrate the efficacy of GDB in effectively reducing TDS concentrations in black liquor.

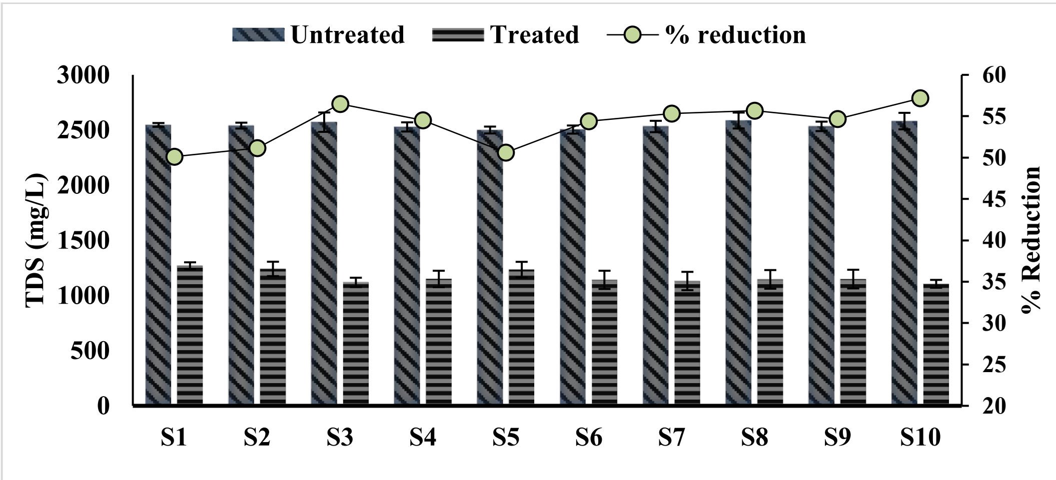


Figure 4.18 TDS of untreated and GDB-treated pulp and paper industry effluent.

4.2.1.10. Total nitrogen

A comparative examination was conducted on ten samples (S1-S10) of untreated and black liquor treated with the GDB. The results indicate a noteworthy decrease in TN levels. The untreated samples had an average concentration of Total Nitrogen of roughly 144.5 mg/L. In contrast, the samples treated with GDB showed a much-reduced average concentration of around 63.76 mg/L, indicating an average decrease of approximately 55.87%, as depicted in Figure 4.19. The results of this study suggest the efficacy of the GDB in effectively reducing Total Nitrogen levels in effluent from the pulp and paper industry.

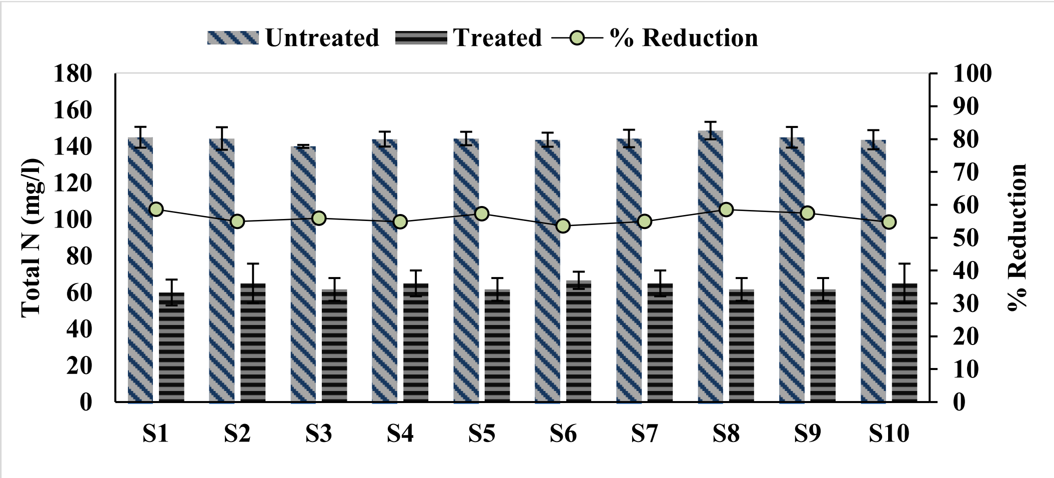


Figure 4.19 Total nitrogen of untreated and GDB-treated pulp and paper industry effluent.

4.2.1.11. Total suspended solids (TSS)

The analysis of ten samples of untreated black liquor, compared to black liquor treated with the GDB, demonstrated a significant decrease in TSS. The untreated samples had an average TSS concentration of about 1477.93 mg/L. In contrast, the samples treated with GDB displayed a significantly reduced average TSS concentration of around 110.67 mg/L, indicating an average decrease of approximately 92.29%. These results, as indicated in Figure 4.20, highlight the effectiveness of the GDB in efficiently reducing TSS levels in black liquor.

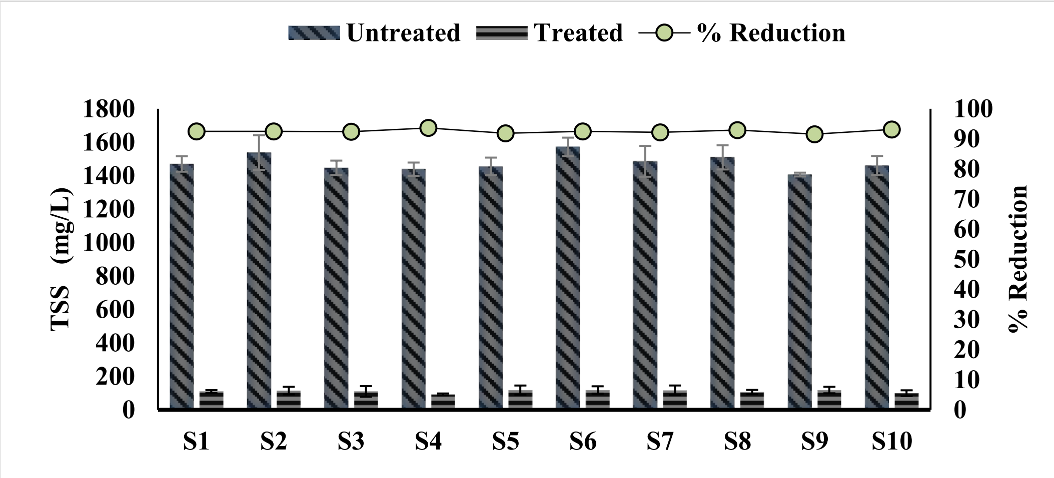


Figure 4.20 Total suspended solids of untreated and GDB-treated pulp and paper industry effluent.

4.2.1.12. Turbidity levels

The present study involves the examination of ten samples of untreated black liquor in comparison to black liquor that has undergone treatment utilizing the GDB. The findings of this research reveal a significant decrease in Turbidity levels, as measured in Nephelometric Turbidity Units (NTU), in the treated black liquor samples. The untreated samples had an average Turbidity value of roughly 120.89 NTU. In contrast, the samples treated with GDB revealed a drastically reduced average Turbidity value of around 14.485 NTU, indicating an average decrease of approximately 87.99%, as depicted in Figure 4.21. The results of this study emphasize the effectiveness of the GDB in effectively reducing turbidity levels in black liquor.

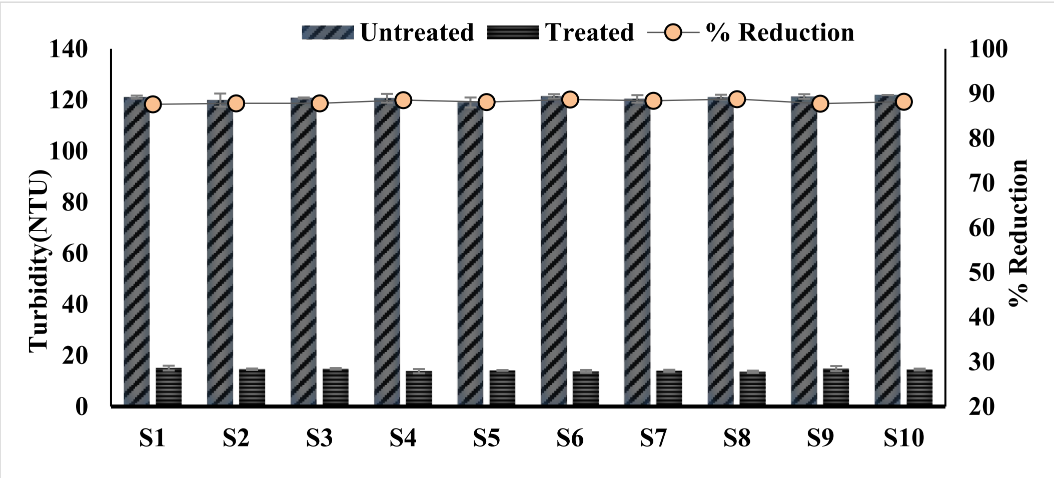


Figure 4.21 Turbidity of untreated and GDB-treated pulp and paper industry effluent.

4.2.2. Performance evaluation of an integrated vertical flow constructed wetland (IVFCW) for the treatment of paper and pulp industry effluent (black liquor)

The effective treatment of industrial effluents is a prominent issue, given its potential detrimental effects on aquatic ecosystems and human well-being. The pulp and paper industry is a notable entity that significantly contributes to water pollution by discharging effluents that possess a multifaceted composition consisting of both organic and inorganic elements. The release of untreated or insufficiently treated effluents from pulp and paper mills gives rise to various ecological concerns, including the depletion of oxygen and the enrichment of nutrients. Constructed wetlands are a viable and environmentally beneficial method for treating industrial effluents. The IVFCWs have gained considerable interest due to their ability to treat industrial wastewater efficiently. IVFCWs are engineered replicas of the natural wetland that facilitate the interaction between wastewater plants and the underlying substrate having biofilm. This design promotes the elimination of pollutants by using a range of physical, chemical, and biological mechanisms, such as sedimentation, filtration, adsorption, microbial degradation, and plant absorption.

The present study evaluated the viability and effectiveness of IVFCWs in treating wastewater from the pulp and paper sector. The present work included the design, fabrication, and assessment of a laboratory-scale IVFCW. The IVFCW was subjected to 10 sampling events in a semicontinuous mode. The effectiveness of IVFCW was measured in terms of physicochemical parameters, such as BOD_5, COD, pH, TDS, TSS, total nitrogen, lignin degradation, phosphates, sulfates, and electrical conductivity. The findings of the present research provide insights into the effectiveness of the IVFCW system in reducing the contaminants found in the effluent, hence leading to the development of sustainable wastewater treatment methods for industrial effluents.

Table 4. 5 Efficacy of IVFCW for the treatment of pulp and paper industry effluent

Sample Code	COD (%Efficiency)	BOD (%Efficiency)	DO (%Increase)	pH (%Variation)	EC (%Efficiency)	TDS (% Efficiency)	TSS (% Efficiency)	PO_4^{3-} (%Efficiency)	SO_4^{2-} (% Efficiency)	TN (%Efficiency)	Lignin (%Efficiency)	Turbidity (%Efficiency)
S1	73.13953	70.75616	188.6403	5.83333	73.78818	59.69721	76.19048	54.65779	62.52159	58.3908	54.73326	87.4883
S2	74.54686	74.95171	248.1293	6.33028	65.99665	64.35077	89.72692	71.25541	56.65674	67.89764	48.28174	88.68955
S3	71.47008	73.82683	215.331	5.261044	72.35421	64.76864	100	46.27492	55.50038	77.38095	51.2987	88.31907
S4	75.96658	74.87415	228.5714	3.2	57.69246	63.02883	100	42.87653	60.88539	79.39815	55.20425	88.41061
S5	69.6875	75.03204	435.2941	3.360656	73.19469	62.46057	100	50.99476	52.51203	76.44287	53.4296	88.5014
S6	69.90084	75.64928	204.7452	2.21739	57.21522	66.46683	100	46.68838	63.75462	59.86172	63.05626	88.3218
S7	70.10173	75.0108	239.9293	3.18584	64.36506	62.15447	100	56.97248	58.72033	73.9024	59.67973	88.37209
S8	74.20074	75.42601	258.0705	3.361702	69.15854	63.20261	100	59.1195	63.25482	77.13056	69.98023	88.77236
SS9	76.28807	74.02143	204.4199	3.49776	71.29246	64.85437	100	46.03464	65.46467	77.93103	72.13534	88.21236
S10	75.77339	75.07119	251.6129	0.421941	70.02141	61.74993	100	56.10948	62.43972	74.71056	58.84867	89.16822

4.2.2.1. Biological oxygen demand (BOD)

The IVFCW system used for effluent remediation from the pulp and paper industry showed notable effectiveness in reducing BOD. During the assessment of 10 samples, the untreated effluent exhibited a range of initial BOD values, ranging from 1486.67 mg/L to 1560.67 mg/L. However, after treatment using the IVFCW system, a significant decrease in BOD was observed, leading to treated effluent BOD levels ranging from 365.33 mg/L to 447.33 mg/L. The observed results demonstrated a noteworthy decrease in BOD levels, with reductions ranging from 70.76% to 75.65%, as shown in Figure 4.22. These findings highlight the IVFCW system's substantial ability to remove pollutants effectively.

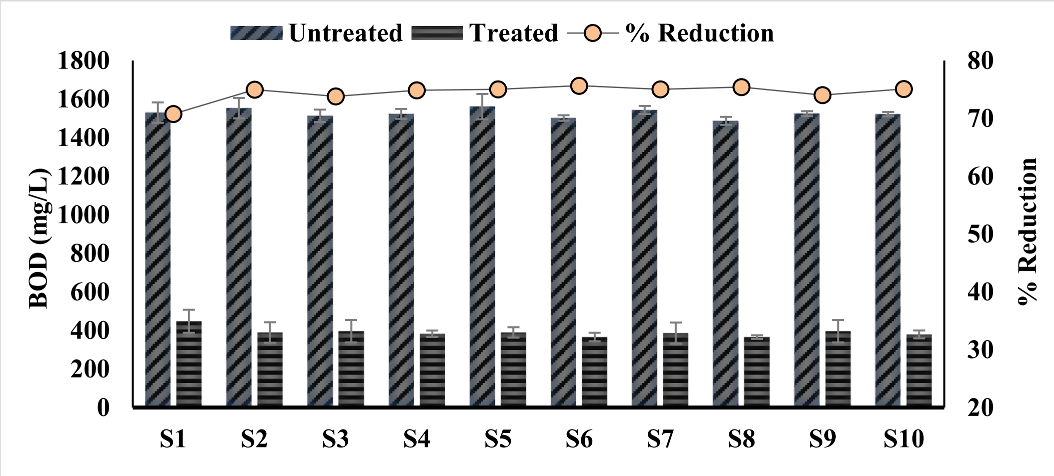


Figure 4.22 BOD_5 of untreated and IVFCW-treated pulp and paper industry effluent.

4.2.2.2. Chemical oxygen demand (COD)

The use of the IVFCW system showed significant efficacy in reducing the COD of pulp and paper industry wastewater. The values of COD in the untreated effluent varied between 1706.67 mg/L and 1793.33 mg/L. Following the IVFCW treatment, a significant reduction in COD level was seen in the effluent. The recorded values ranged from 412.33 mg/L to 529 mg/L. These results demonstrate a noteworthy decrease in COD levels, ranging from 69.69% to 76.29%, as depicted in Figure 4.23, which highlights the adequate capacity of IVFCW to eliminate persistent organic compounds. The findings highlight the efficacy of IVFCWs as a viable strategy for mitigating the high levels of COD in effluent from the pulp and paper industry.

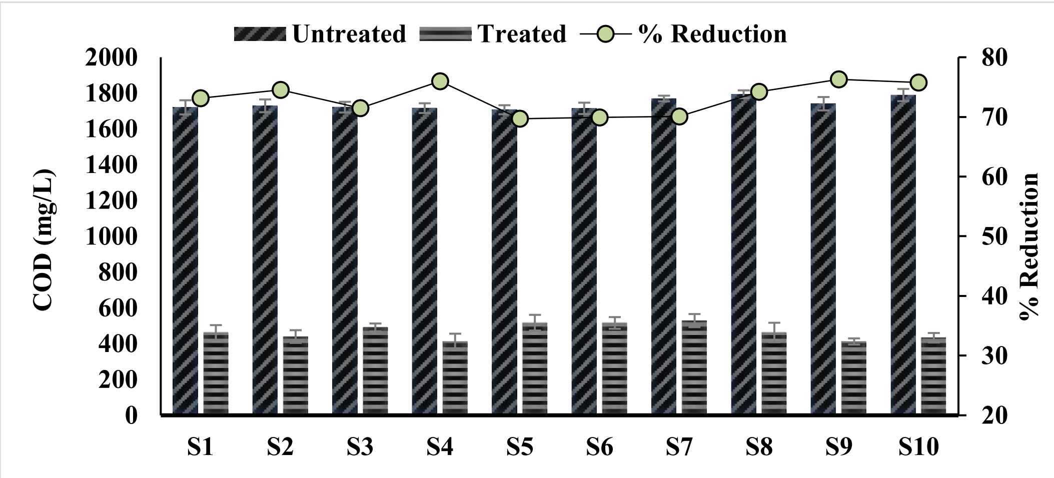


Figure 4.23 COD of untreated and IVFCW-treated pulp and paper industry effluent.

4.2.2.3. Dissolved oxygen content

After treatment through the IVFCW system, BL has shown a significant improvement in dissolved oxygen (DO) levels. The untreated samples' initial DO values varied between 1.19 mg/L and 1.96 mg/L. After undergoing IVFCW treatment, a significant elevation in DO levels was observed, with values ranging from 5.51 mg/L to 6.823 mg/L. The increase in DO content from 188.64% to 435.29% is shown in Figure 4.24. These findings highlight the IVFCW's efficacy in enhancing the effluent's oxygen levels, suggesting its potential to promote the health of aquatic ecosystems and overall improvement in water quality.

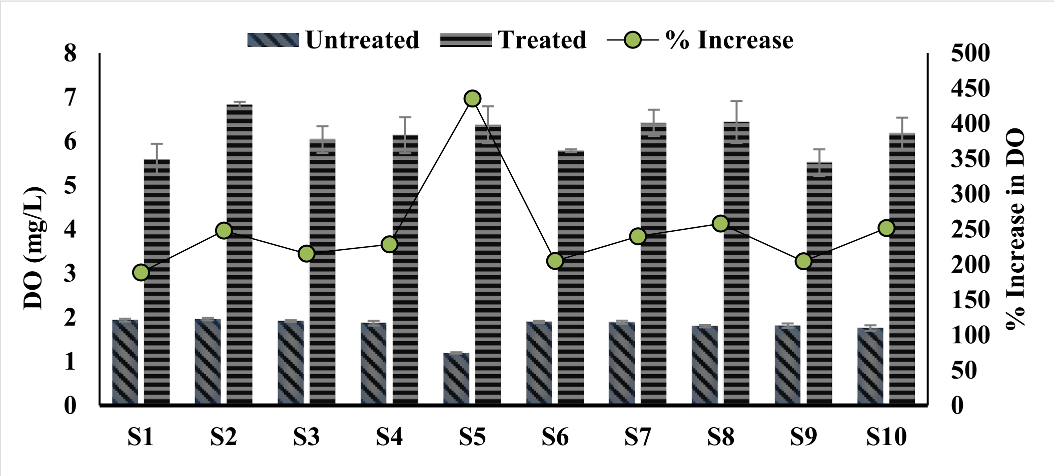


Figure 4.24 Dissolve oxygen of untreated and IVFCW-treated pulp and paper industry effluent.

4.2.2.4. Electrical conductivity (EC)

The IVFCW system has shown significant efficacy in mitigating elevated levels of EC in the effluent generated by the pulp and paper industry effluent. The initial EC values in the untreated samples varied between 910.33 µS/cm and 961.67 µS/cm. After treatment with the IVFCW, a significant reduction in EC was observed, with values ranging from 252.33 µS/cm to 393.33 µS/cm. This data indicates a significant decrease in EC, ranging from 57.22% to 73.79%, as shown in Figure 4.25. The findings highlight the IVFCW's effectiveness in reducing the effluent's conductivity and enhancing water quality.

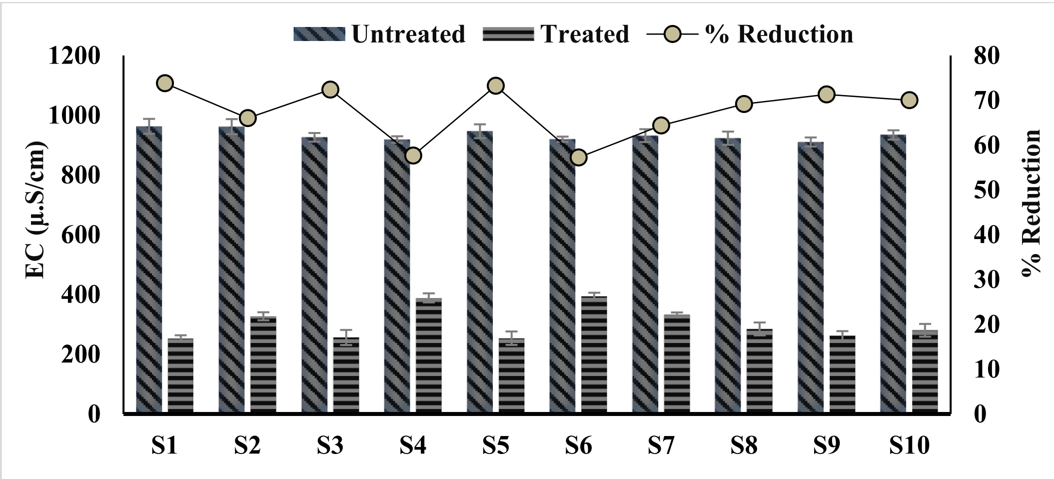


Figure 4.25 Electrical conductivity of untreated and IVFCW-treated pulp and paper industry effluent.

4.2.2.5. Lignin content

The data shown in Figure 4.26 demonstrates the effectiveness of the IVFCW system in decreasing the lignin concentration in effluent generated by the pulp and paper industry. The untreated samples exhibited initial lignin levels ranging from 1165.67 mg/L to 1274.67 mg/L. Following the treatment by IVFCW, a significant decrease in the concentration of lignin was observed, leading to a range of values spanning from 326.67 mg/L to 607 mg/L. The results indicate a significant decrease in the amount of lignin, with reductions ranging from 48.28% to 72.14%. The results highlight the efficacy of IVFCW in the degradation of lignin, a highly resistant organic molecule, and its potential to aid in reducing persistent pollutants found in industrial wastewater.

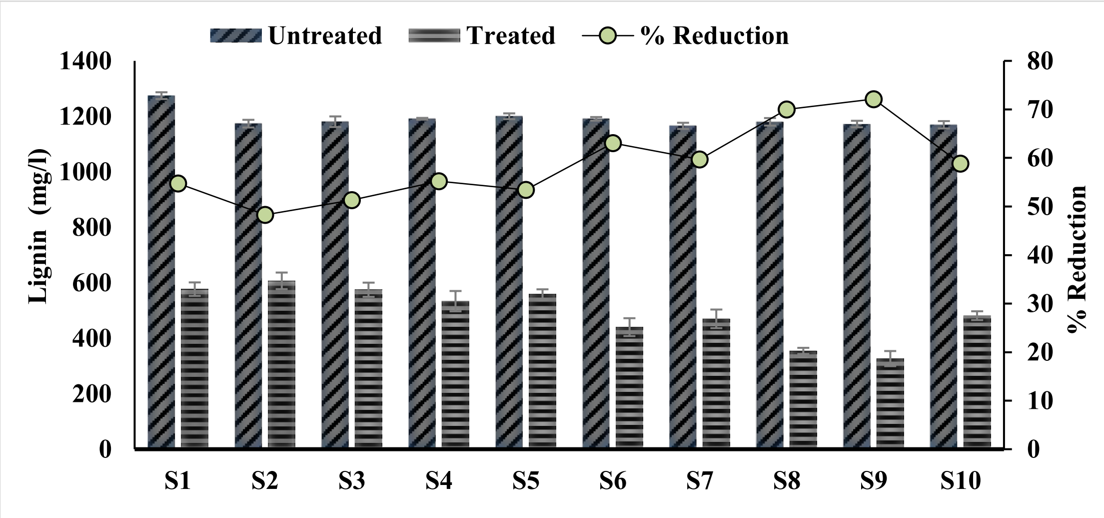


Figure 4.26 Lignin content of untreated and IVFCW-treated pulp and paper industry effluent.

4.2.2.6. pH analysis

The pH variations in the effluent from the pulp and paper industry after it undergoes treatment in the IVFCW system provide intriguing observations. The pH values initially varied between 7.2667 and 8.3. Following treatment, there were modest fluctuations in the pH values, which ranged from 7.57 to 8.0433. Significantly, several samples exhibited slight elevations in pH, whilst others had negligible reductions, as seen in Figure 4.27. After treatment, all the samples have pH under permissible limits. The few fluctuations observed highlight the IVFCW's ability to control and maintain pH levels within a minimal range effectively.

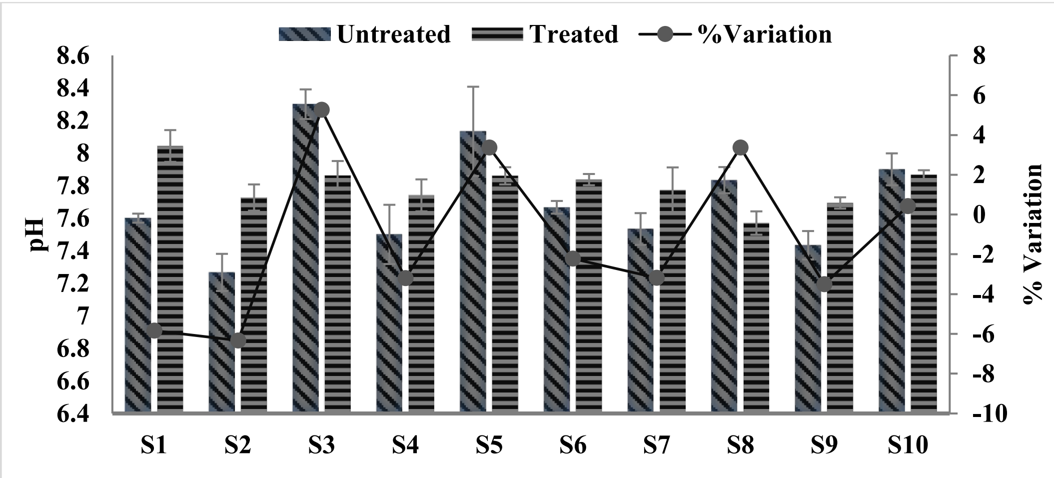


Figure 4.27 pH of untreated and IVFCW-treated pulp and paper industry effluent.

4.2.2.7. Phosphate-Orthophosphates

The IVFCW system demonstrates a significant decrease in phosphate levels in the effluent of the pulp and paper industry. The phosphate concentrations in the untreated samples varied between 2.4567 mg/L and 3.85 mg/L, while the treated effluent exhibited reduced phosphate values ranging from 1.1067 mg/L to 1.9733 mg/L. These results indicate a significant decrease in phosphate concentration, with reductions ranging from 42.88% to 71.26%, as shown in Figure 4.28. The findings highlight the efficacy of IVFCW in efficiently eliminating phosphates from the effluent, hence making a substantial contribution towards mitigating nutrient pollution and the potential for eutrophication in the receiving water bodies.

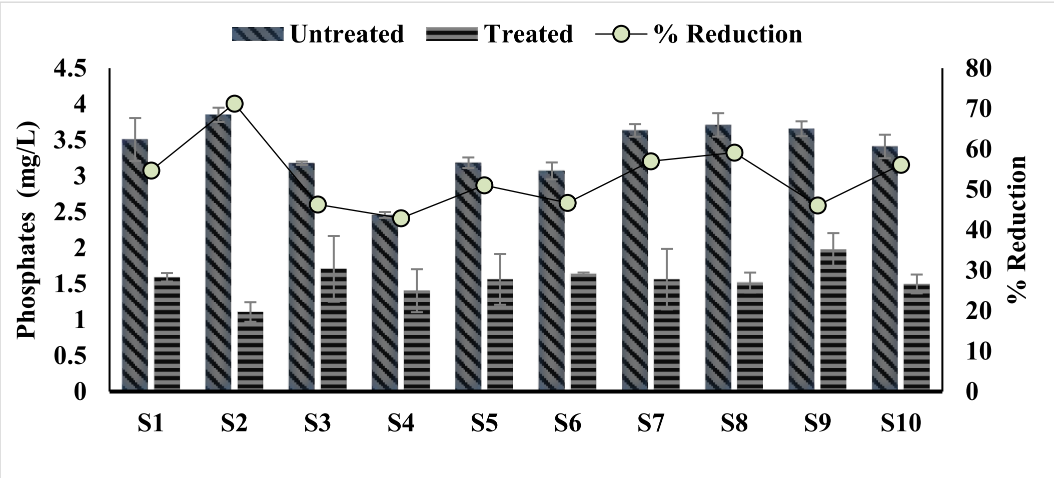


Figure 4.28 Phosphates in untreated and IVFCW-treated pulp and paper industry effluent.

4.2.2.8. Sulfates

The findings of the present study demonstrate the efficacy of the IVFCW system in mitigating sulfate levels in effluent from the pulp and paper industry. The initial sulfate concentration varied between 2215 mg/L and 2351 mg/L, while the treated effluent had decreased sulfate concentrations ranging from 785.33 mg/L to 1052.33 mg/L. These findings indicate a significant decrease in the concentration of sulfates, with reductions ranging from 52.51% to 65.46%, as represented in Figure 4.29. The results of this study emphasize the effectiveness of IVFCW in reducing sulfate concentrations in the effluent.

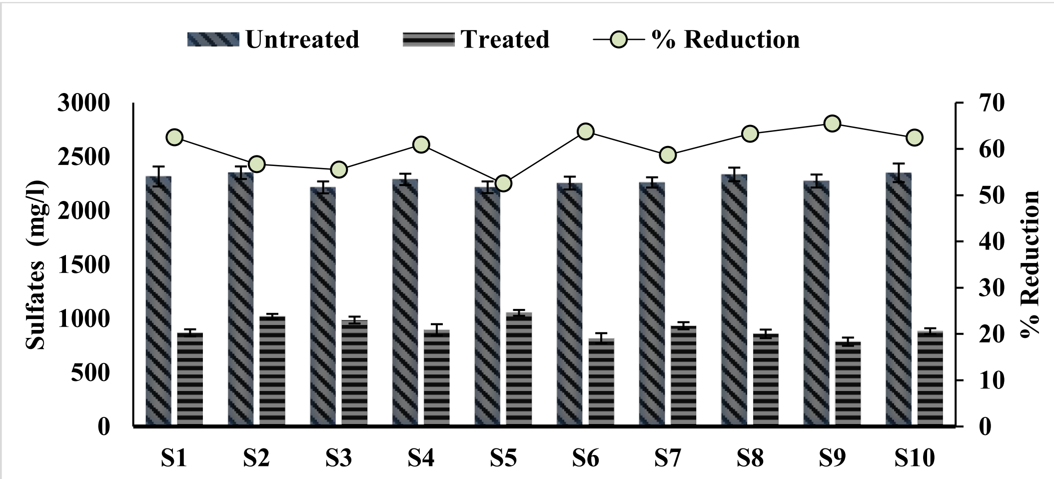


Figure 4.29 Sulfates in untreated and IVFCW-treated pulp and paper industry effluent.

4.2.2.9. Total dissolved solids (TDS)

The results in Figure 4.30 make it clear that the IVFCW system works to lower the total dissolved solids (TDS) in wastewater from the pulp and paper industry. The initial TDS concentrations ranged between 2460 mg/L and 2687 mg/L, while the TDS concentrations in the effluent after treatment varied from 839 mg/L to 1029.33 mg/L. These results indicate a significant decrease in TDS concentration, ranging from 59.70% to 66.47%. The present study shows that IVFCW is very good at removing dissolved particles from the effluent. This makes a big difference in reducing the harmful TDS levels in the wastewater.

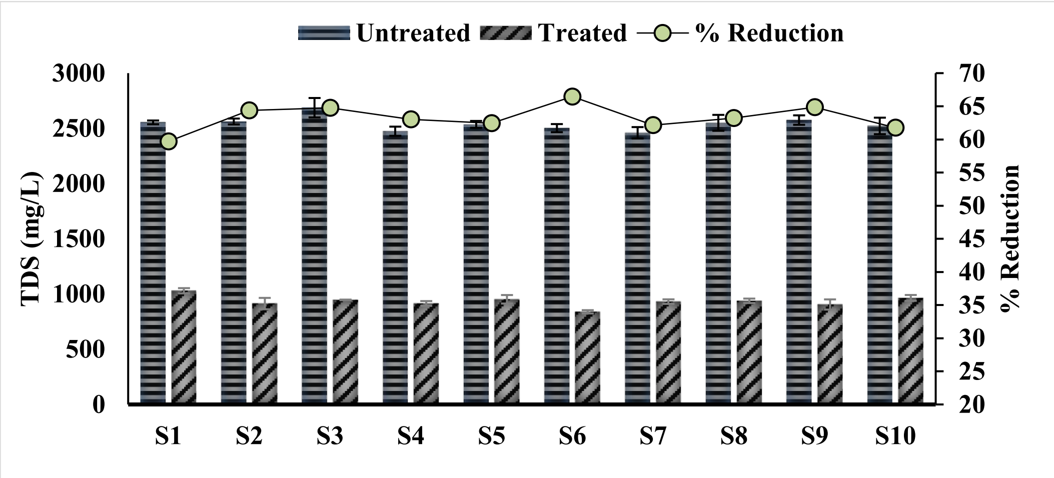


Figure 4.30 Total dissolved solids in untreated and IVFCW-treated pulp and paper industry effluent.

4.2.2.10. Total nitrogen (TN)

The results demonstrate the notable efficacy of the IVFCW system in mitigating the amounts of TN in the effluent produced by the pulp and paper industry. TN concentrations in untreated samples varied between 140 mg/L and 148.67 mg/L. Following the treatment process, the levels of TN in the effluent ranged from 29.67 mg/L to 60.33 mg/L. This signifies a significant decrease in the overall nitrogen concentration, with reductions ranging from 58.39% to 79.40% (Figure 4.31). The findings highlight the effectiveness of IVFCW in effectively reducing nitrogen compounds, leading to a significant reduction in the nutrient load of the effluent and probable ecological consequences in the surrounding ecosystem.

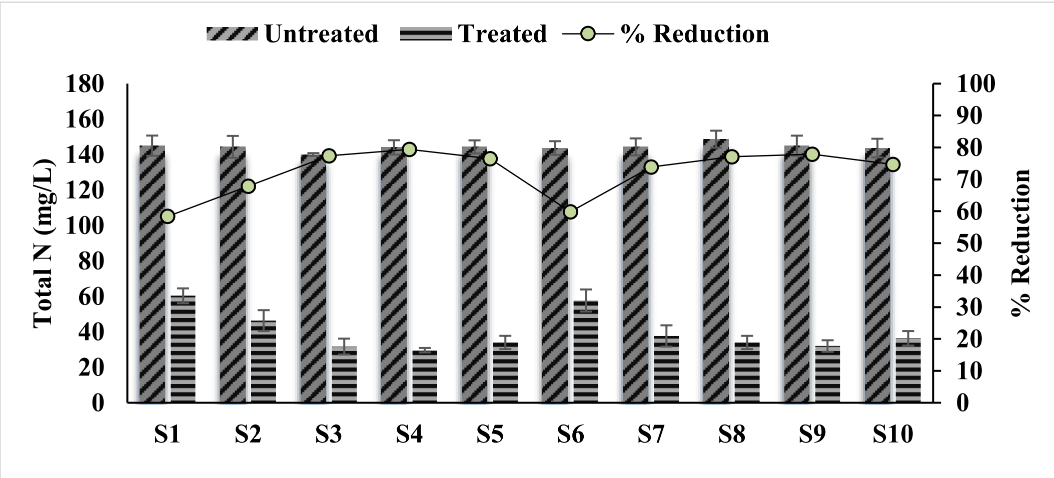


Figure 4.31 Total nitrogen in untreated and IVFCW-treated pulp and paper industry effluent.

4.2.2.11. Total suspended solids (TSS)

The present study's findings demonstrate the effectiveness of the IVFCW system in significantly decreasing the amounts of TSS in the effluent produced by the pulp and paper industry. At the outset, the TSS concentrations ranged from 1406.7 mg/L to 1572.6 mg/L. Following the treatment process via IVFCW, the effluent exhibited a comprehensive elimination of TSS, resulting in a concentration of 0 mg/L in all treated samples after two weeks of operation (S3-S10). The findings (Figure 4.32) demonstrate the IVFCW's outstanding ability to successfully remove suspended particles from the effluent, contributing considerably to the overall improvement in water quality.

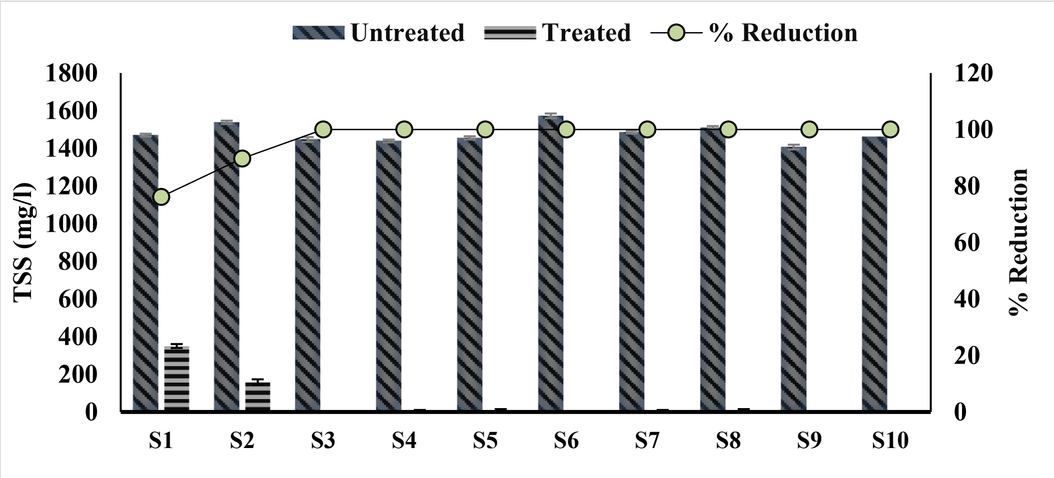


Figure 4.32 Total suspended solids in untreated and IVFCW-treated pulp and paper industry effluent.

4.2.2.12. Turbidity

The present study demonstrates the significant efficacy of the Integrated IVFCW system in mitigating turbidity levels in treated effluent from the pulp and paper units. The initial turbidity readings exhibited a range of 119 NTU to 125.23 NTU, significantly reduced to 13.56 NTU to 15.15 NTU in the effluent after treatment via IVFCW. The observed outcomes indicate a substantial decrease in turbidity, ranging from 87.49% to 89.17%, as shown in Figure 4.33. The findings highlight the effectiveness of IVFCW in effectively clarifying the effluent by removing suspended particles, leading to enhanced visual clarity and overall improvement in water quality.

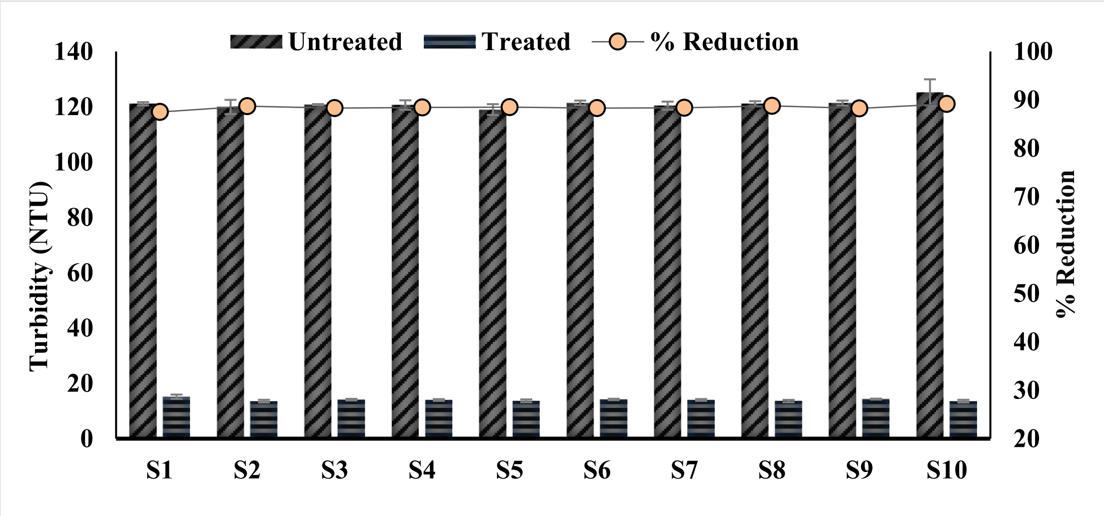


Figure 4.33 Turbidity in untreated and IVFCW-treated pulp and paper industry effluent.

Phase 3

Results-Objective 3

4.3. Efficiency monitoring for GDB and IVFCW for the treatment of black liquor blended with domestic wastewater.

4.3.1. Efficiency monitoring for GDB for the treatment of black liquor blended with domestic wastewater.

The pulp and paper industry has historically been a fundamental pillar of worldwide production, supplying necessary materials for several industries. However, this industry gives rise to a noteworthy environmental predicament in the shape of black liquor (BL), an effluent highly concentrated and resistant to degradation. The disposal and treatment of BL requires additional cost, time, and time-consuming process and increases the value of the final product. The present study presents an innovative methodology that integrates the treatment of BL with household wastewater, using a gravity-driven bioreactor system to address the environmental issue linked to BL. The physicochemical properties of the influent wastewater are shown in Table 4.6. This study aims to investigate the efficacy of GDB to biodegrade the pollutants present in BL and domestic wastewater simultaneously, providing a sustainable and effective resolution to address the environmental consequences associated with wastewater. The present study elucidates the favorable results and potential consequences of this innovative GDB in treating wastewater from the pulp and paper industry.

Table 4. 6 Efficacy of GDB for the treatment of pulp and paper industry effluent and domestic wastewater simultaneously

Sample Code	COD (%Efficiency)	BOD (%Efficiency)	DO (%Increase)	pH (%Variation)	EC (%Efficiency)	TDS (% Efficiency)	TSS (% Efficiency)	PO_4^{3-} (%Efficiency)	SO_4^{2-} (% Efficiency)	TN (%Efficiency)	Lignin (%Efficiency)	Color reduction (%Efficiency)
W1	70.79755	70.12146	164.5796	13.38028	75.2597	69.4338	54.14128	49.54792	71.74888	29.82456	71.0084	36.12824
W2	73.42267	71.40509	142.6891	8.8	73.8006	70.96296	61.98724	44.27558	66.83979	34.5036	67.01903	44.2402
W3	74.83203	66.53144	140.3566	11.84314	73.36066	74.33187	100	38.20704	79.25512	50.84337	76.78161	43.18766
W4	72.64688	65.02964	111.4889	15.01946	67.91882	75.78877	100	38.84211	78.32948	40.42371	76.1807	26.28571
W5	77.91579	63.57843	139.7799	2.671756	67.6076	75.22414	100	40.53224	81.75477	54.40141	70.38647	38.45109
W6	74.7284	68.8172	164.7913	9.465021	54.92237	75.48955	100	43.87318	80.93205	64.31255	66.07539	25.08251
W7	84.54198	69.91943	170.5104	5.15873	62.19701	73.46048	100	44.16058	79.91273	64.08108	78.6747	38.05436
W8	81.69728	68.46307	181.4208	7.058824	65.49515	78.1457	100	30.84622	79.84805	63.95181	75.88106	43.62934
W9	80.49981	69.69571	171.9934	7.45098	63.94052	75.51585	100	67.1193	73.88493	59.801	66.55093	40.98124
W10	81.96256	81.27892	164.8841	10.72797	65.87642	70.96149	100	50.44643	77.43833	61.59905	70.42411	41.56977
W11	81.7644	69.63025	202.7944	16.60232	68.14473	73.48668	100	38.82353	79.88857	59.53086	74.67611	44.48399
W12	79.31716	68.5219	168.8057	14.55224	69.6793	74.3554	100	41.86265	76.56665	65.41717	70.5157	28.46821

4.3.1.1. Biological oxygen demand (BOD)

In evaluating the effectiveness of the GDB for wastewater treatment, the samples of untreated wastewater showed a variation in BOD_5, ranging from a maximum of 835 mg/L to a minimum of 680 mg/L. After treatment through GDB, the wastewater exhibited lower levels of BOD, ranging from a maximum value of 275.33 mg/L to a minimum value of 148.33 mg/L. The sample W10 exhibited the highest degree of BOD decrease, achieving a noteworthy reduction of 81.28% as shown in Figure 4.34. The findings of the present study highlight the efficacy of the GDB in substantially decreasing levels of BOD and improving the efficiency of wastewater treatment processes.

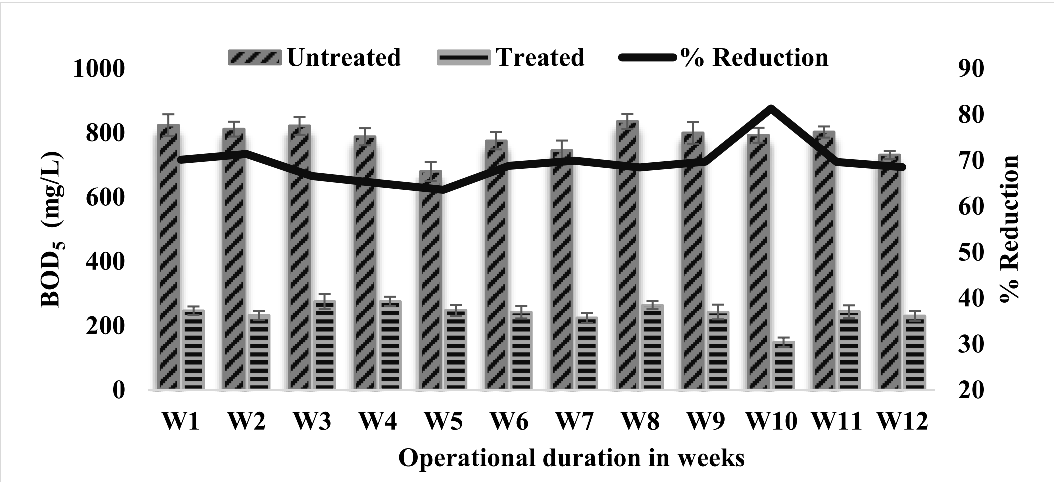


Figure 4.34 BOD_5 in untreated and GDB-treated blended wastewater.

4.3.1.2. Chemical oxygen demand (COD)

In the assessment of a GDB, it was observed that the samples of untreated wastewater exhibited a range of COD values, with the highest recorded at 1397.33 mg/L and the lowest at 1086.67 mg/L, however, after treatment via GDB, a significant decrease in COD levels was observed. The most substantial drop, reaching 84.54%, was recorded in sample W7 as depicted in Figure 4.35. The COD readings of the treated wastewater varied between a high of 327.33 mg/L and a low of 212 mg/L, indicating that the GDB successfully reduced COD levels in various types of wastewaters.

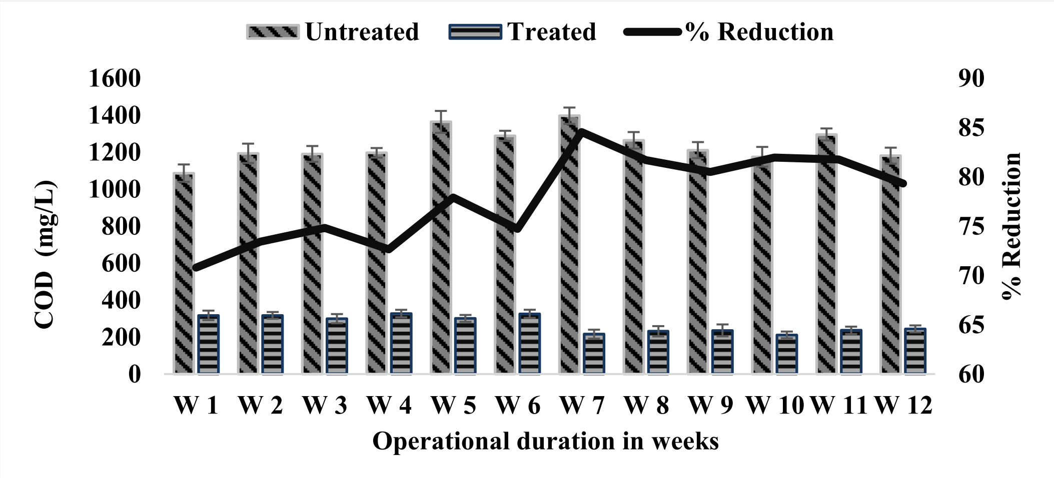


Figure 4.35 COD in untreated and GDB-treated blended wastewater.

4.3.1.3. Dissolve oxygen

The GDB showed promising results for the treatment of blended wastewater. Untreated samples of wastewater displayed an average DO concentration of 2.03 mg/L, while after treatment a significant increase in DO content, reaching 5.07 mg/L was observed. Figure 3.36 depicts an average increase in DO is about 298 times, which underscore the efficacy of GDB in improving water quality.

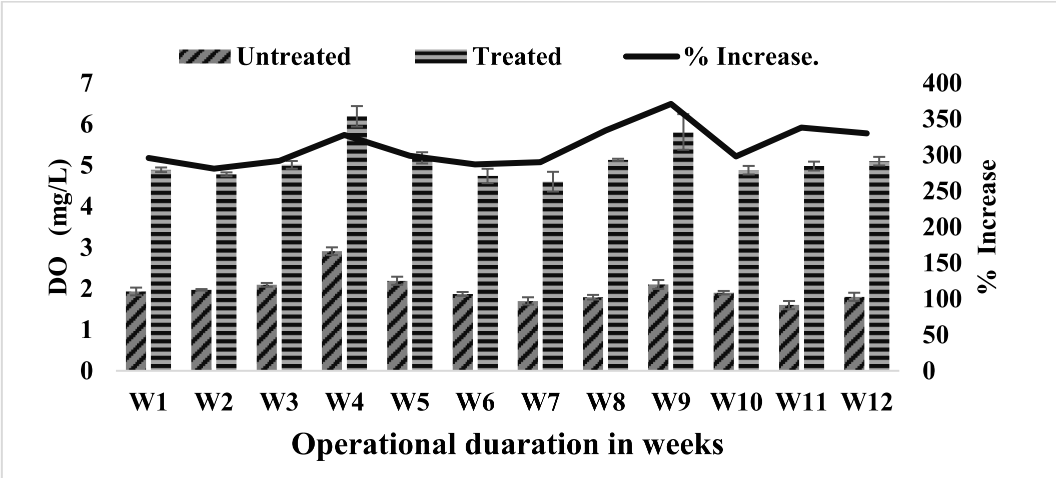


Figure 4.36 Dissolved oxygen content of untreated and GDB-treated blended wastewater.

4.3.1.4. pH analysis

The evaluation of pH fluctuations in wastewater treated by a GDB for treating pulp and paper industry and domestic wastewater revealed that the untreated wastewater displayed a pH range of 8.1 to 9.4. while after treatment via GDB, a pH range of 7.2 to 8.5 was observed. The untreated wastewater exhibited pH values ranging from a high of 9.4 to a low of 8.1. The pH values observed in the treated wastewater ranged from a high of 8.5 to a low of 7.2. It is worth mentioning that the untreated sample W11 exhibited the most significant percentage fluctuation in pH, with a value of 16.6%, as indicated in Figure 4.37. This study's results highlight the bioreactor's potential to control and reduce pH variations in wastewater effectively.

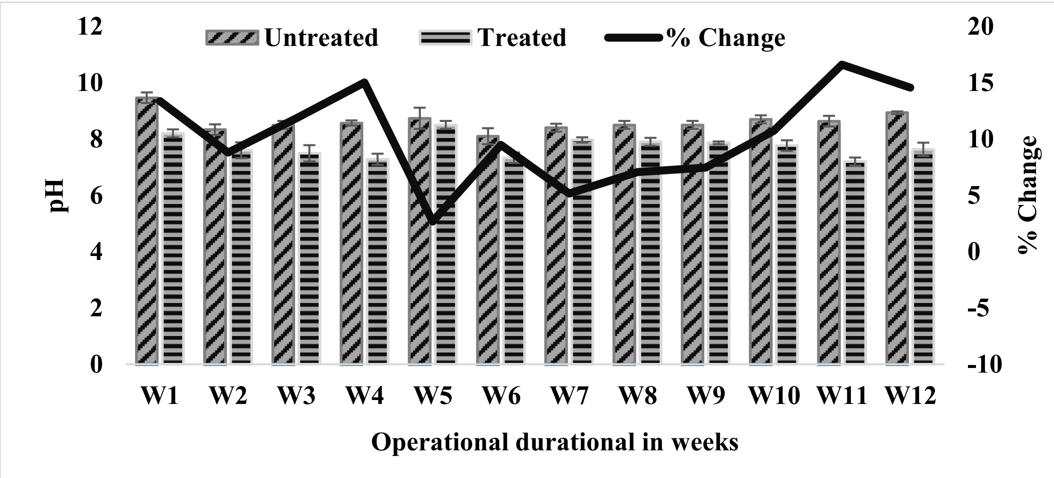


Figure 4.37 pH of untreated and GDB-treated blended wastewater.

4.3.1.5. Electrical conductivity (EC)

In the assessment of GDB intended to treat a mixture of wastewater originating from the pulp and paper industry and residential sources, the initial untreated wastewater showed a variation in EC values ranging from 1778.66 to 1939 µS/cm. After the treatment, the treated wastewater exhibited a significant decrease in electrical conductivity (EC), with values ranging from 476.33 to 851.66 µS/cm. The untreated wastewater exhibited a maximum EC value of 1939 µS/cm and a minimum EC value of 1778.66 µS/cm. In contrast, the treated wastewater displayed a maximum EC value of 851.66 µS/cm and a lowest EC value of 476.33µS/cm. Significantly, the bioreactor demonstrated a noteworthy maximum percentage decrease of 75.259%, as shown in Figure 4.38. This study's results highlight the GDB's effectiveness in significantly reducing EC levels in a mixture of wastewaters.

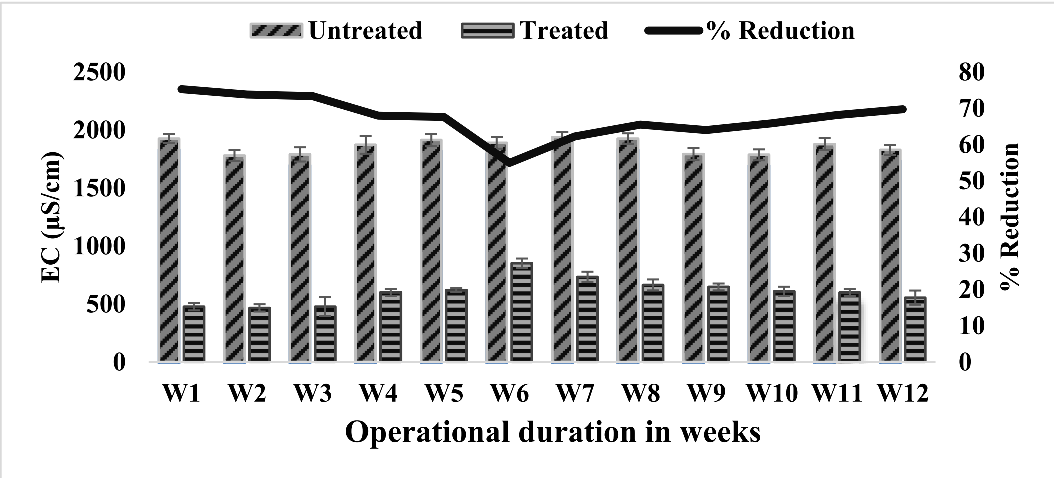


Figure 4.38 Electrical conductivity of untreated and GDB-treated wastewater.

4.3.1.6. Total dissolved solids (TDS)

In the evaluation of GDB, designed to treat a combination of wastewater from the pulp and paper industry and domestic sources, the initial untreated wastewater displayed TDS concentrations varying from 1376.33 to 1671.33 mg/L. After treatment in GDB, the treated wastewater exhibited a significant decrease in TDS, ranging from 341 to 478.66 mg/L. The GDB demonstrated a maximum percentage decrease of 78.14% in TDS, as shown in Figure 4.39, in sample W8. These findings highlight the effectiveness of the GDB in substantially reducing TDS concentrations in wastewater by improving water quality and reducing the environmental consequences.

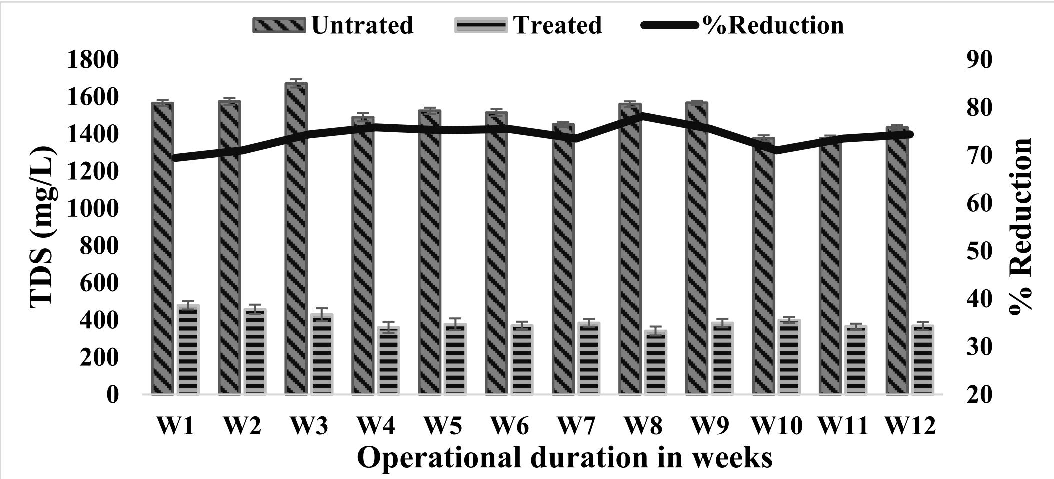


Figure 4.39 Total dissolved solids of untreated and GDB-treated wastewater.

4.3.1.7. Total suspended solids (TSS)

In the assessment of TSS inside a GDB, designed explicitly for the treatment of a combination of wastewater originating from the pulp and paper industry and household sources, the untreated wastewater showed TSS concentrations varying between 2438.33 and 2571.66 mg/L. Significantly, the wastewater that underwent treatment exhibited a substantial decrease in total suspended solids (TSS), as shown by a complete elimination (100% reduction) of TSS in all samples. The total eradication of TSS in the treated wastewater highlights the GDB's exceptional efficacy in suspended particle removal, as shown in Figure 4.40. The results of the present study indicate that the GDB is efficient in eliminating TDS.

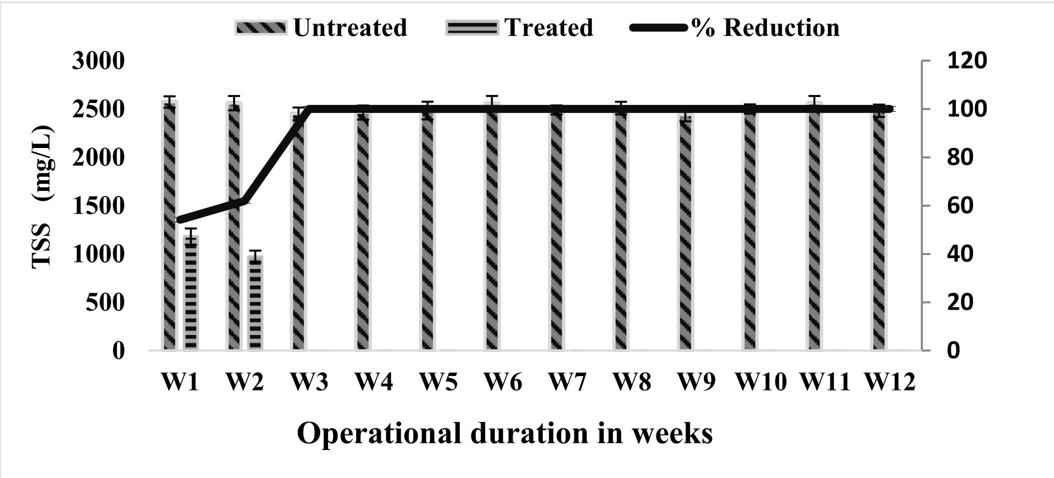


Figure 4.40 TSS of untreated and GDB-treated wastewater.

4.3.1.8. Lignin content

In the analysis of lignin concentration inside a GDB, designed for the simultaneous treatment of mixed wastewater originating from both pulp and paper industry and domestic origins, the initial untreated wastewater exhibited a range of values spanning from 138 to 164.66 mg/L. Following the treatment via GDB, a significant reduction in lignin content, ultimately achieving a minimum value of 29.5 mg/L, was observed. The GDB demonstrated a maximum reduction of 78.67% in lignin concentration in sample W7, as shown in Figure 4.41. These results describe the practical and ecologically friendly approach for addressing the environmental consequences of both pulp and paper industry waste and home sewage.

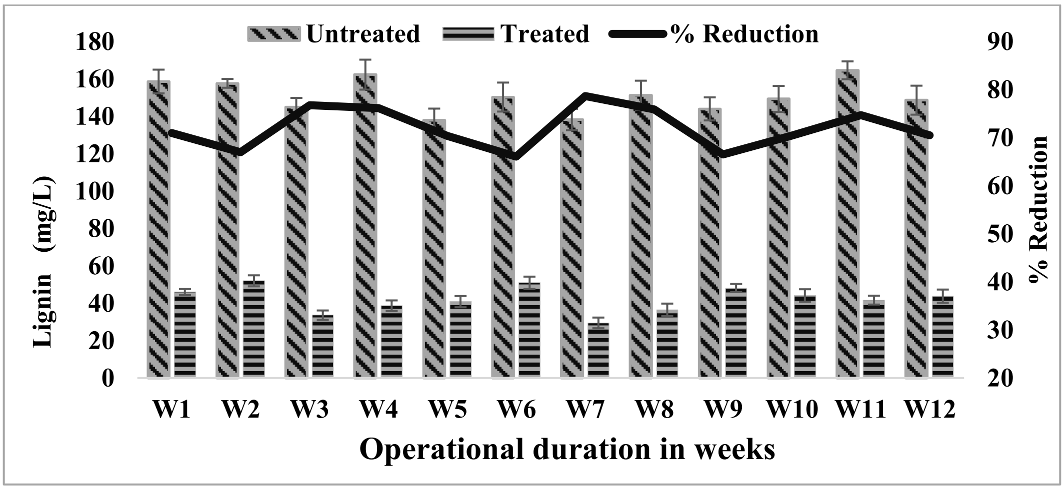


Figure 4.41 Lignin content of untreated and GDB-treated wastewater.

4.3.1.9. Color

In evaluating GDB for color reduction, about 12 samples were analyzed. The initial untreated wastewater displayed a color spectrum ranging from 202 to 281 Pt-Co units. After undergoing the treatment in GDB, there was a significant reduction in color, reaching a minimum value of 134 Pt-Co units. The GDB demonstrated a maximum color reduction of 44.48% in color in sample W11, as shown in Figure 4.42. The findings of the present study underscore the efficacy of the bioreactor in substantially diminishing the presence of color in wastewater, GDB's potential as a viable and environmentally conscious approach to improving the visual appeal of water and mitigating the ecological consequences of combined discharges from the pulp and paper sector and residential sewage.

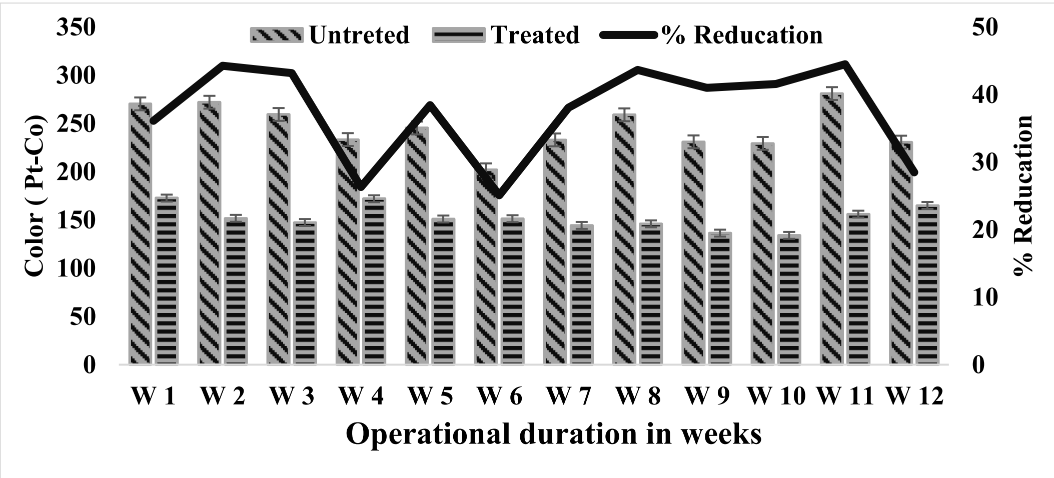


Figure 4.42 Color of untreated and GDB-treated blended wastewater.

4.3.1.10. Phosphate-orthophosphates

In the assessment of GDB, for the bioremediation and phosphate removal of wastewater originating from the pulp and paper industry and domestic origins, 12 samples were collected and analyzed. The untreated wastewater exhibited a range of phosphate levels from 3.12 to 3.89 mg/L, while after undergoing the treatment, a significant reduction in phosphate concentrations of 1.13 mg/L was achieved. GDB significantly decreased the phosphates level, about 67.1% in phosphate in sample W9, as shown in Figure 4.43. These results highlight the effectiveness of the GDB in reducing phosphate levels in mixed wastewater, indicating its promise as an ecologically viable approach for addressing the consequences of phosphate-laden discharges from industrial and residential sources.

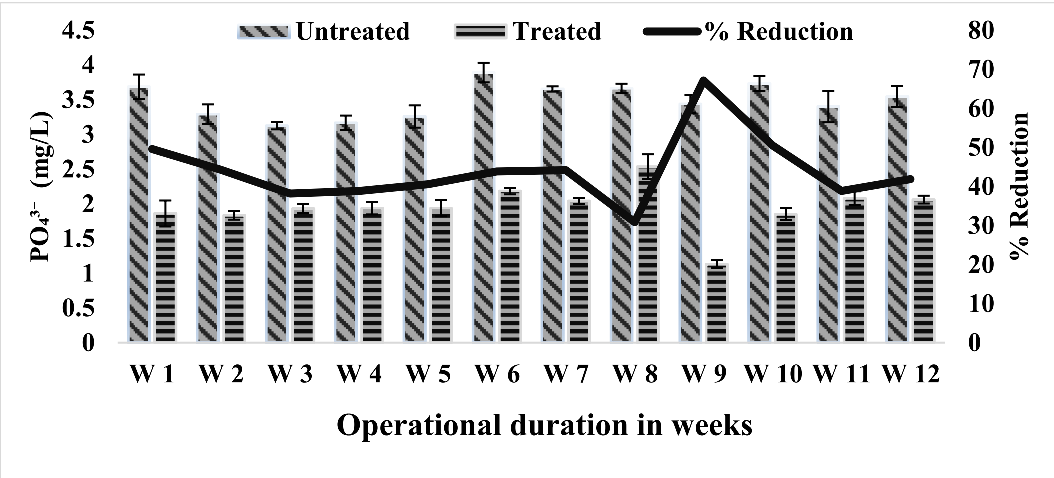


Figure 4.43 Phosphate of untreated and GDB-treated blended wastewater.

4.3.1.11. Sulfate

The analysis of sulfate concentrations (mg/L) in wastewater samples collected from the pulp and paper industry and residential colonies was conducted to monitor the efficacy of GDB. The untreated wastewater displayed sulphate levels between 834.33 and 937 mg/L. After treatment in GDB, there was a significant reduction in sulphate concentrations, reaching a minimum value of 165.6mg/L. The GDB demonstrated a maximum reduction in sulphates of 81.75% in sample W5, as shown in Figure 4.44. These findings affirm the effectiveness of the GDB in reducing sulphate levels in wastewater, highlighting its potential as an ecologically friendly approach to addressing the consequences of sulfate-rich effluents from industrial and residential origins.

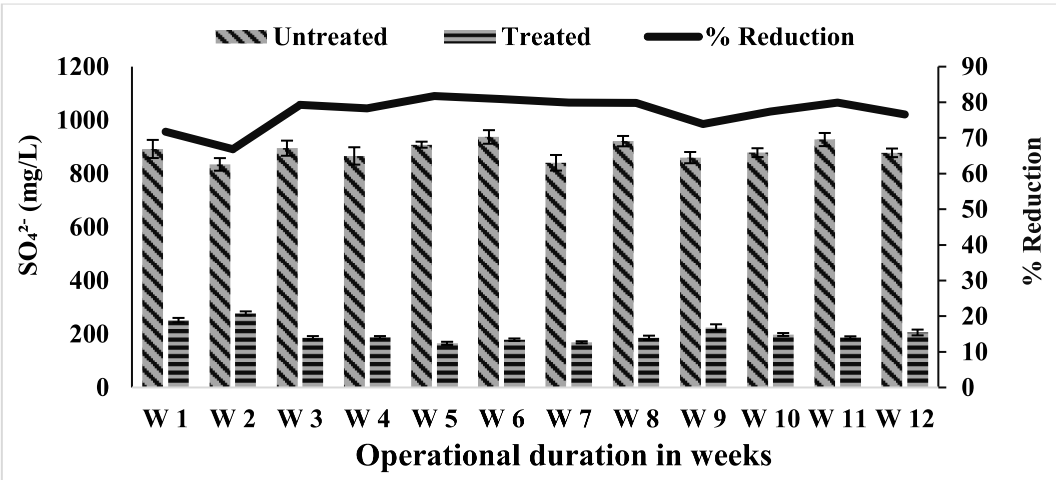


Figure 4.44 Sulfate of untreated and GDB-treated blended wastewater.

4.3.1.12. Total nitrogen (TN)

In evaluating GDB for total nitrogen (TN) removal, wastewater from the pulp and paper industry and domestic sources were collected and analyzed. The untreated wastewater showed TN levels between 123.33 and 151.46 mg/L. After treatment through GDB, a significant reduction in TN content was achieved (44.3 mg/L). GDB presented a maximum reduction of 65.41% in total nitrogen, as shown by the analysis of sample W12 and Figure 4.45. The results emphasized the effectiveness of the GDB in reducing total nitrogen levels in wastewater, indicating its promise as an ecologically viable approach for addressing the effects of nitrogen-rich waste from industrial and residential sources. The significant decrease in overall nitrogen levels may be attributed to the GDB's ability to improve water quality, therefore addressing a critical component of environmental responsibility within wastewater treatment.

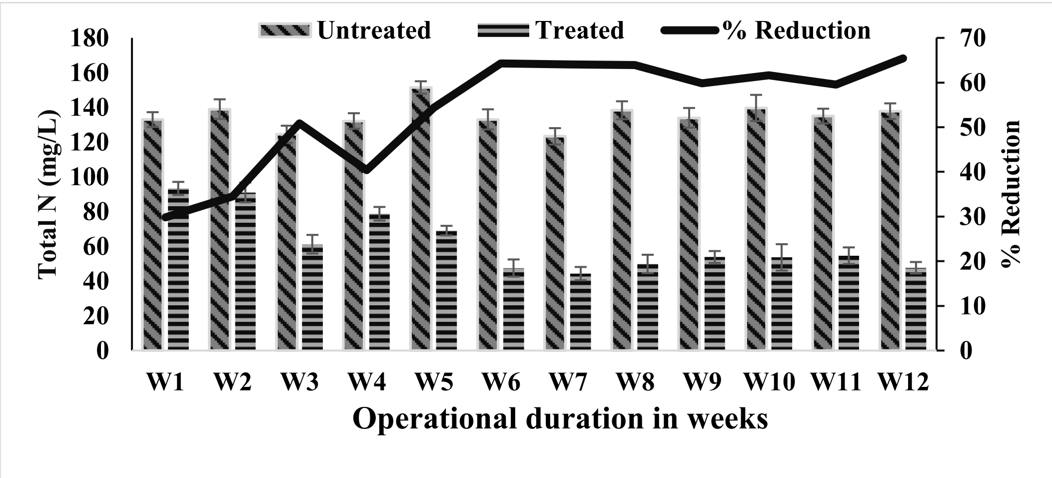


Figure 4.45 Total nitrogen of untreated and GDB-treated wastewater.

4.3.1.13. Colony forming unit (CFU)

The findings of CFU before and after treatment using the GDB show a decline in microbial populations inside the treated samples. The data indicates a significant decrease of 60.3% in CFU after the treatment through GDB as shown in Figure 4.46. The results of this study highlight the efficacy of the GDB in mitigating microbial contamination in wastewater and the practical implementation of the GDB for improving the microbiological characteristics of the effluent. Reducing microbial load is crucial for ensuring environmental integrity and adherence to water quality regulations.

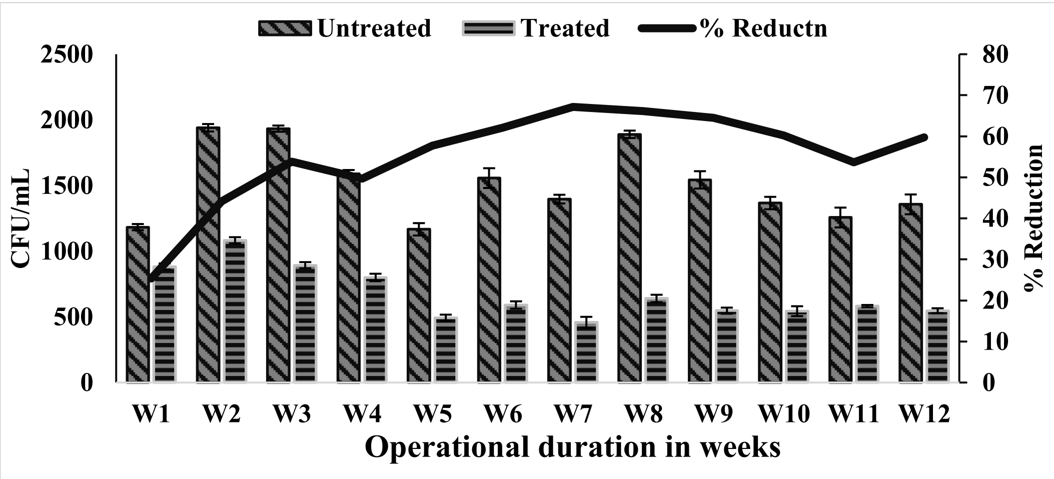


Figure 4.46 CFU/mL of untreated and GDB-treated blended wastewater.

4.3.1.14. MPN Index

In the present study, a significant reduction in microbial contamination, the Most Probable Number (MPN) index, was observed after treatment with GDB. Following the treatment, an average drop of 81% in the MPN index was observed, as shown in Figure 4.47. These results demonstrate the GDB's efficacy in decreasing the microbial burden in the effluent of the pulp and paper sector (black liquor) when co-treated with household wastewater. Furthermore, these results signify the ability of the GDB to improve the microbiological characteristics of the effluent and ensure environmental safety and water quality.

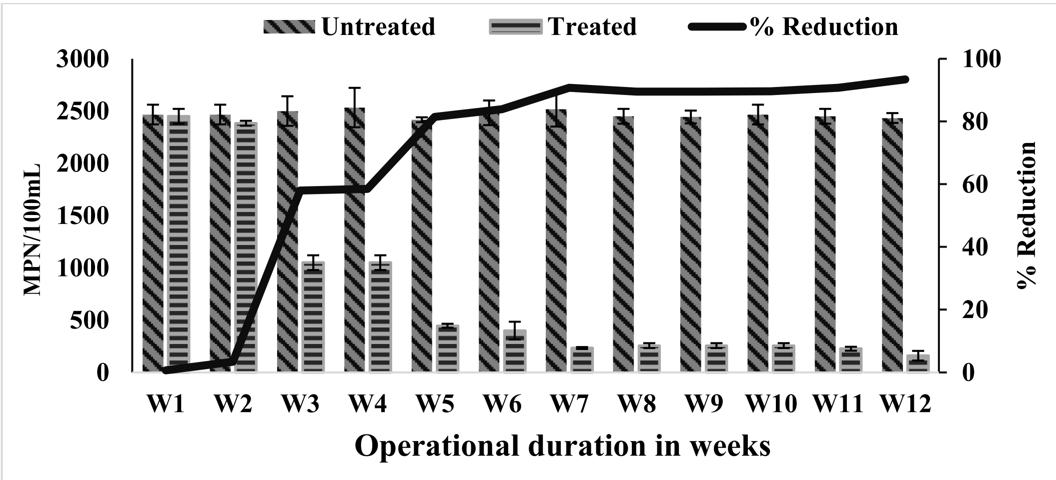


Figure 4.47 MPN index of untreated and GDB-treated blended wastewater.

4.3.2. Efficiency monitoring for IVFCW for the treatment of black liquor blended with domestic wastewater.

Effective treatment of industrial effluents, such as black liquor from the pulp and paper industry, poses a significant environmental issue. The IVFCW has the potential of sustainable solutions for wastewater. The present study was conducted to assess the efficacy of IVFCW in treating black liquor blended with household wastewater by monitoring important physicochemical and microbiological parameters as performance indicators. The comprehensive analysis and results of the present study indicated a significant outcome with the utilization of IVFCW for treating black liquor combined with domestic wastewater, results are indicated in Table 4.7. Thus, the present work contributes to the progress of sustainable wastewater treatment methodologies.

Table 4. 7 Efficacy of IVFCW for the treatment of pulp and paper industry effluent and domestic wastewater simultaneously

Sample Code	COD (%Efficiency)	BOD (%Efficiency)	DO (%Increase)	pH (%Variation)	EC (%Efficiency)	TDS (% Efficiency)	TSS (% Efficiency)	PO_4^{3-} (%Efficiency)	SO_4^{2-} (% Efficiency)	TN (%Efficiency)	Lignin (%Efficiency)	Color reduction (%Efficiency)
W1	79.69325	82.06478	297.3333	13.38028	85.64751	79.58706	54.14128	61.30199	75.85949	39.09774	84.53782	59.80271
W2	81.57454	83.6894	289.3333	8.8	85.08246	81.12169	61.98724	58.76393	70.31562	41.69784	84.73573	52.81863
W3	75.06551	78.62069	299	11.84314	80.84948	84.50339	100	53.57524	82.97952	60.48193	86.09195	59.89717
W4	72.64752	77.73074	338.6667	15.01946	78.56507	83.50861	100	58.73684	81.79369	48.24212	88.80903	56.85714
W5	77.91579	77.9902	320.3333	2.671756	78.13208	84.36475	100	62.88639	86.23348	54.40141	70.38647	54.75543
W6	81.69969	81.72043	289.3333	9.465021	65.40226	82.57426	100	69.58012	84.13376	71.82569	85.67627	39.93399
W7	84.54198	83.34825	266	5.15873	75.12463	83.57077	100	61.04015	84.67275	72.18919	83.87952	50.50072
W8	81.69728	80.35928	351.6667	7.058824	75.88296	84.34095	100	59.05369	82.45297	71.90361	81.47577	57.65766
W9	80.49981	80.53356	374.6667	7.45098	74.31227	84.47139	100	67.1193	84.39206	67.63682	78.33333	59.59596
W10	81.96256	81.27892	296.6667	10.72797	78.8501	84.96004	100	69.91071	77.43833	68.75895	80.53571	55.37791
W11	81.7644	82.17698	351	16.60232	78.75133	84.57627	100	67.35294	79.88857	66.93827	87.02429	60.73547
W12	87.3307	82.57299	336.6667	14.55224	80.55758	84.2741	100	70.08467	84.96012	72.67231	84.97758	55.92486

4.3.2.1. Biological oxygen Demand (BOD)

In the Integrated IVFCW assessment, specifically designed to treat combined wastewater from both pulp and paper industrial and home origins, the untreated wastewater showed BOD concentrations varying between 680 and 835 mg/L. Following the treatment via IVFCW, there was a significant reduction in BOD, 124 mg/L. The IVFCW exhibited a maximum reduction of 83.68% in BOD in sample W2, as shown in Figure 4.48. The results of this study highlight the effectiveness of IVFCW in reducing BOD levels in wastewater and its potential as a sustainable approach to address the environmental consequences of organic pollutants originating from industrial and residential origins. The wetland's ability to improve water quality is evident in the significant decrease in BOD, which plays a crucial role in the overall effectiveness of the wastewater treatment procedure.

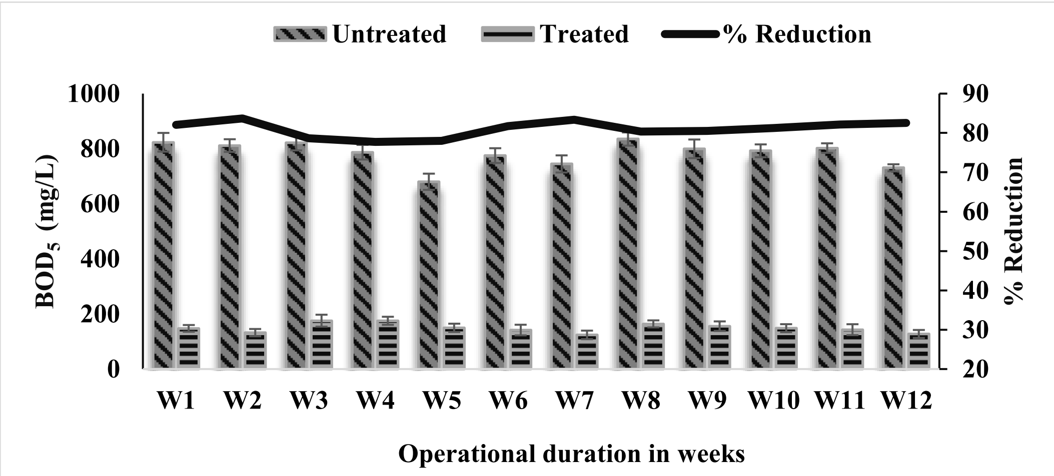


Figure 4.48 BOD_5 of untreated and IVFCW-treated blended wastewater.

4.3.2.2. Chemical oxygen demand (COD)

In evaluating COD in the IVFCW, the untreated wastewater displayed COD concentrations varying from 680 to 835 mg/L. Following the treatment, the IVFCW system effectively decreased the COD, reaching 124 mg/L. The IVFCW exhibited significant efficacy, achieving a maximum COD reduction of 83.68% sample W2, as shown in Figure 4.49. The results of this study highlight the effectiveness of IVFCW in reducing COD levels in wastewater and underscore the potential as a viable and ecologically friendly approach for addressing the adverse effects of chemical pollutants originating from industrial and residential activities.

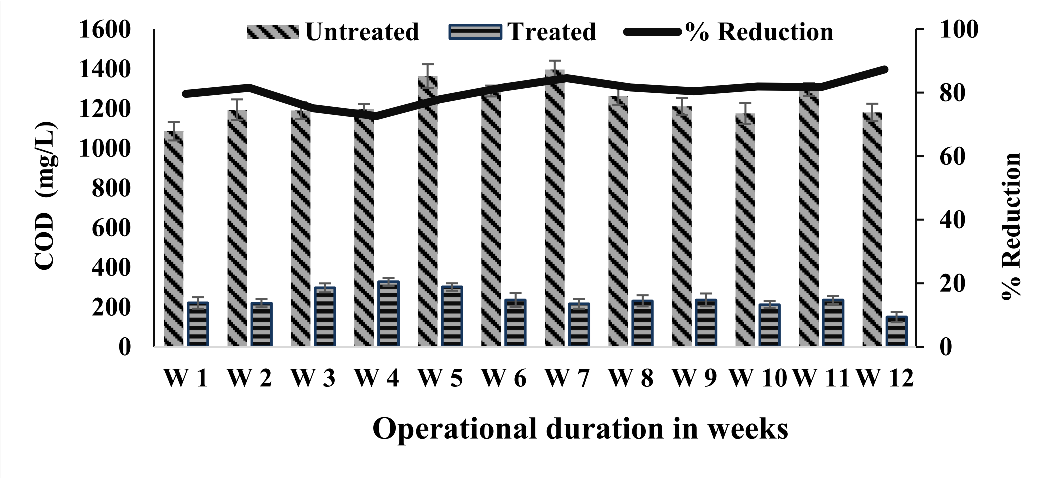


Figure 4.49 COD of untreated and IVFCW-treated blended wastewater.

4.3.2.3. Dissolved oxygen (DO)

When evaluating DO levels in the IVFCW system, specifically developed to treat wastewater from the pulp and paper industry and household sources simultaneously, the untreated wastewater exhibited DO values ranging from 1.55 to 2.79 mg/L. Following the treatment with IVFCW, a substantial rise in DO levels, with values ranging from 4.32 to 6.176 mg/L, was observed. This resulted in a significant increase of 374.66% in sample W9, as shown in Figure 4.50. The enhancement in DO levels highlights the effectiveness of IVFCW in improving the availability of oxygen in the wastewater that has undergone treatment and serves as an indication of the potential of the IVFCW as an ecologically sound approach for enhancing water quality in the treatment of intricate mixtures of wastewater.

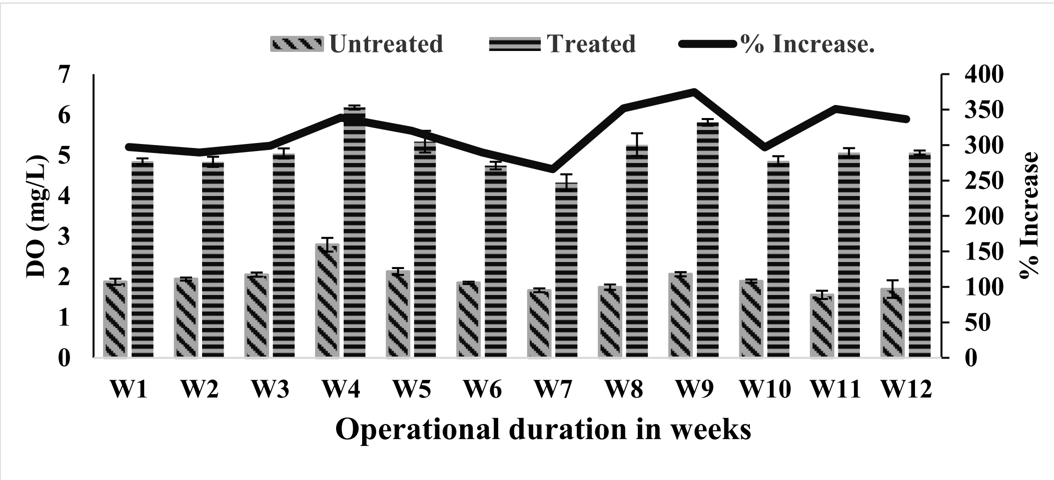


Figure 4.50 Dissolved oxygen of untreated and IVFCW-treated wastewater.

4.3.2.4. Lignin content

In assessing lignin content in the IVFCW designed for treating wastewater from the pulp and paper industry and residential origins, the untreated wastewater displayed lignin concentrations ranging from 138 to 164.66 mg/L. Following the treatment, the IVFCW has shown significant efficacy in reducing lignin content, resulting in values ranging from 18.166 to 40.866 mg/L. The IVFCW exhibited significant effectiveness, with a maximum reduction of lignin of 88.81% in sample W4, as shown in Figure 4.51. The results of this study highlight the effectiveness of the IVFCW in reducing lignin levels in mixed wastewater and underscore its potential as an ecologically friendly approach to addressing the harmful effects of lignin-rich effluents originating from industrial and residential origins.

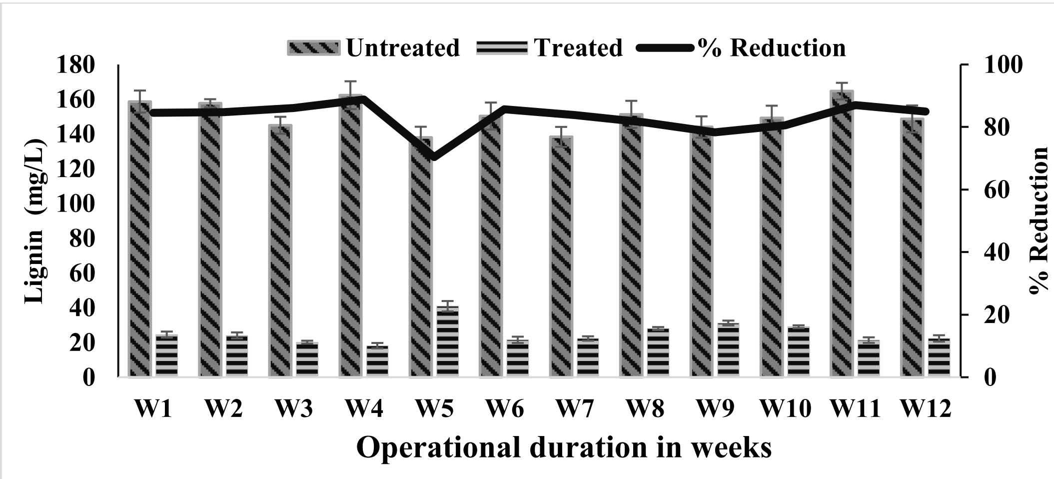


Figure 4.51 Lignin content of untreated and IVFCW-treated wastewater.

4.3.2.5. Color

In evaluating color in the IVFCW designed for treating combined wastewater from the pulp and paper industry and residential origins, the initial untreated wastewater exhibited color concentrations varying from 202 to 281 Pt-Co Units. Following the treatment application, the IVFCW exhibited a significant reduction in color, resulting in values ranging from 93.33 to 121.33 Pt-Co Units. The IVFCW had remarkable effectiveness, as shown by a maximum decrease in color of 60.73% recorded in sample W11, as shown in Figure 4.52. The results of this study emphasize the effectiveness of IVFCW in reducing color in wastewater and the potential of the IVFCW as an ecologically sustainable approach to address the harmful effects of colored effluents originating from industrial and residential origins.

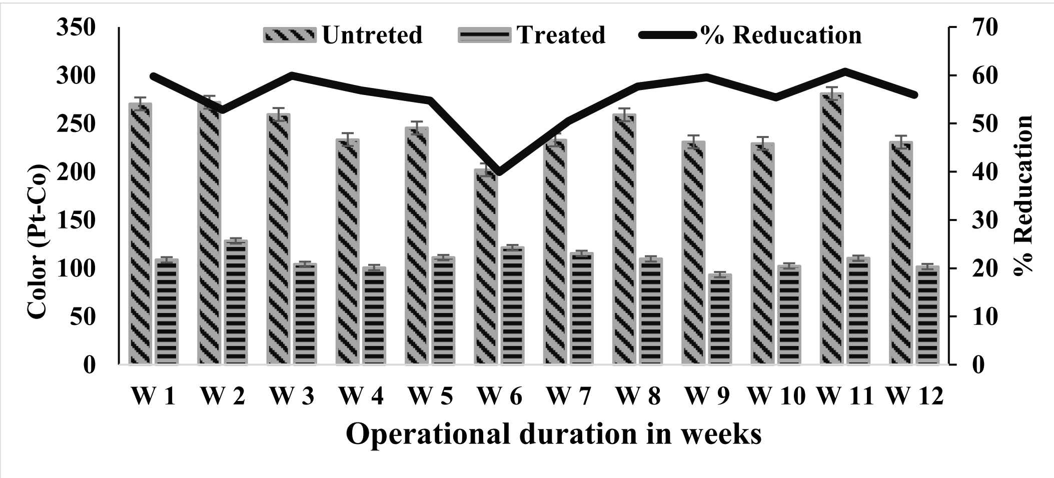


Figure 4.52 Color of untreated and IVFCW-treated blended wastewater.

4.3.2.6. Total dissolved solids (TDS)

The IVFCW was designed to treat blended wastewater from the pulp and paper industry and domestic sources. The untreated wastewater had TDS concentrations of 1376.3 to 1671.3 mg/L. The IVFCW lowered TDS to 207–319.66 mg/L after treatment through IVFCW. In sample W10, the IVFCW reduced TDS by 84.96% as shown in Figure 4.53. Due to its capacity to drastically decrease TDS concentrations in blended wastewater, IVFCW may be an ecologically viable alternative for treating industrial and household wastewater. Furthermore, the significant TDS reduction shows the IVFCW's ability to improve water quality and wastewater treatment success.

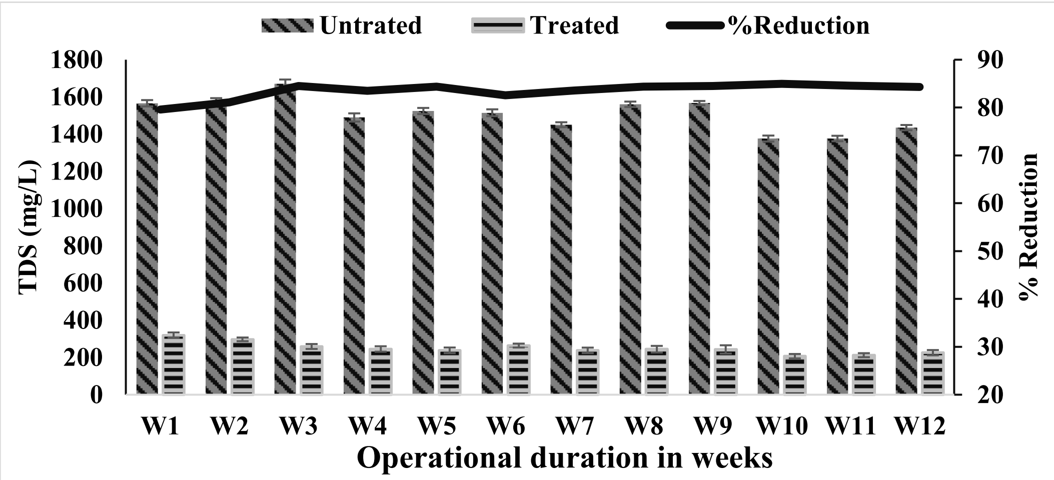


Figure 4.53 Total dissolved solids of untreated and IVFCW-treated wastewater.

4.3.2.7. Total suspended solids (TSS)

The untreated wastewater used as an influent for IVFCW, collected from the pulp and paper industry and domestic outlets, had TSS 2448.33 to 2559.67 mg/L. IVFCW treatment removed suspended solids, reducing TSS to 0 mg/L in samples W3–W12, showing 100% reduction, as depicted in Figure 4.54. These findings highlight that the IVFCW can completely remove TSS from blended wastewater, making it a viable approach to treating industrial and home-utilized wastewater.

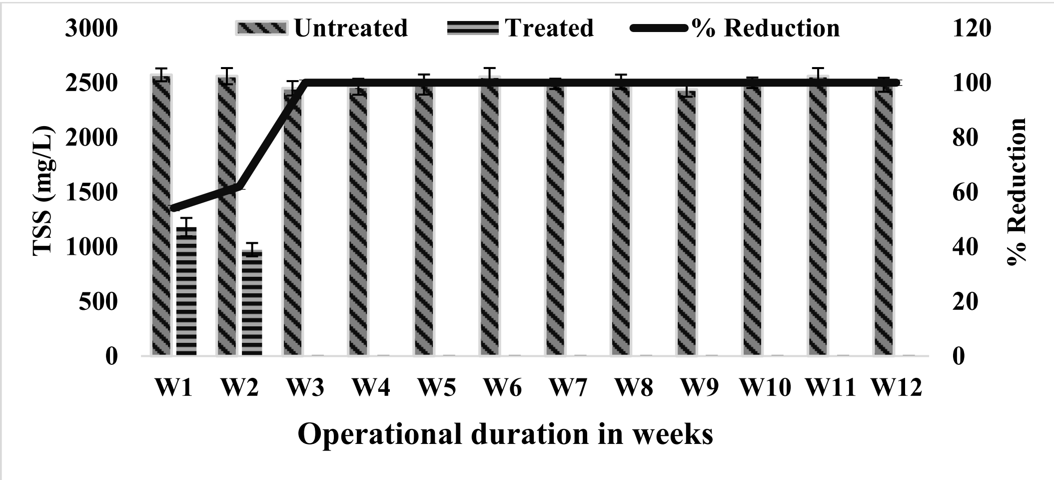


Figure 4.54 Total suspended solids of untreated and IVFCW-treated wastewater.

4.3.2.8. Electrical conductivity (EC)

The untreated wastewater had EC concentrations ranging from 1778.66 to 1925.33 µS/cm in the evaluation of EC within the IVFCW designed for treating blended wastewater from the pulp and paper industry and domestic sources. Following treatment, the IVFCW successfully lowered EC, with values ranging from 265.33 to 653.66 µS/cm. The IVFCW performed well, with a maximum EC reduction of 85.647% in sample W1, as shown in Figure 5.55. These findings illustrate that the IVFCW's capacity considerably reduces the EC of blended wastewater, emphasizing its potential as an ecologically friendly option for treating wastewater from both industrial and household sources. The significant decrease in EC concentration demonstrates the IVFCW's ability to improve water quality, which contributes to the overall effectiveness of the wastewater treatment process.

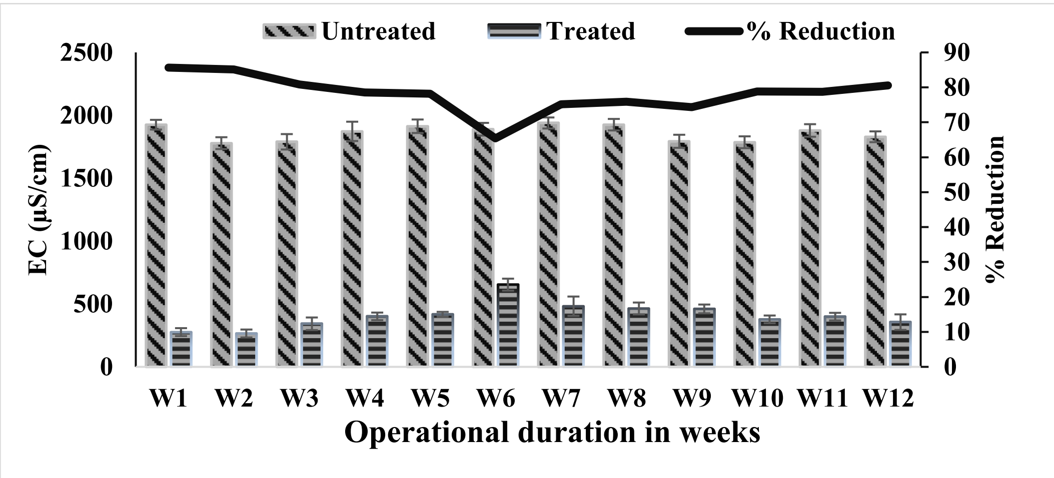


Figure 4.55 Electrical conductivity of untreated and IVFCW-treated blended wastewater.

4.3.2.9. pH analysis

When evaluating the pH fluctuations inside the IVFCW intended to treat combined wastewater from the pulp and paper industry and home origins, the pH levels of the untreated wastewater ranged from 8.1 to 9.46. Following the treatment via IVFCW, pH levels ranged from 7.2 to 8.5. The wetland exhibited a significant decrease in pH levels, with sample W11 showing a maximum drop of 16.66%, as shown in Figure 4.56. This suggests that the wetland is effective in managing and optimizing pH values. This study's findings emphasize the IVFCW's capacity to address and reduce acidity or alkalinity levels in wastewater.

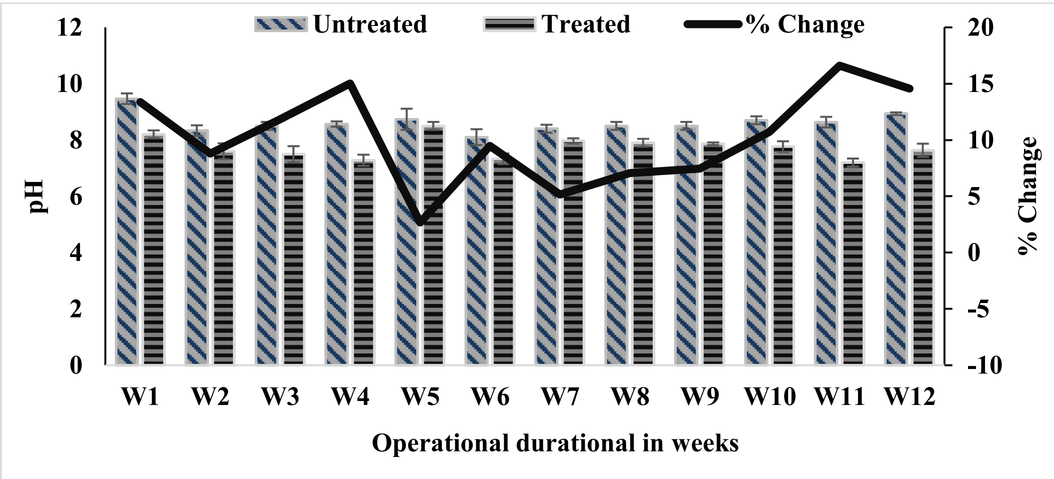


Figure 4.56 pH variations in untreated and IVFCW-treated wastewater.

4.3.2.10. Phosphate-Orthophosphates

During the assessment of phosphates in IVFCW, developed for treating combined wastewater from the pulp and paper industry and residential sources, the initial phosphate levels in the untreated wastewater varied between 3.12 and 3.89 mg/L. After treatment through IVFCW, there was a notable reduction in phosphate levels, ranging from 1.06 to 1.5 mg/L. The IVFCW exhibited effectiveness, with a peak decrease of 70.08% in phosphate levels in sample W12, as shown in Figure 4.57. The findings of the present study demonstrate the effectiveness of the IVFCW in the removal of phosphates from wastewater, underscoring its promise as a sustainable approach for mitigating nutrient pollution in wastewater originating from both industrial and residential sources.

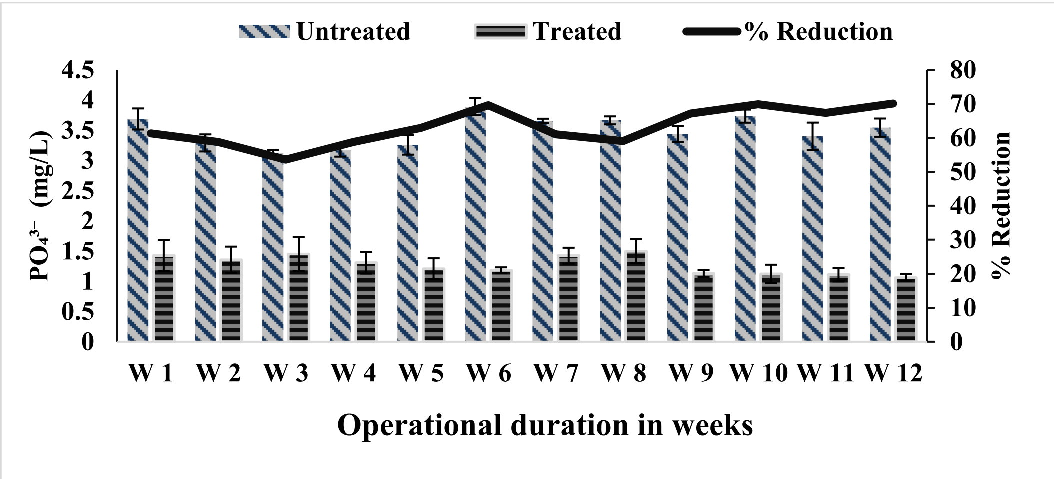


Figure 4.57 Phosphates of untreated and IVFCW-treated wastewater.

4.3.2.11. Sulfate

In evaluating sulphate levels in the IVFCW system, constructed for treating wastewater from the pulp and paper industry and household sources, the untreated wastewater samples showed sulfates ranging from 834.33 to 937 mg/L. After treatment in the IVFCW, there was a significant reduction in sulfate levels, with values ranging from 125 to 215.33 mg/L. The wetland exhibited significant effectiveness, with a maximum decrease of 86.233% in sulphate levels in sample W5, as shown in Figure 4.58. The findings of the present study highlight the efficacy of the IVFCW in addressing elevated levels of sulphate in wastewater, showcasing its potential as an environmentally sustainable approach for mitigating sulphate contaminants originating from industrial and residential sources.

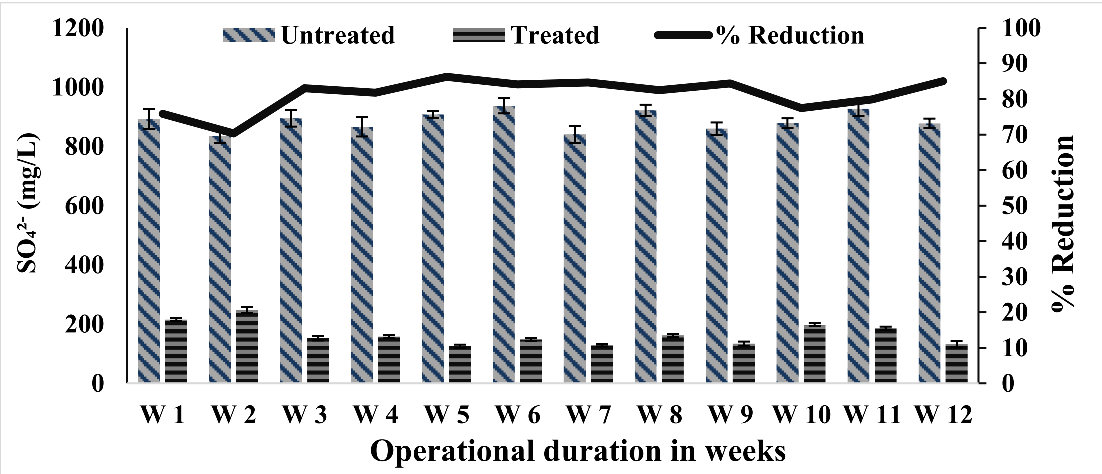


Figure 4.58 Sulfates of untreated and IVFCW-treated blended wastewater.

4.3.2.12. Total nitrogen (TN)

In the TN assessment in the IVFCW, designed for treating combined wastewater from the pulp and paper industry and home sources, the initial untreated wastewater showed TN varying between 123.33 and 151.46 mg/L. Following the treatment in IVFCW, the TN exhibited a significant reduction, with values ranging from 34.3 to 81.04 mg/L. The IVFCW exhibited considerable effectiveness, as shown by a maximum reduction of TN 72.67% in sample W12, as indicated in Figure 4.59. This study's results highlight IVFCW's efficacy in lowering TN levels in wastewater. These findings demonstrate the potential of IVFCW as a sustainable approach for mitigating nitrogen pollutants in wastewater originating from industrial and residential sources.

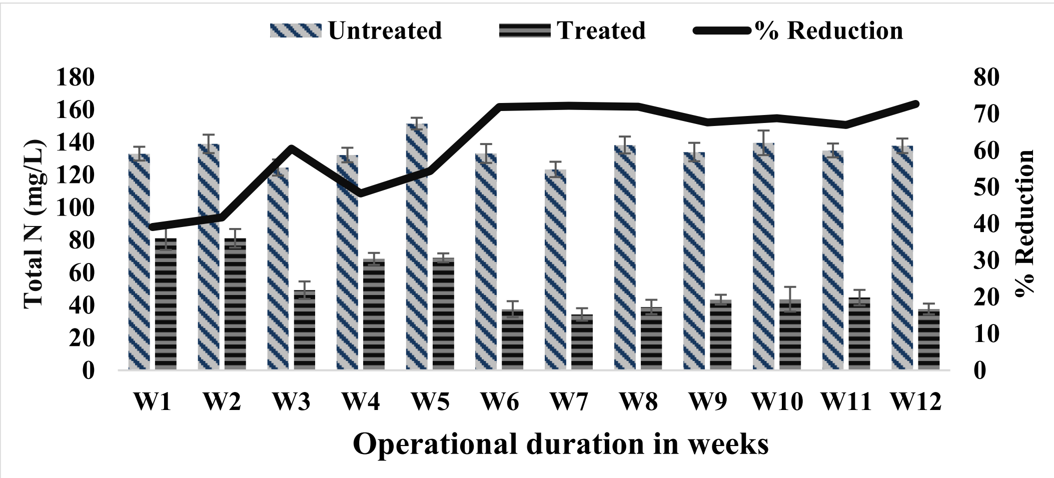


Figure 4.59 Total nitrogen of untreated and IVFCW-treated blended wastewater.

4.3.2.13. Microbiological Analysis: Colony forming unit (CFU)

Examining colony-forming units per millilitre (CFU/mL) in untreated and IVFCW-treated wastewater samples demonstrates a significant decrease in microbial concentration after the treatment process. Results (shown in Figure 4.60) of the present study demonstrate a notable decrease of 60% in colony-forming units per millilitre (CFU/mL) on average and a maximum of 67%, suggesting that the IVFCW can substantially remove microorganisms. These results highlight the treatment system's capacity to provide a more microbiologically sterile effluent, reducing the possibility of waterborne illnesses and possible environmental pollution.

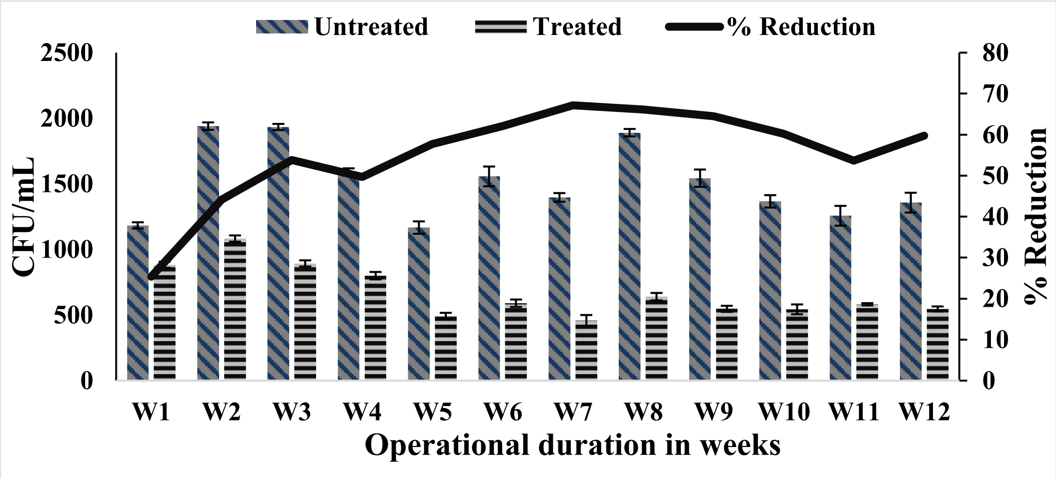


Figure 4.60 CFU/mL of untreated and IVFCW-treated wastewater.

4.3.2.14. Microbiological Analysis: Most probable number (MPN) index

The untreated blended wastewater has MPN values from 2416.6 to 2533.33. The IVFCW reduced MPN to 160 -1050 in treated wastewater. IVFCW reduced MPN by 93.424% in sample W12, as shown in Figure 4.61. These results demonstrate the IVFCW's ability to reduce microbiological pollutants, making it a promising and sustainable method for improving treated wastewater from varied sources' microbial quality. The decrease meets microbiological criteria, demonstrating the IVFCW's potential to enhance water quality.

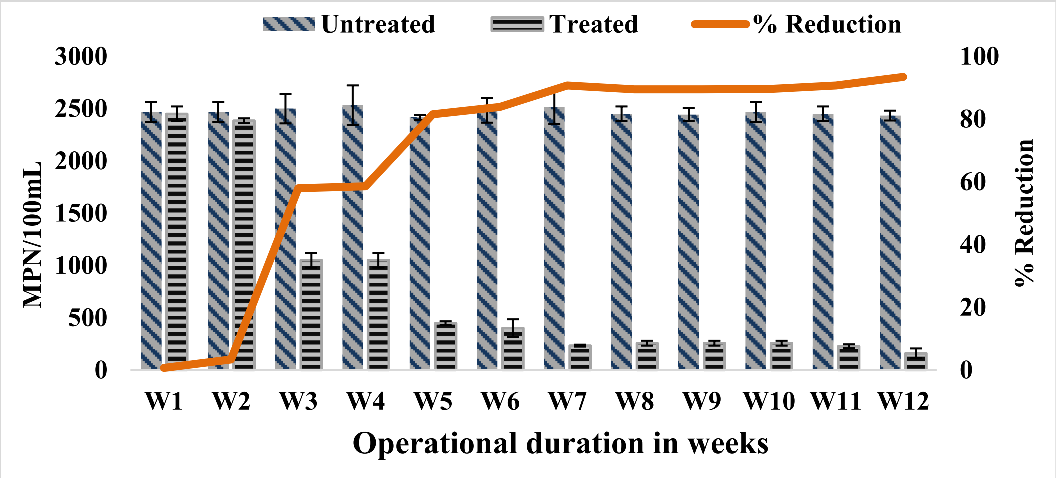


Figure 4.61 Most probable number index of untreated and IVFCW-treated blended wastewater.

Phase 4

Results-Objective 4

4.4. Study of biofilm developed on stone media in GDB and from the rhizosphere of IVFCW.

4.4.1. Study of biofilm developed on stone media in GDB.

Biofilm samples were taken from the aerobic chamber of GDB and were characterized using conventional methods, i.e., microscopy and biochemical characterization. Based on differences in colony morphology, growth pattern, and pigmentation, 14 strains (T1-T14) were selected for further biochemical characterization. The results of microscopy and different biochemical tests are shown in Table 4.8. Based on different biochemical tests like oxidase, catalase, urease, MR-VP, nitrate, citrate utilization, and H_2S production, the selected bacterial strains were identified as *Streptococcus, Aeromonas hydrophilla, E. coli, Vibrio, P.aeruginosa, Alcaligens faecals, Staphylococcus sp., Bacillus subtilis, Micrococcus variens, S.dysentery, Acenetobacter sp., Serratia sp., Citrobacter* and *Salmonella.*

Table 4. 8 Biochemical identification of different strains isolated from the biofilm developed on stone media in GDB.

Strain Code	Morphology	Gram's Staining	Oxidase	Catalase	Urease	MR	VP	Indole	Nitrate	Citrate	TSI	H_2S	Expected Genera
T1	**Cocci**	**G+**	-	-	-	+	-	-	+	-	A	+	*Sterptococcus*
T2	**Rods**	**G+**	-	+	-	+	-	-	+	-	A/K	-	*Aeromonas hydrophilla*
T3	**Rods**	**G-**	-	+	-	+	-	+	+	-	A	-	*E.coli*
T4	**Coma**	**G-**	+	+	-	-	-	+	+	+	K/A	-	*Vibrio*
T5	**Rods**	**G-**	+	+	-	+	-	-	+	+	-	-	*P.aeruginosa*
T6	**Cocci**	**G-**	+	+	-	-	-	-	-	+	-	-	*Alcaligens faecals*
T7	**Rods**	**G-**	+	+	-	+	+	-	+	-	A	-	*Staphylococcus sp.*
T8	**Rods**	**G+**	+	+	-	-	+	-	+	+	A	+	*Bacillus Subtilis*
T9	**Chain**	**G+**	-	+	-	+	+	-	-	-	A	-	*Micrococcus Variens*
T10	**Rods**	**G-**	-	+	-	-	-	+	+	-	K/A	-	*S.dysentery*
T11	**Cocci**	**G_**	-	+	+	+	+	-	-	-	-	+	*Acenetobacter sp.*
T12	**Cocci**	**G+**	-	+	-	+	-	+	+	+	A/K	+	*Serratia sp.*
T13	**Chain**	**G+**	+	+	-	+	+	-	+	+	A	-	*Citrobacter*
T14	**Chain**	**G-**	+	+	+	+	+	+	+	+	A	+	*Salmonella*
Key + = positive test; - = negative test;± = Variables; AG = Acid and gas production;K= alkaline; A = Acidic; NC = No color change; K/A, H2S = Red/yellow with black precipitation; K/A = Red/yellow; A/NC = Acid/no color change													

4.4.2. Study of biofilm developed on stone in the rhizosphere of IVFCW.

The soil sample was taken from the rhizosphere of *Typha latifolia*. Different dilutions (10^{-1} to 10^{-6}) of the sample were made with autoclaved distilled water, spread on the nutrient agar plates under sterile conditions in a laminar flow hood, and incubated at 37°C for bacterial growth. After 24-48 hours, colonies were selected based on differences in morphology, size, coloration, marginal shapes and pigmentation. 17 different strains (S1-S17) were selected for further biochemical characterization. The results of microscopy and different biochemical tests are shown in Table 4.9. Based on different biochemical tests like oxidase, catalase, urease, MR-VP, nitrate, citrate utilization, and H_2S production, the selected bacterial strains were identified as *Klebsiella*, *Bacillus subtilis*, *Micrococcus variens, Salmonella*, *Bacillus cereus, Aeromonas hydrophilla*, *Proteus vulgarius, Acenetobacter sp.*, *Listeria sp.*, *Corynebacterium*, *Sterptococcus, Neisseria sp*., *P. aeruginosa*, *S. dysentery*, *Enterobacter aerogenes*, *Alcaligens faecals* and *E. coli*.

Table 4. 9 Biochemical identification of different strains isolated from the rhizosphere of IVFCW.

Strain Code	Gram's Staining	Morphology	Oxidase	Catalase	Urease	MR	VP	Indole	Nitrate	Citrate	TSI	H_2S	Identified Genera
S1	G^+	Rods	-	+	+	-	+	-	+	-	A	+	***Klebsiella***
S2	G^+	Rods	+	+	-	-	+	-	+	+	A	+	***Bacillus subtilis***
S3	G^+	Chain	-	+	-	+	+	-	-	-	A	-	***Micrococcus variens***
S4	G-	Chain	+	+	+	+	+	+	+	+	A	+	***Salmonella***
S5	G_-	Chain	+	+	-	-	+	-	+	+	A	-	***Bacillus cereus***
S6	G^+	Rods	-	+	-	+	-	-	+	-	A/K	-	***Aeromonas hydrophilla***
S7	G^+	Chain	+	+	-	+	+	-	+	+	A	-	***Proteus vulgarius***
S8	G_-	Cocci	-	+	+	+	+	-	-	-	-	+	***Acenetobacter sp.***
S9	G^+	Rods	-	+	-	-	+	-	+	-	K	-	***Listeria sp.***
S10	G^+	Rods	-	+	-	+	-	-	+	-	A/K	-	***Corynebacterium***
S11	G^+	Cocci	-	-	-	+	-	-	+	-	A	+	***Sterptococcus***
S12	G^-	Rods	-	+	-	-	-	-	+	-	A	+	***Neisseria sp.***
S13	G^-	Rods	+	+	-	+	-	-	+	+	-	-	***P. aeruginosa***
S14	G^-	Rods	-	+	-	-	-	+	+	-	K/A	-	***S. dysentery***
S15	G^-	Rods	-	+	-	-	-	-	+	+	K/A	-	***Enterobacter aerogenes***
S16	G^-	Cocci	+	+	-	-	-	-	-	+	-	-	***Alcaligens faecals***
S17	G^-	Rods	-	+	-	+	-	+	+	-	A	-	***E. coli***

Key: ± = Variables; AG = Acid and gas production; K = alkaline; A = Acidic; NC = No color change; K/A, H_2S = Red/yellow with black precipitation; K/A = Red/yellow; A/NC = Acid/no color change.

CHAPTER 5

DISCUSSION

5. Discussion

The present study aligns with UN Sustainable Development Goal 6, which emphasizes universal access to water and sanitation. Traces of contaminants make vast volumes of water unfit for drinking purposes, thus not only reducing the universal availability of water but also increasing the prevalence of waterborne diseases. Most of such contaminants are released mainly by industrial activities. The extended demand of freshwater for industrial processes and the release of untreated effluent containing harmful contents increase the risk of water contamination and environmental deterioration, underscoring the importance of implementing an efficient wastewater treatment method. Considering the socioeconomic factors, public health concerns, and the need for environmental conservation, there is an urgent need for a sustainable, eco-friendly, and technologically advanced wastewater treatment process practically adoptable by industries for water recycling systems and participation in carbon credit schemes. The aim of this study was to develop an integrated biological reactor that can effectively and economically treat effluent from the pulp and paper industry, while also being environmentally friendly and sustainable. and ensuring water availability while simultaneously conserving ecosystems and public health. The findings of the physicochemical analysis of wastewater, isolation, and characterization of bacterial strains from the BL provide a valuable potential for these indigenous bacterial strains to be used in wastewater treatment. During the initial screening process, TR-1, TR-5, and TR-8 showed exceptional lignin degrading capacity on agar plates amended with lignin. The efficacy of the selected strains was further confirmed in a secondary screening process in a shake flask experiment at a range of pH and temperature. The selected bacterial strain TR-1 showed maximum growth, reaching a peak of 3.12g/l at 41°C and pH 8. Additionally, it showed significant lignin degradation (77%) and color reduction (65%) at a temperature of 37°C and pH level of 8. These results indicate that the selected bacterial strain is slightly thermo-alkaliphile and can be maintained at 41°C and pH eight high temperatures. Likewise, the selected bacterial strains TR-5 showed substantial growth of 2.9g/L at a temperature of 37°C and a pH level of 8, 65% lignin degradation and color reduction. The selected bacterial strain TR-8 exhibited the highest growth (2.34g/L) at a temperature of 37°C, a pH level of 8, 66% lignin degradation, and a 65% reduction in color. After biochemical identification, the selected bacterial strains were identified as TR-1 *B. subtilis*, TR-5 *B. cereus*, and TR-8 *Pseudomonas*. The physicochemical assessment BL shows that it has an alkaline pH, a high lignin content, and significant concentrations of both dissolved and suspended particles. The BL exhibited elevated levels of chemical oxygen demand (COD), biological oxygen demand (BOD), nitrates, and sulfates. When comparing these results to permissible values, it is evident that the BL may pose environmental challenges. This highlights the importance of establishing effective treatment measures. Overall, the selected indigenous bacterial strains analyzed, TR-1, TR-5, and TR-8, exhibited considerable capacity for degrading lignin and diminishing color under different pH and temperature conditions. Bacteria require carbon as a food source. They even take lignin from the paper mill effluents as their carbon source and acquire needed nutrients by breaking it down (up to 98%) into simple forms (Raj *et al.,*2007). To degrade the lignin, bacteria produce a variety of enzymes such as LiP, MnP, and laccases. In addition to these complex enzymatic processes, bacteria may employ bioaccumulation and biosorption (Rasool *et al.,*2023). According to studies by Ojha and Markandeya (2016), lignin was degraded to a maximum of 40.19% in wastewater from the paper industry when three bacterial isolates, *B.subtilis*, *Bacillus endo-physics*, and *Bacillus sp.* were combined. These isolates included *Bacillus subtilis*, *Bacillus endo-phyticus*, and *Bacillus sp.* The rate of lignin breaks and color reduction of 50-97% is achieved through the application of *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus sp.*, and *P.aeruginosa* in investigations of batch scale (Raj *et al.,*2007; Tiku *et al.,*2010; Tyagi *et al.,*2014). Bacteria degrade lignin differently (Chandra *et al.,*2007; Chandra & Bharagava, 2013), for bacteria, the degradation of lignin either aerobically or anaerobically though is a challenging task but bacteria accomplish efficiently due to versatile enzyme production capacity and ability to shift metabolism. Some bacteria, like *Streptomyces viridosporous*, are involved in lignin breakdown (Hossain & Ismail, 2015). Two bacterial species, *Serratia liquefaciens* and *Paenibacillus sp.*, were reported to produce or induce enzymes for lignin breakdown. The enzymes lignin peroxidase and laccase performed the restoration as they were used in the bioremediation of paper and pulp effluent (Raj *et al.,*2014; Haq *et al.,*2016a, 2017). Laccase enzyme efficiently handles cellulose and hemicellulose-based crude products, transforming them into valuable value-added products from pre-hydrolysis liquid (PHL). Wang *et al*. (2015) saw lignin removal from PHL following the modeling of laccase induction in kraft pulp. These findings of the present emphasize the potential application of these bacteria in treating BL. This can facilitate the development of more sustainable and efficient techniques for wastewater treatment in the pulp and paper industry. However, further research is needed to examine the potential of these strains to be scaled up and maintain stability over an extended period and in-situ wastewater remediation system.

The physicochemical characterization of wastewater was done, and it was subjected to treatment in gravity-driven bioreactor (GDB) and integrated vertical flow constructed wetland (IVFCW) planted with *Typha latifolia*. The wastewater had a very pungent and unpleasant odor. It was observed that GDB efficiently removed unpleasant odors due to the prolonged contact time of pollutants with biofilms that extended the degradation of organic contaminants. Previous studies also reported that increased contact time of microbial biofilm with pollutants helps in the degradation of organic and aromatic pollutants, resulting in the removal of unpleasant odors when treated in various types of biological reactors (Rasool *et al.,*2017; Sehar *et al.,*2015). The influent had a broad range of pH values, ranging from 7.28 to 9.47, indicating significant variation in the initial pH. Treatment with GDB resulted in an average pH level change of around 8.82% across all samples, suggesting a successful and uniform pH adjustment in the treated effluent. Notably, sample wastewater exhibited the most significant alteration in pH with a post-treatment increase of 15.02%. This observation highlights the GDB's ability to effectively regulate pH levels, mainly when the original values are more extreme. Notably, the effluent pH levels remained within the permissible limits of WHO (2006) standards of 6.5-8.5 after treatment, indicating the GDB's effectiveness in neutralizing acidic or alkaline constituents. It is essential to highlight that the GDB system has both aerobic and anaerobic biofilms; this combination likely played a pivotal role in pH adjustment by allowing the biodegradation of diverse organic and inorganic constituents found in wastewater (Castro *et al.,*2017). The observed enhancement in pH levels demonstrates the potential of the GDB for accomplishing efficient wastewater treatment, particularly for intricate mixtures of pulp and paper industry effluent and domestic wastewater; however, additional research on the distinct microbial communities and chemical transformations occurring within the GDB holds the potential to elucidate the fundamental processes driving the observed alterations in pH.

In PPI, during different steps of the product-making process, a considerable quantity of chemical compounds is released, causing coloration and a foul smell of the effluent. Biofilm in anaerobic and aerobic chambers and microbial communities in wetland rhizospheres played a crucial role in hazardous chemical degradation, color reduction, and odor reduction. Similar results in odor and color reduction were observed by (Rasool *et al.,*2017). After treatment through IVFCW, it significantly reduced and was under the permissible limits of USEPA. Although pH has no direct or indirect influence on terrestrial or aquatic life, it is one of the critical and essential parameters determining wastewater quality. The decrease in pH in the IVFCW system may be because of nitrate conversion to molecular nitrogen and phosphate and sulfate metabolism (Sakuma *et al*.,2008). DO is considered one of the crucial parameters in determining water quality. Initial DO levels varied from 2.91 to 1.7 mg/L. Throughout the treatment in the GDB, it was observed that the average spike in DO levels was around 153.34%, the highest increase in dissolved oxygen levels, with a notable jump of 186.59% observed when GDB was operated with blended wastewater. This result underscores the significant potential of the GDB to enhance DO levels effectively. The findings highlight the potential of the GDB to improve oxygen levels by reducing the BOD and COD, hence enhancing water quality and the ecological well-being of the receiving water bodies. The increase in DO and reduction in the organic contaminants indicates the active metabolism of organic pollutants in the wastewater. The increase in DO content after treatment through GDB is attributed to an oxygen-rich environment in the aerobic chamber that favours the rapid growth of aerobic microbes. These aerobic bacteria participate in the metabolic process of organic materials, resulting in the liberation of oxygen and, subsequently, increases in DO levels (Luan *et al.,*2022).

In contrast, inside an anaerobic chamber of GDB, the elevation in DO content results from several microbial metabolic activities, such as denitrification. These processes generate oxygen as a byproduct of their reactions, contributing to the observed increase in DO levels (Huang *et al.,*2022). The GDB utilizes both aerobic and anaerobic processes in a mutually beneficial manner to enhance DO, hence improving water quality and promoting a more sustainable ecology in the receiving water bodies.

An increase in DO content (1.9 mg/L to 5.1 mg/L) after treatment through IVFCW confirms the reduction of organic containments and better water quality. Thus, the present design of IVFCW proved very efficient in reducing organic contaminants. As reported by Taylor in 2011 and Ciria in 2005, plants can transfer oxygen from leaves to roots and father into the rhizome, where bacteria take it up as an electron donor to degrade the pollutants. Wetlands promote the growth of aerobic microorganisms, such as bacteria and algae, responsible for the breakdown and decomposition of organic pollutants (Moazzem *et al.,*2023). As these microorganisms metabolize the organic pollutants present in the wastewater, they consume oxygen. This microbial activity and macrophytes in the wetlands promote oxygenation through photosynthesis (Babuponnusami *et al.,*2023). Macrophytes release oxygen as a byproduct of photosynthesis, further contributing to increased D.O. levels. Additionally, the physical processes in wetlands, such as aeration and exposure to air-water interfaces, facilitate the transfer of atmospheric oxygen into the water, enhancing D.O. levels. As a result, wastewater treatment through wetlands can lead to increased dissolved oxygen concentrations (Mittal *et al.,*2023).

Organic pollutants can be characterized by the proxies of COD and BOD_5, which are associated with the amount of DO in water. The untreated wastewater exhibited a considerable initial variation in pollutant contents from 1391.33 to 1189 mg/L in blended wastewater. An average reduction of 76.00% COD after treatment in the GDB was observed, demonstrating the efficacy of the GDB in degrading and removing organic pollutants from wastewater. An incredible 84.48% decrease proves the GDB's capacity to reduce COD concentrations considerably regardless of higher baseline levels. The GDB is beneficial for treating domestic and industrial wastewater, highlighting its potential as an effective technique for simultaneously removing contaminants of different natures. The main factor influencing COD elimination in an aerobic biofilm bioreactor is oxygen, which promotes the development of aerobic bacteria. These microbes use oxidation to break organic pollutants into simpler, less toxic molecules (Mahto *et al.,*2022). The absence of oxygen in anaerobic biofilm bioreactors promotes the development of anaerobic microbes that use organic matter as an electron donor, which makes it easier to break down complex organic molecules into carbon dioxide and methane. Intending to remove COD, both aerobic and anaerobic methods are crucial. They provide flexible wastewater treatment options tailored to the effluent's requirements (Karadag *et al.,*2015). According to Gao *et al*. (2016), a submerged anaerobic membrane bioreactor removed 83% of COD from the pulping effluent (SAnMBR). Similarly, the untreated effluent significantly varied BOD5 from 823.33 to 680 mg/L. Results indicate that the GDB led to an average reduction of approximately 68.87% in BOD_5, demonstrating the efficacy of GDB's treatment process in decomposing and removing biodegradable organic pollutants in wastewater. The GDB displayed a significant BOD_5 reduction, with a decrease of 71.40%. These results highlight the GDB's ability to effectively reduce BOD_5 concentrations of wastewater with different origins and initial containment levels, establishing its significance in industrial and residential wastewater treatment. Naz *et al*. (2016) reported a 59.67% reduction in the BOD5 in a fluidized bed reactor and a sand column filter with a retention time of 96 hours. The reduction in COD and BOD_5 is attributed to the pre-development of biofilm on the stone media. The biofilm's complex and adapted bacterial colonies utilized organic contaminants and oxidized them into CO_2 and H_2O. The GDB consists of aerobic and anaerobic chambers, presenting a complete and adaptable approach for minimizing BOD. The aerobic chamber facilitates the development of aerobic microorganisms by providing an environment abundant in oxygen. These aerobic bacteria play a crucial role in degrading biodegradable organic contaminants via oxidation. At the same time, the anaerobic chamber facilitates the growth of anaerobic bacteria that effectively engage in the process of fermentation and decomposition of organic substances, hence contributing to the further reduction in BOD levels (Lu *et al.,*2022). Using an integrated process (aerobic and anaerobic simultaneously), the GDB system affirms the attainment of effective and comprehensive BOD removal. To achieve compliance with permissible effluent discharge limits of NEQ of Pakistan or USEPA, an extended HRT is a valuable approach. However, subsequent research is required to explore the impact of varying HRT and/or temperature to optimize the GDB's wastewater treatment efficiency. The IVFCW exhibited a maximum reduction of 83.68% in both BOD and COD. The results of this study highlight the effectiveness of IVFCW in reducing BOD levels in wastewater and its potential as a sustainable approach to address the environmental consequences of organic pollutants originating from industrial and residential origins. The results of this study highlight the effectiveness of IVFCW in reducing COD levels in wastewater and underscore the potential as a viable and ecologically friendly approach for addressing the adverse effects of chemical pollutants originating from industrial and residential activities. Organic pollutants, such as BOD and COD, are linked to the concentration of dissolved organic contaminants in wastewater. The high BOD and COD values in pulp and paper industry effluent can be attributed to organic compounds derived from various processes involved in paper production. These compounds include lignin, cellulose, hemicellulose, and other organic substances in the raw materials. These organic compounds are released into the wastewater during manufacturing, resulting in elevated BOD and COD levels. The effluent from PPI often consists of complicated organic molecules resistant to natural degradation, making it challenging to treat and reduce BOD and COD effectively. Similarly, in 2007, Akratos Tsihrintzis reported that 91.9% of COD and BOD were reduced with HRT of 8 days with a gradual increase in dissolved oxygen. This gradual increase can be related to root development over time, which increases the pore formation in the soil and allows more oxygen to reach down (Hench *et al.,*2003).

In the present study, the GDB successfully lowered the COD of all samples of wastewater from the pulp and paper industry, with drops ranging from 68.92% to 77.58%. The IVFCW also showed a significant reduction in COD, with a range of 69.68% to 76.28%. Similarly, the GDB achieved an average BOD reduction of approximately 67.7%, while the IVFCW demonstrated a slightly higher average reduction of around 73.8%. The GDB demonstrated an average increase of approximately 198.8%, while the IVFCW showed a higher average increase of around 239.5%. According to these results, both bioreactors effectively raise the amount of dissolved oxygen, but IVFCW does a slightly better job of oxygenating the treated effluent. Also, Akratos and Tsihrintzis reported in 2007 that a high percentage of COD and BOD could be reduced by 91.9% by using an 8-day hydraulic retention time (HRT) and a steady rise in dissolved oxygen levels. The steady rise can be attributed to the progressive growth of roots, which leads to the production of larger pores in the soil and facilitates the penetration of more oxygen (Hench et al., 2003).

BL contains more recalcitrant pollutants like lignin, chlorinated lignin, and sops (Virkutyte *et al.,*2017). In contrast, the wastewater from domestic effluent contains relatively less concentration of such recalcitrant and higher microbial communities from the gut of humans (Ju *et al.,*2019). The co-digestion of wastewater originating from various sources with distinct substrates has the potential to mitigate toxicity and foster the establishment of a resilient microbial population. In addition, the microbial community can undergo acclimation and adaptation in response to unfavourable conditions (Chen *et al.,*2008). Hence, the commencement of wastewater treatment, which involves the presence of inhibitors or toxicants at high concentrations, necessitates an initial acclimation or adaption phase that may span from a few days to several weeks. With thick layers of aerobic and anaerobic biofilm, GDB contains a higher population of adapted communities, effectively removing organic contaminants and improving water quality. In the present study, the untreated effluent exhibited a range of lignin concentrations from 167.67 to 148.33 mg/L. Following treatment in the GDB, wastewater exhibited the most significant reduction in lignin content, with an 80.11% decrease (118.83 mg/L).

In comparison, the IVFCW showed a maximum reduction of around 88% in the lignin concentration, while the average efficiency of the IVFCW was 83%. These results affirm the potential IVFCW to reduce the lignin concentration. These results illustrate the GDB's and IVFCW's potential to effectively reduce lignin concentration in wastewater, a critical element in mitigating the adverse effects of lignin-rich industrial effluents on receiving water bodies. In another study, COD, lignin, color, and phenol removal rates were documented at 85%, 74%, 96%, and 81%, respectively (Majumdar *et al.,*2019). Lignin degradation in GDB is primarily attributed to bacterial biofilms, as bacteria can metabolize lignin and its aromatic derivatives, yielding intricate lignocellulosic biomass that sustains their growth.

Moreover, these bacteria facilitate the production of ligninolytic enzymes, which are essential for degradation (Rinaldi *et al.,*2016). Diverse bacterial species have been studied for their competence in detoxifying lignin and generating ligninolytic enzymes. Strains like *Bacillus sp*. and *Paenibacillus sp*. have been isolated from pulp and paper mill waste, with their lignin degradation capabilities verified through degradation product analysis (Chandra *et al.,*2007). The leading cause of lignin breakdown in IVFCWs is commonly linked to bacterial biofilms. Bacterial organisms possess the inherent ability to metabolize lignin and its derivative aromatic compounds, developing intricate lignocellulosic biomass that sustains their proliferation. Furthermore, this metabolic pathway promotes the creation of ligninolytic enzymes, which are critical in lignin degradation (Rinaldi *et al.,*2016).

Numerous bacterial species have been studied for their ability to detoxify lignin and produce ligninolytic enzymes. Bacterial strains were obtained from PPI sludge, notably *Bacillus sp*. and *Paenibacillus sp*. their ability to degrade lignin was validated by evaluating degradation products (Chandra *et al.,*2007). Laccase-producing bacteria, such as *Azotobacter*, *B. megatarium*, and *Serratia*, were also obtained from soil samples (Lai *et al*.,2023). These bacteria can break down lignin, and their ability to degrade lignin is associated with their laccase production (Xu *et al.,*2018). A recent study by Khan *et al*. (2022) observed that *B. altitudinis SL7* exhibited a notable capacity for efficient breakdown of lignin, particularly when exposed to increased compound concentrations.

Total solids in wastewater comprise both TDS and TSS. According to WHO (2006) and US-EPA (2007), the recommended values for TDS in wastewater should not exceed 1000 mg/L, while TSS should be 25-80 mg/L. The concentration of TDS in wastewater samples was very high due to many suspended, dissolved and colloidal particles such as sand particles, slit and clay, industrial waste, and different organic and inorganic ions. In the present study, GDB proves to be quite efficient in reducing the levels of TDS and TSS. The lowest TDS observed was 2561.33 mg/L. After treatment, an average decrease of approximately 72.75% in TDS levels and the most significant reduction, with an impressive decrease of 82.04%. This outcome highlights the GDB's remarkable ability to substantially reduce TDS concentrations, even when confronted with elevated initial TDS levels. These findings demonstrate the efficacy of the GDB treatment process in effectively reducing the concentration of dissolved solids in the effluent, which, in turn, leads to enhanced water quality and a decrease in environmental contamination. In GDB, TDS reduction is owed to microbial metabolism and sedimentation processes. In the aerobic chamber, microbial activity consumes dissolved organic molecules, whereas in the anaerobic chamber, specific processes produce insoluble precipitates (Soo *et al.,*2022). These combined methods efficiently lower the concentration of dissolved solids, improving water quality and conforming with regulatory limits for permitted TDS levels in effluent discharge. After the treatment through IVFCW, a 100% reduction in TSS and an 84% reduction (0 mg/L and 150 mg/L) in TDS were observed. TDS also influences EC; in untreated wastewater samples, the average EC was about 1860µS/cm, while after treatment with IVFCW, EC was reduced to 408µS/cm. The current study showed a maximum reduction of 85% EC values of blended wastewater after treatment with IVFCW at an HRT of 3 days. Total solids are the sum of TSS and TDS. Previous studies have documented similar findings for TDS and TSS concentration reduction.

The GDB achieved an average TSS reduction of approximately 92.5%, while the IVFCW demonstrated an even higher average reduction of 96.3%. Similarly, the GDB demonstrated an average TDS reduction of approximately 54.3%, while the IVFCW showed a higher average reduction of around 63.3%. Furthermore, both the GDB and IVFCW were effective in reducing electrical conductivity (EC). The GDB achieved an average EC reduction of approximately 55.2%, while the IVFCW demonstrated a higher average reduction of around 67.1%. These results indicate the successful capability of both bioreactors in lowering the electrical conductivity of the effluent, with the IVFCW showing a slightly superior performance in this regard. Microbial activity in the aerobic chamber utilizes dissolved organic molecules, whereas the anaerobic chamber facilitates the production of insoluble precipitates through certain mechanisms (Soo et al., 2022). By employing these integrated techniques, the concentration of dissolved solids is effectively reduced, leading to enhanced water quality and compliance with regulatory standards for allowable total dissolved solids (TDS) levels in the discharge of wastewater. Prior research has shown comparable results regarding the decrease in concentrations of TDS and TSS. The B. reptans attained removal efficiencies of 58% and 63.42%, respectively, whereas the T. *portulacastrum* produced removal efficiencies of 70.03% and 74% in the created wetlands. These results were seen with a hydraulic retention time of 20 days. The higher rates of removal reported in *T. portulacastrum* can be attributed to its possession of a highly developed network of roots and root hairs. According to Sehar et al. (2015), they observed that higher hormone replacement therapy (HRT) leads to longer periods of interaction between contaminants and bacteria in the rhizosphere. A further investigation has shown that the retention duration of 48 hours in a trickling sand filter resulted in a reduction of EC by about 29.4% (Khan et al., 2014). Rasool et al., 2023 saw comparable outcomes in reducing EC levels when using GDB and constructed wetland for treating a mixture of wastewater from the pulp and paper industry and home sources.

The removal efficiencies achieved with *B. reptans* were 58% (201.88 mg/L) and 63.42% (212.30 mg/L), respectively, while with *T. portulacastrum*, they were 70.03% (144.06 mg/L) and 74% (150.93 mg/L) in the constructed wetlands. These outcomes were seen when the hydraulic retention period was 20 days. The increased rates of removal observed in *T. portulacastrum* can be attributed to possessing a well-developed network of roots and root hairs. Sehar *et al*. (2015) have witnessed that increased HRT increases the interaction time between pollutants and bacteria in the rhizosphere. The proper selection of macrophytes is critical in removing TSS and TDS. Significant expansion of root systems and root hairs, which effectively increase the accessible surface area for microbial adhesion and colonization, and a more significant number of microbial populations leads to effective removal of total solids by biomineralization and bioaccumulation. The IVFCW system, employed for wastewater treatment, can be ascribed to the concurrent processes of physical filtration, adsorption, and microbial degradation to reduce TDS and TSS. The macrophytes' extensive root network and root hairs increase the surface area for microbial attachment. Settlement in PST and IVFCW allows enhanced particulate matter (TSS) and solutes (TDS) removal through biological interactions and absorption onto root surfaces. This interplay of physical and biological mechanisms effectively reduces TDS and TSS in the effluent. Multiple reasons for reducing EC include reduction in TSS, TDS, and conversion of nitrate or nitrite into molecular nitrogen. Another study showed a decrease of EC by about 29.4% due to an increase in retention time of 48 hours in a trickling sand filter (Khan *et al.,*2014). Izadi *et al*. (2020) created a hybrid airlift membrane bioreactor (HAMBR) that efficiently reduced COD (88-99%), ammonium (54-83%), nitrite (70-90%), nitrate (65-95%), and total nitrogen (61-90%). Poojamnong *et al*. (2020) recently reported submerged polyvinylidene fluoride MBR having 73% COD and 79% color removal efficiency from the pulp and paper sector effluent.

Wastewater from various sources generally comprises different ions and nutrients such as $PO4^{3-}$, $SO4^{2-}$ and TN. While WHO (2006) does not specify a standard limit for phosphate in clean water, the US EPA (2007) sets the limit for phosphorous at 0.05 mg/L. The phosphate concentrations in influent samples were between 3.4-4.0 mg/L. Such high PO43 levels in the wastewater stream can result in eutrophication. The concentrations of phosphates decreased considerably ($P < 0.05$) with a treatment efficiency of 68% (1.13 mg/L) after passing through GDB. This reduction in phosphate level could be attributed to polyphosphorous accumulating bacteria (PAO) in the biofilm (Ni *et al.,*2022). About 58% phosphate reduction was also reported in attached and suspended growth bioreactors. It was attributed to the physiological activities of microbes present in the biofilm (Naz *et al.,*2015). $SO4^{2-}$ is another essential nutrient in almost all types of wastewaters, including industrial effluent, domestic wastewater, and natural runoff. The raw effluent showed varying sulfate levels, with sample W5 having the highest concentration at 908 to 842.33 mg/L. After treatment in the GDB, the results showed an average reduction of approximately 76.98% in sulfate levels. The highest reduction in sulfate concentration, with a significant decrease of 81.75%. These results highlight the GDB's ability to effectively decrease sulfate levels in wastewater, hence playing a crucial role in meeting environmental regulations and mitigating the ecological risks associated with elevated sulfate discharges. Several factors contribute to this reduction, including oxidation, increased DO levels, reduced activities of sulfate-reducing bacteria (SRB) (Stein *et al.,*2007), and precipitation of sulfide with metals and elemental sulfur (Bottrell*et al.,*2009). The reduction in sulfate levels observed in GDB can be ascribed to the synergistic action of various microbial activities. Within the aerobic chamber, SRB engages in the process of sulfate ion consumption, wherein these ions are transformed into comparatively less detrimental substances, such as hydrogen sulfide (Stillger *et al.,*2022). Within the anaerobic chamber, SRB reduces sulfate by employing organic materials as electron donors, thus facilitating sulfate removal. The effective reduction of sulfate in the bioreactor is vital for maintaining environmental compliance and preventing adverse impacts on aquatic ecosystems. This is achieved through the synergistic interaction between aerobic and anaerobic processes (Daraz *et al.,*2022). TN is the sum of all forms of nitrogen in water, including nitrates, nitrites, and ammonia nitrogen. The permissible limits for each form of nitrogen are different, e.g., the nitrates limit is 50 mg/L, and the nitrite limit is 3 mg/L (US-EPA, 2007). The untreated effluent demonstrated a range of TN, with the highest concentration observed at 161.47 to 114.5 mg/L. After treatment, the average reduction in TN levels was approximately 45.58%—the most significant reduction in TN concentration, with a decrease of 68.97% (47 mg/L).

The reduction is essential for ensuring environmental compliance and mitigating nitrogen pollution in water bodies that receive treated wastewater. Our findings are consistent with those of Rasool *et al.,*2017 who reported a ruction in TN of up to 65 % in a trickling filter system for domestic wastewater treatment. TN reduction in the GDB was accomplished by utilizing a synergistic interaction of several processes. For instance, nitrifying bacteria convert ammonia (NH_3) to nitrate (NO^{3-}) in the aerobic chamber, and denitrifying bacteria in the anaerobic chamber convert nitrate into nitrogen gas (N_2). Anammox bacteria may also convert ammonium and nitrite to N2 (Khanthong *et al.,*2023). These synchronized processes successfully decrease TN concentrations, essential for preventing nutrient contamination in effluent and preserving the ecological equilibrium in receiving waters. Thus, the present study indicates that attached growth bioreactors consisting of stone as a filter medium are proficient in nitrogen removal. Another benefit is its budget-friendly and low-maintenance characteristics. Izadi *et al*. (2019) reduced COD (92-99%), ammonium (59-97%), nitrite (78-97%), nitrate (59-98%), and total nitrogen (62-92%) using a fixed-bed membrane bioreactor (FBMBR) with a hydraulic retention time (HRT).

IVFCW showed a significant reduction in removing these nutrients, indicating that the *Typha latifolia,* along with aerobic and anaerobic treatment through microbial biofilm, played a significant role. The efficiency of IVFCW for the treatment of blended wastewater showed an average of about 81% reduction in sulfates, 63% reduction in phosphates, and about 61% reduction in total nitrogen. Essential nutrients such as sulfates (SO_4^{2}-), phosphates (PO_4^{3-}), and nitrates (NO^{-3}) (total nitrogen TN) are commonly found in domestic wastewater and pulp and paper industry effluent. However, their concentrations are much higher in the latter due to detergents, agriculture runoff, and the use of sodium sulfite during the pulping process (Khan *et al.,*2022). Several variables contribute to the decrease in sulfates. First, dissolved oxygen concentration rises; second, sulfate-reducing bacteria reduce it (Stein *et al.,*2007). Third, some of the sulfates are trapped by the different layers of the wetland; fourth, there is chemical reduction of sulfates into elemental sulphur. Fifth, some Sulphur is taken up by the plants into their body through roots (Bottrell *et al.,*2009). Roots also increase the efficiency of removing ions by entrapping sulfate ions on their surface, providing additional time for bacterial reduction (Moazzem *et al.,*2023). Phosphates are also found to be significant in wastewater. Our study showed a significant reduction in the phosphates level, consistent with prior results (Chung *et al.,*2008). Phosphorous is removed in two ways, i.e., plant uptake as a nutrient and phosphorous utilizing bacteria in biofilms (Sehar *et al.,*2014) (Mateus *et al.,*2014).

The GDB showed an average decrease of sulfate of roughly 54.8%, whilst the IVFCW had a little greater average reduction of around 59.5%. The GDB achieved an average reduction in phosphate levels by roughly 54.2%, while the IVFCW showed a larger average reduction of around 56.8%. Likewise, the GDB obtained an average decrease of 56.0% for the total nitrogen (TN) content, whereas the IVFCW showed a larger average reduction of around 70.2%.

The BL emitted a highly noxious and repulsive smell, and its appearance was cloudy. Both the GDB) and IVFCW successfully decreased turbidity levels in the treatment of pulp and paper mill wastewater. The GDB had an average decrease of roughly 88.3%, whilst the IVFCW displayed a little greater average decrease of around 88.8%. The results demonstrate the effective performance of both bioreactors in purifying the wastewater, with IVFCW showing a little advantage in reducing turbidity. Prior research has also indicated that prolonging the exposure of microbial biofilm to pollutants aids in the breakdown of organic and aromatic pollutants, leading to the elimination of unpleasant odors and turbidity when processed in different biological reactors (Rasool et al., 2017; Sehar et al., 2015).

GDB and IVFCW caused changes in pH levels during the treatment of pulp and paper mill wastewater. The GDB had an average pH fluctuation of roughly 2.54%, whilst the IVFCW displayed a significantly lesser average fluctuation of around 1.67%. The results suggest that both bioreactors had a negligible effect on pH levels, with the GDB creating a somewhat greater fluctuation compared to the IVFCW in the treated effluent.

Both the GDB and IVFCW successfully decreased the amount of lignin in pulp and paper mill effluent during treatment. The GDB achieved an average decrease in lignin of roughly 52.4%, but the IVFCW exhibited a slightly greater average reduction of around 58.4%. The results demonstrate the GDB's capacity to efficiently decrease the amount of lignin in wastewater, which is crucial for minimizing the negative impact of lignin-rich industrial waste on water bodies. Majumdar et al. (2019) reported removal rates of 85% for COD, 74% for lignin, 96% for color, and 81% for phenol in a separate investigation.

Furthermore, for phosphates removal, filtration and precipitation are also employed (Arias *et al.,*2001). An integrated system exhibits superior performance due to its utilization of alternating anaerobic and aerobic conditions, facilitating microorganisms' degradation of organic phosphates (Bonomo, 1997). Nitrogen, when mingled with phosphate in an aquatic environment, may generate algal blooms. The sources of these nitrogenous wastes in PPI wastewater are hydrolytic products of plants' cell walls and detergents used during pulp washing (Bhandari *et al.,*2023). The reduction in TN content was due to the assimilation of NO^{-3} by the plant roots and the presence of denitrifying bacteria, which convert these ions into atmospheric nitrogen. The results of the present study are similar to Chang *et al.,*2013.

The presence of any coloration or odor of water is aesthetically unpleasant and serves as an indicator of contamination. In the case of BL, color primarily arises from lignin-containing compounds, while odors are associated with aromatics and the oxidation of aldehydes or ketones. The removal of odor and color can be attributed to the biodegradation of these pollutants. Studies also indicate that biofilms play a significant role in facilitating the biodegradation of organic compounds and the removal of odors (Collivignarelli *et al.,*2019). In the present study, the untreated effluent showed varying levels of color, with the highest intensity of 265.33 Pt-Co and the lowest of 212 Pt-Co. However, following treatment using the GDB process, the average reduction in color levels was approximately 42.36%. The maximum reduction in color was 57.53%. In IVFCW, biodegradation of different organic contaminants that impart color to wastewater, like lignin, thus causes a reduction in the intensity of color by approximately 56 %. The reduction of color in aerobic and anaerobic chambers of the GDB for pulp and paper industry effluent and domestic wastewater is primarily attributed to the biodegradation of colored organic compounds. Aerobic microorganisms metabolize and break down complex organic molecules responsible for color in the aerobic chamber. In contrast, in the anaerobic chamber, anaerobic processes contribute to the removal of color by promoting the transformation of recalcitrant compounds (Dick *et al.,*2022). This dual-process approach efficiently reduces color in the effluent, mitigating the visual and environmental impacts associated with color-rich wastewater, thereby promoting better water quality and adherence to regulatory standards.

The bacterial population in effluent and effluent samples was quantified using the colony-forming unit (CFU) method. Wastewater samples were found to be highly contaminated, containing a considerable number of different types of bacteria. A significant reduction (77.98 %) was observed after treatment through GDB and IVFCW, showing the average reduction in CFU/mL was about 55% and a maximum reduction of 67%. This higher treatment efficiency of the GDB could be attributed to the extended hydraulic retention time in the reactor, which significantly enhances the bacterial adsorption onto the stone surfaces, contributing to biofilm. This metabolically active biofilm is critical in removing organic and inorganic contaminants from wastewater, increasing nitrification and DO levels (Sehar *et al.,*2016 b).

Moreover, the decrease in bacterial population can be attributed to various mechanisms, including sedimentation, filtration, aggregation, biofilm formation on filter media, competition, oxidation, solar irradiation, natural decay and predation (Sehar *et al.,*2015; Rehman *et al.,*2021; Rasool *et al.,*2018). The presence of faecal coliforms in influent and effluent samples was assessed using the MPN test. The untreated wastewater samples contained high values of 2400 MPN index/100 ml due to high concentrations of nutrients and other contaminants. However, treatment through the GDB resulted in a significant reduction with a treatment efficiency of 90%. This significant reduction may be attributed to the natural die-off of pathogens during their passage through the filter media and sand bed filtration. In the case of IVFCW, initially treated samples do not show a significant reduction in MPN/100mL; however, after plant growth and maturation of biofilm on stone media in aerobic and anaerobic chambers, a significant reduction, about 90%, was observed. Constructed wetlands are also reported (Rehman *et al.,*2020) to reduce microbiological parameters, i.e., MPN index and CFU, making the wastewater suitable for irrigation. Rasool *et al.,*2017 reported similar results of about 92% CFU and 90% MPN/100mL in reducing microbial and pathogenic populations. The reduction in MPN/100mL and CFU/mL after treatment through IVFCW is attributed to several factors, such as optimal habitat conditions for diverse microbial communities in aerobic and anaerobic chambers. These microbial communities are essential in organic pollutant reduction, organic matter decomposition, and a considerable reduction in MPN and CFU (Shakira *et al.,*2023). Wetlands substrate(stone/pebbles/gravels) provides a physical filter to trap solids and microbes. In the present study, the sand bed as the final filtration unit enhanced the physical filtration, leading to a 90% reduction of the MPN index. Typha latifolia, present as a wetland plant, not only oxygenates the rhizosphere but also affects nutrient uptake, thus affecting microbial populations indirectly. Other important factors such as competition, predation (by protozoa), and nutrient removal significantly reduce MPN and CFU.

A significant number of bacterial species have been reported attached to soil particles Torsvik & Ovreas (2002), stones media (Rehman *et al.*2022) and roots of the *Typha latifolia* and stone media of GDB which are involved in biofilm formation as well as the removal of nutrients/containments from wastewater (Saher *et al.,*2015). In the present study, microbial profiling of *Typha latifolia* and stone media from the aerobic chamber was done. Isolation and characterization of a number of bacterial species indicated and affirmed the role bacterial biofilm in wastewater treatment process. Several bacterial species were identified, and their involvement in lignin biodegradation/biotransformation was also reported several times, such as *Bacillus sp.* and *Paenibacillussp* (Rinaldi *et al.,*2016), (Chandra *et al.,*2007), *Azotobacter, Bacillus spp.* and *Serratia* were reported by Xu *et al.,*2018.

Conclusions

In its entirety, the present research work demonstrates the capacity of native bacterial strains to treat BL effectively. The Gravity-Driven Bioreactor (GDB) is a sustainable solution that effectively removes BL's COD, BOD, and other pollutants. The Integrated Vertical Flow Constructed Wetland (IVFCW) efficiently mitigates environmental consequences by decreasing Chemical COD, BOD, and pollutants in water. GDB employs biofilms, while IVFCW utilizes biofilm and plants (Typha) to improve treatment efficiency, rendering them environmentally sustainable choices for wastewater remediation. The following conclusions are deduced,

1. This study involved isolation of bacterial strains from black liquor. Among different strains, three (TR-1, TR-5, and TR-8) demonstrated significant capabilities in breaking down lignin. The shake flask experiments validated the substantial increase in TR-1's growth (3.12g/L) at a temperature of 41°C and a pH of 8, along with a 77% reduction in lignin and a 65% color reduction. The biochemical characterization revealed that TR-1 as *B. subtilis*, TR-5 as *B. cereus*, and TR-8 as *Pseudomonas*. From these findings, we concluded that the ingenious bacterial strains have the capacity to treat BL efficiently.

2. The present study demonstrates the effective treatment of BL utilizing a Gravity-Driven Bioreactor (GDB). Significant removal efficiencies were observed, including 73.35% COD, 67.47% BOD, a notable increase of 197.49% in DO, and a 51% reduction in lignin content, highlighting the potential of GDB as an environmentally sustainable and economically feasible biological treatment option for BL.

3. The evaluation of the Integrated Vertical Flow Constructed Wetland (IVFCW) to treat BL revealed significant decreases of 73.84% in COD and 70.76% in BOD, as well as substantial declines in EC, TDS, TSS, PO_4^{3-}, SO_4^{2-}, TN, lignin, and turbidity. The results affirm that IVFCW is effective in treating BL and mitigating the environmental impact of untreated BL.

4. The GDB proves to be an environmentally sustainable and efficient solution for simultaneously treating BL and domestic wastewater. The GDB effectively decreases several pollution indicators, including color (57% reduction), COD (84.5% reduction), BOD5 (71.80% reduction), and TDS (82.8% reduction). There

has been a notable decline in microbial load, with CFU decreasing by 77.98% and MPN by 90%. The results highlight the GDB's capacity to handle intricate wastewater streams efficiently while being cost-effective. The study provides evidence for the practical application of GDB in wastewater treatment, seamlessly incorporating it into current industrial processes without incurring extra costs.

5. The IVFCW effectively removed most of the contaminants from the blended wastewater, such as BOD (81%), COD (80%), and TSS (100%). The IVFCW exhibited its capacity as an environmentally friendly, economically efficient, and self-sustaining wastewater treatment system for addressing the environmental issues associated with wastewater release.
6. Biofilm played a critical role in both GDB and IVFCW as an aggregation of dynamic bacterial communities, significantly contributing to the treatment process's overall effectiveness. Biofilms of both aerobic zones, as they actively contribute to the breakdown of organic materials and anaerobic parts, play a role in the process of nutrient removal by aiding in the biodegradation/biotransformation of nitrogen molecules, hence improving the system's overall efficiency. The coexistence of aerobic and anaerobic biofilms developed using blended wastewater played a more crucial role in attaining elevated treatment efficiencies. Thus, biofilm-based bioreactors are sustainable and ecologically friendly for the remediation of wastewater generated by the pulp and paper industry.
7. The comparative analysis of GDB and IVFCW shows that both systems are successful in treating pulp and paper sector wastewater. While GDBs are effective in biodegradation processes, IVFCWs provide a more diverse approach through physical, chemical, and biological treatment mechanisms. The decision between GDB and IVFCW is determined by site-specific considerations such as space availability, treatment goals, and operational preferences. Long-term performance monitoring and more research are required to optimize these systems and assure their long-term viability in treating complicated industrial wastewaters.

Future Prospects

1. Further research should be conducted to explore the potential of GDB & IVFCW in efficiently removing pollutants at different temperatures and loading rates of pulp and paper industry effluent.
2. Assessment of different types of vegetation (two or more) in IVFCW and different filter media in GDB could also be done to determine the most effective type of vegetation and filter media for pilot scale studies.
3. The lab-scale bioreactors should be run in natural, open environmental conditions throughout the year to evaluate the seasonal variability of wastewater treatment efficacy.
4. A detailed study should be carried out to elucidate the interaction of pollutants like lignin with biofilm and the biodegradation kinetics of such recalcitrant contaminates.
5. The pro-type GBD and IVFCE with selected media and vegetation with modern designs should be studied at a pilot scale for the efficient simultaneous treatment of wastewater.
6. Microbial diversities in the biofilm/rhizosphere could be studied using advanced molecular techniques to understand microbial transformations.

References

Abdelaziz, O.Y., Brink, D.P., Prothmann, J., Ravi, K., Sun, M., García-Hidalgo, J., Sandahl, M., Hulteberg, C.P., Turner, C., Lidén, G. and Gorwa-Grauslund, M.F., (2016). Biological valorization of low molecular weight lignin. Biotechnology advances, 34(8), pp.1318-1346.

Abdelhakeem, S. G., Aboulroos, S. A., & Kamel, M. M. (2016). Performance of a vertical subsurface flow constructed wetland under different operational conditions. *Journal of Advanced Research*, *7*(5), 803-814.

Abd-Elsalam, H. E., & El-Hanafy, A. A. (2009). Lignin biodegradation with ligninolytic bacterial strain and comparison of Bacillus subtilis and Bacillus sp. isolated from Egyptian soil. *Am Eurasian J Agric Environ Sci*, *5*(1), 39-44.

Abedinzadeh, N., Shariat, M., Monavari, S. M., & Pendashteh, A. (2018). Evaluation of color and COD removal by Fenton from biologically (SBR) pre-treated pulp and paper wastewater. *Process Safety and Environmental Protection*, *116*, 82-91.

Akratos, C. S., Tatoulis, T. I., & Tekerlekopoulou, A. G. (2020). Biotreatment of winery wastewater using a hybrid system combining biological trickling filters and constructed wetlands. *Applied Sciences*, *10*(2), 619.

Ali, I., Hadi, F., & Bano, A. (2012). Microbial assisted phytoextraction of metals and growth of soybean (Glycine max L. merrill) on industrial wastewater contaminated soil. Pak. J. Bot, 44(5), 1593-1599.

Ali, I., Khan, Z., Peng, C., Naz, I., Sultan, M., Ali, M., ... & Niaz, Y. (2017). Identification and elucidation of the designing and operational issues of trickling filter systems for wastewater treatment. *Polish Journal of Environmental Studies*, *26*(6).

Ali, M., Rousseau, D. P., & Ahmed, S. (2018). A full-scale comparison of two hybrid constructed wetlands treating domestic wastewater in Pakistan. *Journal of Environmental Management*, *210*, 349-358.

Al-Tohamy, R., Ali, S. S., Li, F., Okasha, K. M., Mahmoud, Y. A. G., Elsamahy, T., ... & Sun, J. (2022). A critical review on the treatment of dye-containing wastewater: Ecotoxicological and health concerns of textile dyes and possible remediation approaches for environmental safety. *Ecotoxicology and Environmental Safety*, *231*, 113160.

Amen, R., Yaseen, M., Mukhtar, A., Klemeš, J. J., Saqib, S., Ullah, S., ... & Bokhari, A. (2020). Lead and cadmium removal from wastewater using eco-friendly biochar adsorbent derived from rice husk, wheat straw, and corncob. *Cleaner Engineering and Technology*, 1, 100006.

An, X., Cheng, Y., Zang, H., & Li, C. (2023). Biodegradation characteristics of lignin in pulping wastewater by the thermophilic Serratia sp. AXJ-M: performance, genetic background, metabolic pathway and toxicity assessment. *Environmental Pollution*, 322, 121230.

Arias, C. A., Del Bubba, M., & Brix, H. (2001). Phosphorus removal by sands for use as media in subsurface flow constructed reed beds. *Water research*, *35*(5), 1159–1168.

Arya, V., Philip, L., & Bhallamudi, S. M. (2016). Performance of suspended and attached growth bioreactors for the removal of cationic and anionic pharmaceuticals. *Chemical Engineering Journal*, *284*, 1295-1307.

Asami, H., Golabi, M., & Albaji, M. (2021). Simulation of the biochemical and chemical oxygen demand and total suspended solids in wastewater treatment plants: data-mining approach—Journal *of Cleaner Production*, *296*, 126533.

Ashrafi, O., Yerushalmi, L. & Haghighat, F. (2015). Wastewater treatment in the pulp-and-paper industry: A review of treatment processes and the associated greenhouse gas emission. *Journal of Environmental Management*, *158*, pp.146–157.

Asina, F., Brzonova, I., Voeller, K., Kozliak, E., Kubátová, A., Yao, B., & Ji, Y. (2016). Biodegradation of lignin by fungi, bacteria and laccases. *Bioresource Technology*, *220*, 414-424.

Ávila, C., Reyes, C., Bayona, J. M., & García, J. (2013). Emerging organic contaminant removal depending on primary treatment and operational strategy in horizontal subsurface flow constructed wetlands: influence of redox. *Water Research*, *47*(1), 315-325.

Azizullah, A., Khattak, M. N. K., Richter, P., & Häder, D. P. (2011). Water pollution in Pakistan and its impact on public health—a review. *Environment International, 37(2)*, 479-497.

Babuponnusami, A., Sinha, S., Ashokan, H., Paul, M. V., Hariharan, S. P., Arun, J., ... & Pugazhendhi, A. (2023). Advanced oxidation process (AOP) combined biological process for wastewater treatment: A review on advancements, feasibility and practicability of combined techniques. *Environmental Research*, 116944.

Baghel, S., Sahariah, B. P., & Anandkumar, J. (2020). Bioremediation of lignin-rich pulp and paper industry effluent. *Combined Application of Physico-Chemical & Microbiological Processes for Industrial Effluent Treatment Plant*, 261-278.

Bajpai, P. and Bajpai, P.K., 1994. Biological colour removal of pulp and paper mill wastewaters. Journal of Biotechnology, 33(3), pp.211-220.

Bajpai, P., & Bajpai, P. (2018). Brief description of the pulp and papermaking process. *Biotechnology for pulp and paper processing*, 9-26.

Bajpai, P., (2016). Structure of lignocellulosic biomass. Pretreatment of lignocellulosic biomass for biofuel production (pp. 7–12). Springer, Singapore.

Baldovi, A. A., de Barros Aguiar, A. R., Benassi, R. F., Vymazal, J., & de Jesus, T. A. (2021). Phosphorus removal in a pilot scale free water surface constructed wetland: hydraulic retention time, seasonality and standing stock evaluation. *Chemosphere*, *266*, 128939.

Barwal, A., & Chaudhary, R. (2014). To study the performance of biocarriers in moving bed biofilm reactor (MBBR) technology and kinetics of biofilm for retrofitting the existing aerobic treatment systems: a review. *Reviews in Environmental Science and Bio/Technology*, *pp. 13*, 285–299.

Bengtsson, S., Werker, A., Christensson, M., & Welander, T. (2008). Production of polyhydroxyalkanoates by activated sludge treating a paper mill wastewater. *Bioresource Technology*, *99*(3), 509-516.

Bhais, B. J. (2016). *Polishing of an aerobically pre-treated domestic sewage by constructed wetlands in Qarawit Bani-Zeid village* (Doctoral dissertation).

Bhambore, N., & Kumar, M. S. (2022). Municipal solid waste generation, management scenarios, and leachate treatment using sequencing batch biofilter granular reactor. *Process Safety and Environmental Protection*.

Bhandari, M., Kumar, P., Bhatt, P., Simsek, H., Kumar, R., Chaudhary, A., ... & Prajapati, S. K. (2023). An integration of algae-mediated wastewater treatment and resource recovery through anaerobic digestion. *Journal of Environmental Management*, *342*, 118159.

Blanchette-Mackie, E. J., Dwyer, N. K., Barber, T., Coxey, R. A., Takeda, T., Rondinone, C. M., ... & Londos, C. (1995). Perilipin is located on the surface layer of intracellular lipid droplets in adipocytes. *Journal of lipid research*, *36*(6), 1211–1226.

Blumenthal, U.J., Mara, D.D., Peasey, A., Ruiz-Palacios, G. and Stott, R., 2000. Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. Bulletin of the World Health Organization, 78, pp.1104-1116.

Bonomo, L., Pastorelli, G., & Zambon, N. (1997). Advantages and limitations of duckweed-based wastewater treatment systems. *Water Science and Technology*, *35*(5), 239-246.

Bottrell, S. H., Hatfield, D., Bartlett, R., Spence, M. J., Bartle, K. D., & Mortimer, R. J. (2010). Concentrations, sulfur isotopic compositions and origin of organosulfur compounds in pore waters of a highly polluted raised peatland. *Organic Geochemistry*, *41*(1), 55–62.

Brix, H. (1994). Functions of macrophytes in constructed wetlands. *Water Science and Technology*, *29*(4), 71–78.

Broch-Due, A., Andersen, R., & Opheim, B. (1997). Treatment of integrated newsprint mill wastewater in moving bed biofilm reactors. *Water Science and Technology*, *35*(2-3), 173-180.

Buheji, M., & Al-Muhannadi, K. (2023). Mitigating Risks of Environmental Impacts on Gaza-Review of Precautions & Solutions post (2023 War). International Journal of Advanced Research in Engineering and Technology, 14(7), 15-47.

Buzzini, A. P., & Pires, E. C. (2007). Evaluation of a upflow anaerobic sludge blanket reactor with partial recirculation of effluent used to treat wastewaters from pulp and paper plants. *Bioresource Technology*, *98*(9), 1838-1848.

Cárcamo, E. A. B., & Peñabaena-Niebles, R. (2022). Opportunities and challenges for the waste management in emerging and frontier countries through industrial symbiosis. Journal of Cleaner Production, 363, 132607.

Carty, A., Scholz, M., Heal, K., Gouriveau, F., & Mustafa, A. (2008). The universal design, operation and maintenance guidelines for farm constructed wetlands (FCW) in temperate climates. *Bioresource Technology*, *99*(15), 6780–6792.

Castro, F. D., Bassin, J. P., & Dezotti, M. (2017). Treatment of a simulated textile wastewater containing the Reactive Orange 16 azo dye by a combination of ozonation and moving-bed biofilm reactor: evaluating the performance, toxicity, and oxidation by-products. Environmental Science and Pollution Research, 24, 6307-6316.

Celis-García, L. B., Razo-Flores, E., & Monroy, O. (2007). Performance of a down-flow fluidized bed reactor under sulfate reduction conditions using volatile fatty acids as electron donors. *Biotechnology and Bioengineering*, *97*(4), 771-779.

Chan, Y. J., Chong, M. F., Law, C. L., & Hassell, D. G. (2009). A review on anaerobic–aerobic treatment of industrial and municipal wastewater. *Chemical engineering journal,* 155(1-2), 1-18.

Chan, Y. J., Chong, M. F., Law, C. L., & Hassell, D. G. (2009). A review on anaerobic–aerobic treatment of industrial and municipal wastewater. *Chemical engineering journal*, *155*(1-2), pp. 1-18.

Chandra, R., & Bharagava, R. N. (2013). Bacterial degradation of synthetic and kraft lignin by axenic and mixed culture and their metabolic products. *Journal of Environmental Biology*, *34*(6), 991.

Chandra, R., Abhishek, A., & Sankhwar, M. (2011). Bacterial decolorization and detoxification of black liquor from rayon grade pulp manufacturing paper industry and detection of their metabolic products. *Bioresource Technology*, *102*(11), 6429-6436.

Chandra, R., Raj, A., Purohit, H. J., & Kapley, A. (2007). Characterisation and optimisation of three potential aerobic bacterial strains for kraft lignin degradation from pulp paper waste. *Chemosphere*, *67*(4), 839–846.

Chang, J. J., Wu, S. Q., Dai, Y. R., Liang, W., & Wu, Z. B. (2013). Nitrogen removal from nitrate-laden wastewater by integrated vertical-flow constructed wetland systems. *Ecological Engineering*, *58*, 192-201.

Chanworrawoot, K., & Hunsom, M. (2012). Treatment of wastewater from pulp and paper mill industry by electrochemical methods in membrane reactor. *Journal of Environmental Management*, *113*, 399-406.

Chaudhry, S., & Paliwal, R. (2018). Techniques for remediation of paper and pulp mill effluents: processes and constraints. *Handbook of environmental materials management*, *12*.

Chen, S., Sun, D., & Chung, J. S. (2008). Simultaneous removal of COD and ammonium from landfill leachate using an anaerobic–aerobic moving-bed biofilm reactor system. *Waste Management*, *28*(2), 339-346.

Chen, Y., Cheng, J. J., & Creamer, K. S. (2008). Inhibition of anaerobic digestion process: a review. Bioresource Technology, 99(10), 4044-4064.

Chen, Z. M., Chen, B., Zhou, J. B., Li, Z., Zhou, Y., Xi, X. R., ... & Chen, G. Q. (2008). A vertical subsurface-flow constructed wetland in Beijing. *Communications in Nonlinear Science and Numerical Simulation*, *13*(9), 1986-1997.

Chinnaraj, S., & Rao, G. V. (2006). Implementation of an UASB anaerobic digester at bagasse-based pulp and paper industry. *Biomass and Bioenergy*, *30*(3), 273-277.

Chong, S., Sen, T.K., Kayaalp, A. and Ang, H.M., (2012). The performance enhancements of up-flow anaerobic sludge blanket (UASB) reactors for domestic sludge treatment–a state-of-the-art review. Water Research, 46(11), pp.3434-3470.

Choudhary, A. K., Kumar, S., & Sharma, C. (2011). Removal of chlorinated resin and fatty acids from paper mill wastewater through constructed wetland. *International Journal of Environmental and Ecological Engineering*, *5*(8), 406–410.

Chung, A. K. C., Wu, Y., Tam, N. Y., & Wong, M. H. (2008). Nitrogen and phosphate mass balance in a sub-surface flow constructed wetland for treating municipal wastewater. *Ecological Engineering*, *32*(1), 81-89.

Ciria, M. P., Solano, M. L., & Soriano, P. (2005). Role of macrophyte *Typha latifolia* in a constructed wetland for wastewater treatment and assessment of its potential as a biomass fuel. *Biosystems Engineering*, *92*(4), 535-544.

Collivignarelli, M.C., Abbà, A., Miino, M.C. and Damiani, S., (2019). Treatments for colour removal from wastewater: State of the art. Journal of environmental management, 236, pp.727-745.

Comino, E., Riggio, V., & Rosso, M. (2011). Mountain cheese factory wastewater treatment with the use of a hybrid constructed wetland. *Ecological Engineering*, *37*(11), 1673-1680.

Costa, S., Dedola, D. G., Pellizzari, S., Blo, R., Rugiero, I., Pedrini, P., & Tamburini, E. (2017). Lignin biodegradation in pulp-and-paper mill wastewater by selected white rot fungi. *Water*, *9*(12), 935.

Covinich, L. G., Bengoechea, D. I., Fenoglio, R. J., & Area, M. C. (2014). Advanced oxidation processes for wastewater treatment in the pulp and paper industry: a review.

Cullen, D. (1997). Recent advances on the molecular genetics of ligninolytic fungi. *Journal of Biotechnology*, *53*(2-3), pp. 273-289.

Dakua, M. (2015). Treatment of domestic wastewater by subsurface flow constructed wetland.

Daraz, U., Li, Y., Ahmad, I., Iqbal, R., & Ditta, A. (2022). Remediation technologies for acid mine drainage: Recent trends and future perspectives. *Chemosphere*, 137089.

Das, A., & Naga, R. N. (2011). Activated sludge process with MBBR technology at ETP. *Ippta J*, *23*(2), 135-137.

Das, A., Kundu, P., & Adhikari, S. (2022). Biological treatment of dairy industry wastewater in a suspended growth batch reactor: performance evaluation and biodegradation kinetics. *Bioremediation Journal*, *26*(4), 341-359.

Das, P. P., Sharma, M., & Purkait, M. K. (2022). Recent progress on electrocoagulation process for wastewater treatment: A review. *Separation and Purification Technology*, *292*, 121058.

De la Varga, D., Ruiz, I., & Soto, M. (2013). Winery wastewater treatment in subsurface constructed wetlands with different bed depths. *Water, Air, & Soil Pollution*, *224*, 1-13.

Del Pozo, R., & Diez, V. (2005). Integrated anaerobic–aerobic fixed-film reactor for slaughterhouse wastewater treatment. *Water research*, *39*(6), 1114-1122.

Deng, L., Guo, W., Ngo, H. H., Zhang, X., Chen, C., Chen, Z., ... & Wang, Q. (2022). Recent advances in attached growth membrane bioreactor systems for wastewater treatment. *Science of the Total Environment*, *808*, 152123.

Dereli, R. K., Ersahin, M. E., Ozgun, H., Ozturk, I., Jeison, D., van der Zee, F., & van Lier, J. B. (2012). Potentials of anaerobic membrane bioreactors to overcome treatment limitations induced by industrial wastewaters. *Bioresource technology*, *122*, 160-170.

Dey, R., & Raghuwanshi, R. (2021). Aspects of Microbial Biofilms in Water Treatment. *Removal of emerging contaminants through microbial processes*, 1-21.

Dick, J. G., & Malvessi, E. (2022). Strategies for reuse and recycling of water and effluents in pulp and paper industries. *Research, Society and Development*, *11*(13), e568111335950-e568111335950.

Dick, J. G., & Malvessi, E. (2022). Strategies for reuse and recycling of water and effluents in pulp and paper industries. Research, Society and Development, 11(13), e568111335950-e568111335950.

Domingos, S., Germain, M., Dallas, S., & Ho, G. (2007). Nitrogen removal from industrial wastewater by hybrid constructed wetland systems. In *2nd IWA-ASPIRE conference and exhibition*.

Duan, J., Huo, X., Du, W.J., Liang, J.D., Wang, D.Q. and Yang, S.C., (2016). Biodegradation of kraft lignin by a newly isolated anaerobic bacterial strain, Acetoanaerobium sp. WJDL-Y2. Letters in applied microbiology, 62(1), pp.55-62.

Duan, X., Liu, T., Duan, W., & Hu, H. (2010, June). Adsorption and coagulation tertiary treatment of pulp & paper mills wastewater. In *2010 4th International Conference on Bioinformatics and Biomedical Engineering* (pp. 1-4). IEEE.

Dunne, E. J., Coveney, M. F., Marzolf, E. R., Hoge, V. R., Conrow, R., Naleway, R., ... & Battoe, L. E. (2012). Efficacy of a large-scale constructed wetland to remove phosphorus and suspended solids from Lake Apopka, Florida. *Ecological engineering*, *42*, 90–100.

EPA Environmental Protection Agency. (2014). Sediments. In Water: Pollution prevention & control. Retrieved from http://water.epa.gov/polwaste/sediments.

EPA, U. (2000). Wastewater technology fact sheet: Trickling filter. Environmental ProtectionAgency.Availableat:http://water.epa.gov/scitech/wastetech/upload/2002_06_28_mtb_trickling_filter.pdf.

Ergüder, T. H., & Demirer, G. N. (2008). Low-strength wastewater treatment with combined granular anaerobic and suspended aerobic cultures in upflow sludge blanket reactors. *Journal of Environmental Engineering*, *134*(4), 295-303.

Esmaeeli, A., Sarrafzadeh, M. H., Zeighami, S., Kalantar, M., Bariki, S. G., Fallahi, A., ... & Ghaffari, S. B. (2023). A Comprehensive Review on Pulp and Paper Industries Wastewater Treatment Advances. *Industrial & Engineering Chemistry Research*.

Faubert, P., Barnabé, S., Bouchard, S., Côté, R. and Villeneuve, C., (2016). Pulp and paper mill sludge management practices: What are the challenges to assessing the impacts on greenhouse gas emissions? Resources, Conservation and Recycling, 108, pp.107-133.

Federation, W. E., & Aph Association. (2005). Standard methods for the examination of water and wastewater. *American Public Health Association (APHA): Washington, DC, USA*, *p. 21*.

Ghoreishi, S. M., & Haghighi, M. R. (2007). Chromophores removal in pulp and paper mill effluent via hydrogenation-biological batch reactors. *Chemical Engineering Journal*, *127*(1-3), 59-70.

Ghosh, A., Gao, L., Thakur, A., Siu, P. M., & Lai, C. W. (2017). Role of free fatty acids in endothelial dysfunction. *Journal of Biomedical Science*, *24*(1), 1–15.

Giacobbo, A., Feron, G. L., Rodrigues, M. A. S., Ferreira, J. Z., Meneguzzi, A., & Bernardes, A. M. (2015). Integration of membrane bioreactor and advanced oxidation processes for water recovery in leather industry. *Desalination and water treatment*, *56*(7), 1712-1721.

G. nder, Z. B., Arayici, S., & Barlas, H. (2012).

Guo, H., Yao, H. Y., Huang, Q. Q., Li, T., Show, D. Y., Ling, M., ... & Lee, D. J. (2023). Anaerobic–anoxic–oxic biological treatment of high-strength, highly recalcitrant polyphenylene sulfide wastewater. *Bioresource Technology*, *371*, 128640.

Guo, W., Ngo, H. H., & Li, J. (2012). A mini-review on membrane fouling. *Bioresource technology*, *pp. 122*, 27–34.

Gupta, A., & Gupta, R. (2019). Treatment and recycling of wastewater from pulp and paper mill. *Advances in biological treatment of industrial wastewater and their recycling for a sustainable future*, pp. 13-49.

Gupta, G. K., & Shukla, P. (2020). Insights into the resources generation from pulp and paper industry wastes: challenges, perspectives and innovations. *Bioresource Technology*, *297*, 122496.

Gupta, V. K., Nayak, A., Agarwal, S., & Tyagi, I. (2014). Potential of activated carbon from waste rubber tire for the adsorption of phenolics: effect of pre-treatment conditions. *Journal of colloid and interface science*, *pp. 417*, 420–430.

Hamza, R. A., Iorhemen, O. T., & Tay, J. H. (2016). Advances in biological systems for the treatment of high-strength wastewater. *Journal of Water Process Engineering*, *10*, 128-142.

Haq, I., & Raj, A. (2018). Biodegradation of Azure-B dye by Serratia liquefaciens and its validation by phytotoxicity, genotoxicity and cytotoxicity studies. *Chemosphere*, *pp. 196*, 58–68.

Haq, I., & Raj, A. (2020). Pulp and paper mill wastewater: ecotoxicological effects and bioremediation approaches for environmental safety. *Bioremediation of Industrial*

Waste for Environmental Safety: Volume II: Biological Agents and Methods for Industrial Waste Management, pp. 333–356.

Haq, I., & Raj, A. (2020). Pulp and paper mill wastewater: ecotoxicological effects and bioremediation approaches for environmental safety. *Bioremediation of Industrial Waste for Environmental Safety: Volume II: Biological Agents and Methods for Industrial Waste Management*, pp. 333–356.

Haq, I., Kumar, S., Raj, A., Lohani, M., & Satyanarayana, G. N. V. (2017). Genotoxicity assessment of pulp and paper mill effluent before and after bacterial degradation using Allium cepa test. *Chemosphere*, *169*, 642-650.

Haq, I., Mazumder, P., & Kalamdhad, A. S. (2020). Recent advances in removal of lignin from paper industry wastewater and its industrial applications–A review. *Bioresource Technology*, *312*, 123636.

Harb, M., Xiong, Y., Guest, J., Amy, G., & Hong, P. Y. (2015). Differences in microbial communities and performance between suspended and attached growth anaerobic membrane bioreactors treating synthetic municipal wastewater. *Environmental Science: Water Research & Technology*, *1*(6), 800–813.

Heinz, O. L., Cunha, M. A., Amorim, J. S., Barbosa-Dekker, A. M., Dekker, R. F., & Barreto-Rodrigues, M. (2019). Combined fungal and photo-oxidative Fenton processes for the treatment of wood-laminate industrial waste effluent. *Journal of hazardous materials*, *379*, 120790.

Hench, K. R., Bissonnette, G. K., Sexstone, A. J., Coleman, J. G., Garbutt, K., & Skousen, J. G. (2003). Fate of physical, chemical, and microbial contaminants in domestic wastewater following treatment by small constructed wetlands. *Water research*, *37*(4), 921-927.

Hermosilla, D., Merayo, N., Gascó, A., & Blanco, Á. (2015). The application of advanced oxidation technologies to the treatment of effluents from the pulp and paper industry: a review. *Environmental Science and Pollution Research*, *22*, 168-191.

Hermosilla, T., Wulder, M. A., White, J. C., Coops, N. C., & Hobart, G. W. (2015). Regional detection, characterization, and attribution of annual forest change from 1984 to 2012 using Landsat-derived time-series metrics. *Remote Sensing of Environment*, *pp. 170*, 121–132.

Holt, J.G., Krieg, N.R., Sneath, P.H., Staley, J.T. and Williams, S.T., (1994). Bergey's manual of determinative bacteriology. 9th. Baltimor: William & Wilkins.

Hossain, K., & Ismail, N. (2015). Bioremediation and detoxification of pulp and paper mill effluent: A review. *Research Journal of Environmental Toxicology*, *9*(3), 113.

Hou, R., Li, H., Chen, H., Yuan, R., Wang, F., Chen, Z., & Zhou, B. (2020). Tertiary treatment of biologically treated effluents from pulp and paper industry by microwave-modified activated carbon adsorption. *Desalination Water Treat*, *pp. 182*, 118–126.

Huang, S., Huang, D., Qitang, W. U., Meifang, H. O. U., Xiaoyan, T. A. N. G., & Jian, Z. H. O. U. (2020). Effect of environmental C/N ratio on activities of lignin-degrading enzymes produced by Phanerochaete chrysosporium. *Pedosphere*, *30*(2), 285-292.

Huang, Y., Ding, W., Zhou, X., Jin, W., Han, W., Chi, K., ... & Jiang, G. (2022). Sub-pilot scale cultivation of Tetradesmus dimorphus in wastewater for biomass production and nutrients removal: Effects of photoperiod, CO2 concentration and aeration intensity. Journal of Water Process Engineering, 49, 103003.

Hubbe, M. A., Metts, J. R., Hermosilla, D., Blanco, M. A., Yerushalmi, L., Haghighat, F., ... & Elliott, A. (2016). Wastewater treatment and reclamation: a review of pulp and paper industry practices and opportunities. *BioResources*, *11*(3), 7953–8091.

Izadi, A., Hosseini, M., Najafpour Darzi, G., Nabi Bidhendi, G., Pajoum Shariati, F., & Mosaddeghi, M. R. (2018). Perspectives on membrane bioreactor potential for treatment of pulp and paper industry wastewater: A critical review. *Journal of Applied Biotechnology Reports*, *5*(4), 139-150.

Izadi, A., Hosseini, M., Pajoum Shariati, F., Najafpour Darzi, G., & Nabi Bidhendi, G. (2020). Treatment of real paper-recycling wastewater in a novel hybrid airlift membrane bioreactor (HAMBR) for simultaneous removal of organic matter and nutrients. *Iranian Journal of Chemistry and Chemical Engineering*, *39*(3), 203–211.

Jagaba, A. H., Kutty, S. R. M., Baloo, L., Birniwa, A. H., Lawal, I. M., Aliyu, M. K., ... & Usman, A. K. (2022). Combined treatment of domestic and pulp and paper industry wastewater in a rice straw-embedded activated sludge bioreactor to achieve sustainable development goals. *Case Studies in Chemical and Environmental Engineering*, *6*, 100261.

Jagaba, A. H., Kutty, S. R. M., Baloo, L., Birniwa, A. H., Lawal, I. M., Aliyu, M. K., ... & Usman, A. K. (2022). Combined treatment of domestic and pulp and paper industry wastewater in a rice straw-embedded activated sludge bioreactor to achieve sustainable development goals. *Case Studies in Chemical and Environmental Engineering*, *6*, 100261.

Jahren, S. J., Rintala, J. A., & Ødegaard, H. (2002). Aerobic moving bed biofilm reactor treating thermomechanical pulping whitewater under thermophilic conditions. *Water research*, *36*(4), 1067-1075.

Janshekar, H., & Fiechter, A. (1982). On the bacterial degradation of lignin. *European journal of applied microbiology and biotechnology*, *14*, 47-50.

Janusz, G., Pawlik, A., Sulej, J., Świderska-Burek, U., Jarosz-Wilkołazka, A., & Paszczyński, A. (2017). Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution. *FEMS microbiology reviews*, *41*(6), 941-962.

Ju, F., Beck, K., Yin, X., Maccagnan, A., McArdell, C. S., Singer, H. P., ... & Bürgmann, H. (2019). Wastewater treatment plant resistomes are shaped by bacterial composition, genetic exchange, and upregulated expression in the effluent microbiomes. The ISME journal, 13(2), 346-360.

Kamali, M., & Khodaparast, Z. (2015). Review on recent developments on pulp and paper mill wastewater treatment. *Ecotoxicology and environmental safety*, *114*, 326-342.

Kamali, M., Suhas, D. P., Costa, M. E., Capela, I., & Aminabhavi, T. M. (2019). Sustainability considerations in membrane-based technologies for industrial effluents treatment. *Chemical Engineering Journal*, *368*, 474-494.

Karadag, D., K.roğlu , O. E., Ozkaya, B., & Cakmakci, M. (2015). A review on anaerobic biofilm reactors for the treatment of dairy industry wastewater. Process Biochemistry, 50(2), 262-271.

Karajić, M. (2015). *Water salinity and the efficiency of constructed wetlands* (Doctoral dissertation, Univerza v Novi Gorici, Fakulteta za podiplomski študij).

Karam, A., Zaher, K., & Mahmoud, A. S. (2020). Comparative studies of using nano zerovalent iron, activated carbon, and green synthesized nano zerovalent iron for textile wastewater color removal using artificial intelligence, regression analysis, adsorption isotherm, and kinetic studies. *Air, Soil and Water Research*, *13*, 1178622120908273.

Khalifa, O., Banat, F., & Hasan, S. W. (2022). Electrochemical treatment of petroleum wastewater: standalone and integrated processes. In *Petroleum Industry Wastewater* (pp. 171-183). Elsevier.

Khan, S. I., Zarin, A., Ahmed, S., Hasan, F., Belduz, A. O., Çanakçi, S., & Shah, A. A. (2022). Degradation of lignin by Bacillus altitudinis SL7 isolated from pulp and paper mill effluent. Water Science and Technology, 85(1), 420-432.

Khan, S., Ahmad, I., Shah, M. T., Rehman, S., & Khaliq, A. (2009). Use of constructed wetland for the removal of heavy metals from industrial wastewater. *Journal of Environmental Management*, *90*(11), 3451–3457.

Khan, S.I., Zada, N.S., Sahinkaya, M., Colak, D.N., Ahmed, S., Hasan, F., Belduz, A.O., Çanakçi, S., Khan, S., Badshah, M. and Shah, A.A., (2021). Cloning, expression and biochemical characterization of lignin-degrading DyP-type peroxidase from Bacillus sp. Strain BL5. Enzyme and Microbial Technology, 151, p.109917.

Khanthong, K., Jang, H., Kadam, R., Jo, S., Lee, J., & Jungyu, P. (2023). Bioelectrochemical system for nitrogen removal: Fundamentals, current status, trends, and challenges. *Chemosphere*, 139776.

Khin, T., & Annachhatre, A. P. (2004). Novel microbial nitrogen removal processes. *Biotechnology advances*, *22*(7), 519-532.

Khorsandi, H., Alizadeh, R., Tosinejad, H., & Porghaffar, H. (2014). Analysis of nitrogenous and algal oxygen demand in effluent from a system of aerated lagoons followed by polishing pond. *Water science and technology*, *70*(1), 95-101.

Kim, T. W., Michniewicz, M., Bergmann, D. C., & Wang, Z. Y. (2012). Brassinosteroid regulates stomatal development by GSK3-mediated inhibition of a MAPK pathway. *Nature*, *482*(7385), 419-422.

Kinnarinen, T., Golmaei, M., Jernström, E. and Häkkinen, A., (2016). Separation, treatment and utilization of inorganic residues of chemical pulp mills. Journal of Cleaner Production, 133, pp.953-964.

Knight, D., Pearce, C. L., Smith, K. G., Olian, J. D., Sims, H. P., Smith, K. A., & Flood, P. (1999). Top management team diversity, group process, and strategic consensus. *Strategic management journal*, *20*(5), 445–465.

Knowles, P., Dotro, G., Nivala, J., & García, J. (2011). Clogging in subsurface-flow treatment wetlands: occurrence and contributing factors. *Ecological Engineering*, *37*(2), 99-112.

Kong, W., Chen, H., Lyu, S., Ma, F., Yu, H., & Zhang, X. (2016). Characterization of a novel manganese peroxidase from white-rot fungus Echinodontium taxodii 2538 and its use for the degradation of lignin-related compounds. *Process Biochemistry*, *51*(11), 1776-1783.

Kumar, D., Gaurav, V. K., & Sharma, C. (2018). Ecofriendly remediation of pulp and paper industry wastewater by electrocoagulation and its application in agriculture. *American Journal of Plant Sciences*, *9*(12), 2462.

Kumar, K., Dasgupta, C. N., & Das, D. (2014). Cell growth kinetics of Chlorella sorokiniana and nutritional values of its biomass. *Bioresource Technology*, *pp. 167*, 358–366.

Kumar, R., Ahmed, M., Bhadrachari, G., & Thomas, J. P. (2018). Desalination for agriculture: water quality and plant chemistry, technologies and challenges. *Water Science and Technology: Water Supply*, *18*(5), 1505–1517.

Kumar, R., Singh, A., Maurya, A., Yadav, P., Yadav, A., Chowdhary, P., & Raj, A. (2022). Effective bioremediation of pulp and paper mill wastewater using Bacillus cereus as a possible kraft lignin-degrading bacterium. *Bioresource Technology*, *p. 352*, 127076.

Kumar, V., & Verma, P. (2023). A critical review on environmental risk and toxic hazards of refractory pollutants discharged in chlorolignin waste of pulp and paper mills and their remediation approaches for environmental safety. Environmental Research, 116728.

Kumla, J., Suwannarach, N., Sujarit, K., Penkhrue, W., Kakumyan, P., Jatuwong, K., ... & Lumyong, S. (2020). Cultivation of mushrooms and their lignocellulolytic enzyme production through the utilization of agro-industrial waste. *Molecules*, *25*(12), 2811.

Kuokkanen, V., & Kuokkanen, T. (2013). Recent applications of electrocoagulation in treatment of water and wastewater—a review.

Lai, C. M. T. (2023). *Production and Characterisation of Lignin Peroxidase from Newly Isolated Thermophilic Bacillus licheniformis from Empty Fruit Bunch* (Doctoral dissertation, Curtin University).

Lappalainen, J., Baudouin, D., Hornung, U., Schuler, J., Melin, K., Bjelić, S., Vogel, F., Konttinen, J. and Joronen, T., (2020). Sub-and supercritical water liquefaction of kraft lignin and black liquor derived lignin. Energies, 13(13), p.3309.

Leiviskä, T., Nurmesniemi, H., Pöykiö, R., Rämö, J., Kuokkanen, T., & Pellinen, J. (2008). Effect of biological wastewater treatment on the molecular weight distribution of soluble organic compounds and on the reduction of BOD, COD and P in pulp and paper mill effluent. *Water research*, *42*(14), 3952-3960.

Leiviskä, T., Rämö, J., Nurmesniemi, H., Pöykiö, R., & Kuokkanen, T. (2009). Size fractionation of wood extractives, lignin and trace elements in pulp and paper mill wastewater before and after biological treatment. *Water research*, *43*(13), 3199-3206.

Leyva-Díaz, J. C., Monteoliva-García, A., Martín-Pascual, J., Munio, M. M., García-Mesa, J. J., & Poyatos, J. M. (2020). Moving bed biofilm reactor as an alternative wastewater treatment process for nutrient removal and recovery in the circular economy model. *Bioresource Technology*, *299*, 122631.

Li, J. (2020). *Biomass pretreatment by metal oxides for reducing sugar degradation and water consumption in biofuel production*. Kansas State University.

Li, J., Yuan, H., & Yang, J. (2009). Bacteria and lignin degradation. *Frontiers of biology in China*, *4*, 29-38.

Li, S., & Zhang, X. (2011). The study of PAFSSB on RO pre-treatment in pulp and paper wastewater. *Procedia Environmental Sciences*, *pp. 8*, 4-10.

Lindholm-Lehto, P. C., Knuutinen, J. S., Ahkola, H. S., & Herve, S. H. (2015). Refractory organic pollutants and toxicity in pulp and paper mill wastewaters. *Environmental Science and Pollution Research*, *22*, 6473-6499.

Liu, Q., Hou, J., Zeng, Y., Xia, J., Miao, L., & Wu, J. (2023). Integrated photocatalysis and moving bed biofilm reactor (MBBR) for treating conventional and emerging organic pollutants from synthetic wastewater: Performances and microbial community responses. *Bioresource Technology*, *370*, 128530.

Liu, X., Shen, F., Smith Jr, R. L., & Qi, X. (2019). Black liquor-derived calcium-activated biochar for recovery of phosphate from aqueous solutions. *Bioresource technology*, *p. 294*, 122198.

Lokeshwari, N., Srinikethan, G., Joshi, S. G., Shasikala, I., Srikanth, B., & Sushma, L. (2013). Isolation and screening of fungi for aerobic delignification and reduction of AOX of pulp and paper mill effluent. *International Journal of Environmental and Ecological Engineering*, *7*(12), 1167–1171.

Loupasaki, E., & Diamadopoulos, E. (2013). Attached growth systems for wastewater treatment in small and rural communities: a review. *Journal of Chemical Technology & Biotechnology*, *88*(2), 190-204.

Lu, J. J., Zhang, H., Li, W., Yi, J. B., Sun, F. Y., Zhao, Y. W., ... & Dong, W. Y. (2022). Biofilm stratification in counter-diffused membrane biofilm bioreactors (MBfRs) for aerobic methane oxidation coupled to aerobic/anoxic denitrification: Effect of oxygen pressure. Water Research, 226, 119243.

Luan, Y. N., Yin, Y., An, Y., Zhang, F., Wang, X., Zhao, F., ... & Liu, C. (2022). Investigation of an intermittently-aerated moving bed biofilm reactor in rural wastewater treatment under low dissolved oxygen and C/N condition. Bioresource Technology, 358, 127405.

Madan, S., Sachan, P., & Singh, U. (2018). A review on bioremediation of pulp and paper mill effluent–An alternative to conventional remedial technologies. *Journal of Applied and Natural Science*, *10*(1), 367-374.

Madhav, S., Ahamad, A., Singh, P., & Mishra, P. K. (2018). A review of textile industry: Wet processing, environmental impacts, and effluent treatment methods. *Environmental Quality Management*, *27*(3), 31–41.

Mahapatra, K., Ramteke, D. S., & Paliwal, L. J. (2012). Production of activated carbon from sludge of food processing industry under controlled pyrolysis and its application for methylene blue removal. *Journal of Analytical and Applied Pyrolysis*, *95*, 79-86.

Mahto, K. U., & Das, S. (2022). Bacterial biofilm and extracellular polymeric substances in the moving bed biofilm reactor for wastewater treatment: A review. Bioresource Technology, 345, 126476.

Majumdar, S., Priyadarshinee, R., Kumar, A., Mandal, T., & Mandal, D. D. (2019). Exploring Planococcus sp. TRC1, a bacterial isolate, for carotenoid pigment production and detoxification of paper mill effluent in immobilized fluidized bed reactor. *Journal of Cleaner Production*, *p. 211*, 1389–1402.

Mänttäri, M., Pihlajamäki, A., & Nyström, M. (2006). Effect of pH on hydrophilicity and charge and their effect on the filtration efficiency of NF membranes at different pH. *Journal of Membrane Science*, *280*(1-2), 311-320.

Martin, P., Nishida, J., Afzal, J., Akbar, S., Damania, R., & Hanrahan, D. (2006). Pakistan strategic country environmental assessment. South Asia Region, World Bank, 1, 1-80.

Masi, F., & Martinuzzi, N. (2007). Constructed wetlands for the Mediterranean countries: hybrid systems for water reuse and sustainable sanitation. *Desalination*, *215*(1-3), 44-55.

Mateus, D. M., Vaz, M. M., Capela, I., & Pinho, H. J. (2014). Sugarcane as constructed wetland vegetation: Preliminary studies. *Ecological engineering*, *62*, 175-178.

Merayo, N., Hermosilla, D., Blanco, L., Cortijo, L., & Blanco, Á. (2013). Assessing the application of advanced oxidation processes and their combination with biological treatment to effluents from the pulp and paper industry. *Journal of hazardous materials*, *262*, 420-427.

Mishra, R. K. (2023). Fresh water availability and its global challenge. British Journal of Multidisciplinary and Advanced Studies, 4(3), 1-78.

Misra, M., Akansha, K., Sachan, A., & Sachan, S. G. (2020). Removal of dyes from industrial effluents by application of combined biological and physicochemical treatment approaches. *Combined application of physico-chemical & microbiological processes for industrial effluent treatment plant*, 365-407.

Mittal, M., Bhuwal, A., Sharma, P., & Aggarwal, N. K. (2023). Utilization of pulp and paper industrial wastewater for production of polyhydroxybutyrate by *Bacillus sonorensis* NAM5. *Systems Microbiology and Biomanufacturing*, pp. 1–14.

Moazzem, S., Bhuiyan, M., Muthukumaran, S., Fagan, J., & Jegatheesan, V. (2023). Microbiome Wetlands in Nutrient and Contaminant Removal. *Current Pollution Reports*, 1-16.

Mosse, K. P. M., Patti, A. F., Christen, E. W., & Cavagnaro, T. R. (2011). Winery wastewater quality and treatment options in Australia. *Australian Journal of Grape and Wine Research*, *17*(2), 111-122.

Munir, H. M. S., Feroze, N., Ramzan, N., Sagir, M., Babar, M., Tahir, M. S., & Khoo, K. S. (2022). Fe-zeolite catalyst for ozonation of pulp and paper wastewater for sustainable water resources. Chemosphere, 297, 134031.

Murthy, N. S., Curran, S. A., Aharoni, S. M., & Minor, H. (1991). Premelting crystalline relaxations and phase transitions in nylon 6 and 6, 6. *Macromolecules*, *24*(11), 3215–3220.

Naz, I., Khan, Z. U., Ali, N., & Ahmed, S. (2013). Removal of pollution indicators from sewage by fixed film bioreactor and sand filter at low temperature.

Naz, I., Rehman, A., Sehar, S., Perveen, I., & Ahmed, S. (2017). Assessment of an integrated tire-derived rubber media-fixed biofilm reactor (TDR-FBR) and sand column filter (SCF) for wastewater treatment at low temperature. *Desalin. Water Treat*, *99*, 185-195.

Naz, I., Seher, S., Perveen, I., Saroj, D.P. and Ahmed, S., (2015). Physiological activities associated with biofilm growth in attached and suspended growth bioreactors under aerobic and anaerobic conditions. Environmental technology, 36(13), pp.1657-1671.

Naz, I., Ullah, W., Sehar, S., Rehman, A., Khan, Z.U., Ali, N. and Ahmed, S., (2016). Performance evaluation of stone-media pro-type pilot-scale trickling biofilter system for municipal wastewater treatment. Desalination and Water Treatment, 57(34), pp.15792-15805.

Negi, G. C., Sharma, S., Vishvakarma, S. C., Samant, S. S., Maikhuri, R. K., Prasad, R. C., & Palni, L. M. (2019). Ecology and use of Lantana camara in India. *The Botanical Review*, *85*(2), 109-130.

Neoh, C. H., Noor, Z. Z., Mutamim, N. S. A., & Lim, C. K. (2016). Green technology in wastewater treatment technologies: Integration of membrane bioreactor with various wastewater treatment systems. *Chemical Engineering Journal*, *283*, 582–594.

Ni, M., Chen, Y., Pan, Y., Huang, Y., Li, D. P., Li, L., ... & Song, Z. (2022). Study on community structure and metabolic mechanism of dominant polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) in suspended biofilm based on phosphate recovery. *Science of The Total Environment*, *815*, 152678.

Ni, M., Chen, Y., Pan, Y., Huang, Y., Li, D. P., Li, L., ... & Song, Z. (2022). Study on community structure and metabolic mechanism of dominant polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs) in suspended biofilm based on phosphate recovery. Science of The Total Environment, 815, 152678.

Nicolle, L. E., Gupta, K., Bradley, S. F., Colgan, R., DeMuri, G. P., Drekonja, D., ... & Siemieniuk, R. (2019). Clinical practice guideline for the management of asymptomatic bacteriuria: 2019 update by the Infectious Diseases Society of America. *Clinical Infectious Diseases*, *68*(10), e83-e110.

Nivala, J., Knowles, P., Dotro, G., García, J., & Wallace, S. (2012). Clogging in subsurface-flow treatment wetlands: measurement, modeling and management. *Water research*, *46*(6), 1625-1640.

Obotey Ezugbe, E., & Rathilal, S. (2020). Membrane technologies in wastewater treatment: a review. *Membranes*, *10*(5), 89.

Ojha, A. K., & Tiwari, M. (2016). Lignin decolorization and degradation of pulp and paper mill effluent by ligninolytic bacteria. *Iranian (Iranica) Journal of Energy & Environment*, *7*(3), 282-293.

Oleszczuk, P. (2008). Phytotoxicity of municipal sewage sludge composts related to physico-chemical properties, PAHs and heavy metals. *Ecotoxicology and Environmental Safety*, *69*(3), 496-505.

Oller, I., Malato, S., & Sánchez-Pérez, J. (2011). Combination of advanced oxidation processes and biological treatments for wastewater decontamination—a review. *Science of the total environment*, *409*(20), 4141-4166.

Ortega-Clemente, A., Ponce-Noyola, M. T., Montes-Horcasitas, M. C., Vicent, M. T., Barrera-Cortés, J., & Poggi-Varaldo, H. M. (2007). Semi-continuous treatment of recalcitrant anaerobic effluent from pulp and paper industry using hybrid pellets of Trametes versicolor. *Water Science and Technology*, *55*(6), 125-133.

Paliwal, R., Uniyal, S. and Rai, J.P.N., 2015. Evaluating the potential of immobilized bacterial consortium for black liquor biodegradation. Environmental Science and Pollution Research, 22(9), pp.6842-6853.

Paraskeva, P., & Diamadopoulos, E. (2006). Technologies for olive mill wastewater (OMW) treatment: a review. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, *81*(9), 1475-1485.

Parveen, S., Ali, M. I., Aslam, M., Ali, I., Jamal, A., Al-Ansari, M. M., ... & Huang, Z. (2022). Optimizing biocatalytic potential of Dipodascus australiensis M-2 for degrading lignin under laboratory conditions. *Microbiological Research,* 265, 127179.

Patel, K., Patel, N., Vaghamshi, N., Shah, K., Duggirala, S. M., & Dudhagara, P. (2021). Trends and strategies in the effluent treatment of pulp and paper industries: A review highlighting reactor options. *Current Research in Microbial Sciences*, *p. 2*, 100077.

Pathak, C., Chopra, A.K. and Srivastava, S., (2013). Accumulation of heavy metals in Spinacia oleracea irrigated with paper mill effluent and sewage. Environmental monitoring and assessment, 185(9), pp.7343-7352.

Pereira, M. D. G., Cardoso de Souza Neta, L., Fontes, M. P. F., Souza, A. N., Carvalho Matos, T., de Lima Sachdev, R., ... & Verónica Flores Nardy Ribeiro, A. (2014). An overview of the environmental applicability of vermicompost: From wastewater treatment to the development of sensitive analytical methods. *The Scientific World Journal*, *2014*.

Pickard, B. R., Daniel, J., Mehaffey, M., Jackson, L. E., & Neale, A. (2015). EnviroAtlas: A new geospatial tool to foster ecosystem services science and resource management. *Ecosystem Services*, *14*, 45-55.

Poe, A. C., Piehler, M. F., Thompson, S. P., & Paerl, H. W. (2003). Denitrification in a constructed wetland receiving agricultural runoff. *Wetlands*, *23*(4), 817–826.

Poggi-Varaldo, H. M., Gutierrez-Saravia, A., Fernández-Villagómez, G., Martínez-Pereda, P., & Rinderknecht-Seijas, N. (1999, November). A full-scale system with wetlands for slaughterhouse wastewater treatment. In *Wetlands & Remediation II: Second International Conference on Wetlands & Remediation* (pp. 213-223).

Pointing, S. (2001). Feasibility of bioremediation by white-rot fungi. *Applied microbiology and biotechnology*, *57*, 20-33.

Pokhrel, D., & Viraraghavan, T. (2004). Treatment of pulp and paper mill wastewater—a review. *Science of the total environment*, *333*(1-3), pp. 37–58.

Pokhrel, D., & Viraraghavan, T. (2004). Treatment of pulp and paper mill wastewater—a review. *Science of the total environment*, *333*(1-3), pp. 37–58.

Poojamnong, K., Tungsudjawong, K., Khongnakorn, W., & Jutaporn, P. (2020). Characterization of reversible and irreversible foulants in membrane bioreactor (MBR) for eucalyptus pulp and paper mill wastewater treatment using fluorescence regional integration. *Journal of Environmental Chemical Engineering*, *8*(5), 104231.

Portela, A. M. K., Rehman, U., & Wicks, J. (2022). Suspended growth tanks. *CFD Modelling for Wastewater Treatment Processes*, 51.

Pritchard, M., Mkandawire, T. and O'neill, J.G., (2007). Biological, chemical and physical drinking water quality from shallow wells in Malawi: Case study of Blantyre, Chiradzulu and Mulanje. Physics and Chemistry of the Earth, Parts A/B/C, 32(15-18), pp.1167–1177.

Qamar, K., Nchasi, G., Mirha, H. T., Siddiqui, J. A., Jahangir, K., Shaeen, S. K., ... & Essar, M. Y. (2022). Water sanitation problem in Pakistan: A review on disease prevalence, strategies for treatment and prevention. Annals of Medicine and Surgery, 104709.

Ram, C., Rani, P., Gebru, K. A., & Mariam Abrha, M. G. (2020). Pulp and paper industry wastewater treatment: use of microbes and their enzymes. *Physical Sciences Reviews*, *5*(10), 20190050.

Ramesh, A., Lee, D. J., Wang, M. L., Hsu, J. P., Juang, R. S., Hwang, K. J., ... & Tseng, S. J. (2006). Biofouling in membrane bioreactor. *Separation science and technology*, *41*(7), 1345-1370.

Rasool, T., Rehman, A., Naz, I., Ullah, R. and Ahmed, S., (2017). Efficiency of a locally designed pilot-scale trickling biofilter (TBF) system in natural environment for the treatment of domestic wastewater. Environmental technology, 39(10), pp.1295-1306.

Ravindran, B., Kumari, S. S., Stenstrom, T. A., & Bux, F. (2016). Evaluation of phytotoxicity effect on selected crops using treated and untreated wastewater from different configurative domestic wastewater plants. *Environmental technology*, *37*(14), 1782-1789.

Rehman, A., Ali, H., Naz, I., Saroj, D. P., & Ahmed, S. (2021). Domestic wastewater treatment efficiency of the pilot-scale trickling biofilter system with variable flow rates and hydraulic retention times. *Environmental Technology*, *42*(6), 972-983.

Rehman, A., Anees, M., Sehar, S., Alhewairini, S. S., Saroj, D. P., & Ahmed, S. (2021). Simulated Modelling, Design, and Performance Evaluation of a Pilot-Scale Trickling Filter System for Removal of Carbonaceous Pollutants from Domestic Wastewater. *Water*, *13*(22), 3210.

Rehman, A., Ayub, N., Naz, I., Perveen, I., & Ahmed, S. (2020). Effects of Hydraulic Retention Time (HRT) on the Performance of a Pilot-Scale Trickling Filter System Treating Low-Strength Domestic Wastewater. *Polish Journal of Environmental Studies*, *29*(1).

Rehman, A., Naz, I., Khan, Z.U., Rafiq, M., Naeem, A. and Ahmad, S., (2012). Sequential application of plastic media-trickling filter and sand filter for domestic wastewater treatment at low temperature condition. British Biotechnology Journal, 2(4), 179.

Rehman, A., Zakir, B., Anees, M., Naz, I., Alhewairini, S.S. and Sehar, S., (2022). Bio-purification of domestic wastewater through constructed wetland planted with Paspalidiumflavidum. Water Environment Research, 94(1), p.e1685.

Ren, Z., Ma, P., Lv, L., Zhang, G., Li, W., Wang, P., ... & Gao, W. (2022). Application of exogenous redox mediators in anaerobic biological wastewater treatment: A critical review. *Journal of Cleaner Production*, 133527.

Rice, E.W. ed., (2012). Standard methods for the examination of water and wastewater (Vol. 10). Washington, DC: American Public Health Association.

Rinaldi, R., Jastrzebski, R., Clough, M. T., Ralph, J., Kennema, M., Bruijnincx, P. C., & Weckhuysen, B. M. (2016). Paving the way for lignin valorisation: recent advances in bioengineering, biorefining and catalysis. *Angewandte Chemie International Edition*, *55*(29), 8164-8215.

Ritchie, J., Lewis, J., Nicholls, C. M., & Ormston, R. (Eds.). (2013). *Qualitative research practice: A guide for social science students and researchers*. sage.

Rusten, B., & Ødegaard, H. (2018). Primary treatment: Particle separation by rotating belt sieves. *Adv Wastewater Treat*, *1*, 1-28.

Rusten, B., Eikebrokk, B., Ulgenes, Y., & Lygren, E. (2006). Design and operations of the Kaldnes moving bed biofilm reactors. *Aquacultural Engineering*, *34*(3), 322–331.

Saeed, T., Afrin, R., Al Muyeed, A., & Sun, G. (2012). Treatment of tannery wastewater in a pilot-scale hybrid constructed wetland system in Bangladesh. *Chemosphere*, *88*(9), 1065–1073.

Safari, S., Azadi Aghdam, M., & Kariminia, H. R. (2016). Electrocoagulation for COD and diesel removal from oily wastewater. *International Journal of Environmental Science and Technology*, *pp. 13*, 231–242.

Sahu, O., Mazumdar, B., & Chaudhari, P. K. (2014). Treatment of wastewater by electrocoagulation: a review. *Environmental science and pollution research*, *21*, 2397–2413.

Saif, Y., Ali, M., Jones, I. M., & Ahmed, S. (2021). Performance evaluation of a field-scale anaerobic baffled reactor as an economic and sustainable solution for domestic wastewater treatment. *Sustainability*, *13*(18), 10461.

Sakuma, T., Jinsiriwanit, S., Hattori, T., & Deshusses, M. A. (2008). Removal of ammonia from contaminated air in a biotrickling filter–denitrifying bioreactor combination system. *Water research*, *42*(17), 4507–4513.

Santos, E., Carvalho, M., & Martins, S. (2023). Sustainable Water Management: Understanding the Socioeconomic and Cultural Dimensions. Sustainability, 15(17), 13074.

Saritha, V., Maruthi, Y. A., & Mukkanti, K. (2010). Potential fungi for bioremediation of industrial effluents. *BioResources*, *5*(1).

Sasi, P. K. C., Viswanathan, A., Mechery, J., Thomas, D. M., Jacob, J. P., & Paulose, S. V. (2020). Phycoremediation of paper and pulp mill effluent using planktochlorella nurekis and Chlamydomonas reinhardtii–a comparative study. *Journal of Environmental Treatment Techniques*, *8*(2), 809–817.

Savant, D. V., Abdul-Rahman, R., & Ranade, D. R. (2006). Anaerobic degradation of adsorbable organic halides (AOX) from pulp and paper industry wastewater. *Bioresource Technology*, *97*(9), 1092-1104.

Savant, D. V., Abdul-Rahman, R., & Ranade, D. R. (2006). Anaerobic degradation of adsorbable organic halides (AOX) from pulp and paper industry wastewater. *Bioresource Technology*, *97*(9), 1092-1104.

Sayadi, M. H., Kargar, R., Doosti, M. R., & Salehi, H. (2012). Hybrid constructed wetlands for wastewater treatment: A worldwide review. *Proceedings of the international academy of ecology and environmental sciences*, *2*(4), 204.

Sayadi, M. H., Kargar, R., Doosti, M. R., & Salehi, H. (2012). Hybrid constructed wetlands for wastewater treatment: A worldwide review. *Proceedings of the international academy of ecology and environmental sciences*, *2*(4), 204.

Sehar, S., Aamir, R., Naz, I., Ali, N., & Ahmed, S. (2013). Reduction of contaminants (physical, chemical, and microbial) in domestic wastewater through hybrid constructed wetland. *International Scholarly Research Notices*, *2013*.

Sehar, S., Naeem, S., Perveen, I., Ali, N. and Ahmed, S., (2015). A comparative study of macrophytes influence on wastewater treatment through subsurface flow hybrid constructed wetland. Ecological Engineering, 81, pp.62-69.

Sehar, S., Naz, I., Das, T. and Ahmed, S., (2016). Evidence of microscopic correlation between biofilm kinetics and divalent cations for enhanced wastewater treatment efficiency. RSC advances, 6(18), pp.15112-15120.

Sehar, S., Naz, I., Khan, S., Naeem, S., Perveen, I., Ali, N., & Ahmed, S. (2016b). Performance Evaluation of Integrated Constructed Wetland for Domestic Wastewater Treatment. Water and Environment Research.88(3):280-7. doi: 10.2175/106143016X14504669767814.

Setiawan, Y., & Hardiani, H. (2020, December). The potential of anaerobic-constructed wetland system for wastewater treatment of rice straw pulping. In *IOP Conference Series: Materials Science and Engineering* (Vol. 980, No. 1, p. 012070). IOP Publishing.

Shakira, A., Mullah, A., & Lubis, S. S. (2023). Efektivitas Metode Multi Soil Layering (MSL) dalam Penurunan Total Koliform Limbah Cair Domestik. *Dampak*, *20*(2), 83-92.

Shakthi, A. S., Mullasseri, S., Vijisha, P., Manjari, K., Sahoo, N. R., Nagaleekar, V. K., ... & Naik, P. (2018). Degrading Paper Mill Sludge Fungi and earthworms as bioagents.

Shalaby, I. M., Altalhy, A. D., & Mosallam, H. A. (2008). Preliminary field study of a model plant for sewage water treatment using gravel bed hydroponics method. *World Applied Science Journal*, *4*(2), 238-243.

Shankar, R., Singh, L., Mondal, P., & Chand, S. (2014). Removal of COD, TOC, and color from pulp and paper industry wastewater through electrocoagulation. *Desalination and Water Treatment*, *52*(40-42), pp. 7711-7722.

Shin, C., Lee, E., McCarty, P. L., & Bae, J. (2011). Effects of influent DO/COD ratio on the performance of an anaerobic fluidized bed reactor fed low-strength synthetic wastewater. *Bioresource Technology*, *102*(21), 9860-9865.

Sigoillot, J. C., Berrin, J. G., Bey, M., Lesage-Meessen, L., Levasseur, A., Lomascolo, A., ... & Uzan-Boukhris, E. (2012). Fungal strategies for lignin degradation. *Advances in botanical research*, *61*, 263-308.

Singh, A. K., & Chandra, R. (2019). Pollutants released from the pulp paper industry: Aquatic toxicity and their health hazards. *Aquatic toxicology*, *pp. 211*, 202–216.

Singh, A. K., Bilal, M., Iqbal, H. M., Meyer, A. S., & Raj, A. (2021). Bioremediation of lignin derivatives and phenolics in wastewater with lignin modifying enzymes: Status, opportunities and challenges. *Science of the Total Environment*, *777*, 145988.

Singh, A.K. and Chandra, R., (2019). Pollutants released from the pulp paper industry: Aquatic toxicity and their health hazards. Aquatic toxicology, 211, pp.202–216.

Singh, S. K. (2018). Sustainable people, process and organization management in emerging markets. *Benchmarking: An International Journal*, *25*(3), 774–776.

Singh, S.P., Bansal, M.C., Singh, S.P., Singh, A.M. and Dixit, A.K., (2015). Rheological properties and statistical analysis of wheat straw soda black liquor. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 37(24), 2639–2646.

Solano, M. L., Soriano, P., & Ciria, M. P. (2004). Constructed wetlands as a sustainable solution for wastewater treatment in small villages. *Biosystems engineering*, *87*(1), 109-118.

Soloman, P. A., Basha, C. A., Velan, M., Ramamurthi, V., Koteeswaran, K., & Balasubramanian, N. (2009). Electrochemical degradation of Remazol Black B dye effluent. *CLEAN–soil, air, water*, *37*(11), 889–900.

Song, H. L., Nakano, K., Taniguchi, T., Nomura, M., & Nishimura, O. (2009). Estrogen removal from treated municipal effluent in small-scale constructed wetland with different depth. *Bioresource Technology*, *100*(12), 2945-2951.

Soo, P. L., Bashir, M. J., & Wong, L. P. (2022). Recent advancements in the treatment of palm oil mill effluent (POME) using anaerobic biofilm reactors: Challenges and future perspectives. *Journal of Environmental Management*, *p. 320*, 115750.

Steephen, A., Preethi, V., Annenewmy, B., Parthasarathy, R., & Sairam, M. (2023). Solar photocatalytic hydrogen production from pulp and paper wastewater. International Journal of Hydrogen Energy.

Stein, O. R., Borden-Stewart, D. J., Hook, P. B., & Jones, W. L. (2007). Seasonal influence on sulfate reduction and zinc sequestration in subsurface treatment wetlands. *Water research*, *41*(15), 3440-3448.

Stillger, L., & Müller, D. (2022). Peptide-coating combating antimicrobial contaminations: a review of covalent immobilization strategies for industrial applications. Journal of Materials Science, 57(24), 10863-10885.

Stokke, D.D., Wu, Q. and Han, G., (2013). Introduction to wood and natural fiber composites. John Wiley & Sons.

Strategy, P. W. S. (2002). National water sector profile, volume 5, October 2002, Ministry of Water and Power, Office of the Chief Engineering Advisor. Available at http://waterinfo. net. pk/cms/pdf/vol5. pdf.

Sudarsan, J. S., Roy, R. L., Baskar, G., Deeptha, V. T., & Nithiyanantham, S. (2015). Domestic wastewater treatment performance using constructed wetland. *Sustainable Water Resources Management*, *1*, 89-96.

Tanner, C. C., Clayton, J. S., & Upsdell, M. P. (1995). Effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands—I. Removal of oxygen demand, suspended solids and faecal coliforms. *Water Research*, *29*(1), 17–26.

Tawfik, A., Bakr, M. H., Nasr, M., Haider, J., Lim, H., Qyyum, M. A., & Lam, S. S. (2022). Economic and environmental sustainability for anaerobic biological treatment of wastewater from paper and cardboard manufacturing industry. *Chemosphere*, *289*, 133166.

Taylor, C. R., Hook, P. B., Stein, O. R., & Zabinski, C. A. (2011). Seasonal effects of 19 plant species on COD removal in subsurface treatment wetland microcosms. *Ecological Engineering*, *37*(5), 703–710.

Tettleton, R. P., Howell, F. G., & Reaves, R. P. (1993). *Performance of a constructed marsh in the tertiary treatment of bleach kraft pulp mill effluent: results of a 2-year pilot project* (pp. 437–440). CRC Press/Lewis Publishers: Boca Raton, FL, USA.

Tezel, U., Guven, E., Erguder, T. H., & Demirer, G. N. (2001). Sequential (anaerobic/aerobic) biological treatment of Dalaman SEKA pulp and paper industry effluent. *Waste management*, *21*(8), 717-724.

Thamke, S. B. A. K., & Khan, A. (2021). Constructed wetlands-Natural treatment of wastewater. *Int. J. Eng. Res. Technol*, *10*(06), 204-212.

Thut, R. N. (1989). *Utilization of artificial marshes for treatment of pulp mill effluents* (pp. 239-244). Lewis Publishers, Michigan.

Toczyłowska-Mamińska, R. (2017). Limits and perspectives of pulp and paper industry wastewater treatment–A review. *Renewable and Sustainable Energy Reviews*, *78*, 764-772.

Torsvik, V., & Øvreås, L. (2002). Microbial diversity and function in soil: from genes to ecosystems. *Current opinion in microbiology*, *5*(3), 240-245.

Treatment of pulp and paper mill wastewater using ultrafiltration process: Optimization of the fouling and rejections. *Industrial & engineering chemistry research*, *51*(17), 6184-6195.

Tribot, A., Amer, G., Alio, M. A., de Baynast, H., Delattre, C., Pons, A., ... & Dussap, C. G. (2019). Wood-lignin: Supply, extraction processes and use as bio-based material. *European Polymer Journal*, *112*, 228-240.

Turner, T., Wheeler, R., & Oliver, I. W. (2022). Evaluating land application of pulp and paper mill sludge: A review. *Journal of Environmental Management*, *p. 317*, 115439.

Uğurlu, M., Gürses, A., Doğar, Ç., & Yalçın, M. (2008). The removal of lignin and phenol from paper mill effluents by electrocoagulation. *Journal of Environmental Management*, *87*(3), 420-428.

US-EPA (2007). Bureau of Water Supply and Wastewater Management: department of Environmental Protection Agency, Wastewater Treatment Plant Operator Training (Module 20: Trickling filters

Vijayakumar, V., Keerthi, & Balasubramanian, N. (2015). Heavy metal removal by electrocoagulation integrated membrane bioreactor. *Clean–soil, air, water*, *43*(4), 532-537.

Vikrant, K., Kim, K. H., Szulejko, J. E., Pandey, S. K., Singh, R. S., Giri, B. S., ... & Lee, S. H. (2017). Bio-filters for the Treatment of VOCs and Odors-A Review. *Asian Journal of Atmospheric Environment*, *11*(3), 139-152.

Virkutyte, J. (2017). Aerobic treatment of effluents from pulp and paper industries. In Current Developments in Biotechnology and Bioengineering (pp. 103-130).

Vymazal, J. (2001). Types of constructed wetlands for wastewater treatment: their potential for nutrient removal. *Transformations of nutrients in natural and constructed wetlands*, pp. 1–93.

Vymazal, J. (2013). Emergent plants used in free water surface constructed wetlands: a review. *Ecological engineering*, *pp. 61*, 582–592.

Vymazal, J. (2014). Constructed wetlands for treatment of industrial wastewaters: A review. *Ecological engineering*, *pp. 73*, 724–751.

Vymazal, J., Greenway, M., Tonderski, K., Brix, H., & Mander, Ü. (2006). 5 Constructed Wetlands for Wastewater Treatment. *Wetlands and natural resource management*, *190*, 69.

Wallace, S., & Kadlec, R. (2005). BTEX degradation in a cold-climate wetland system. *Water Science and Technology*, *51*(9), 165–171.

Wang, W., Wang, S., Ma, X., & Gong, J. (2011). Recent advances in catalytic hydrogenation of carbon dioxide. *Chemical Society Reviews*, *40*(7), 3703-3727.

Wang, Y., Chen, X., Wu, B., Ma, T., Jiang, H., Mi, Y., ... & Li, C. (2022). Potential and mechanism for bioremediation of papermaking black liquor by a psychrotrophic lignin-degrading bacterium, Arthrobacter sp. C2. Journal of Hazardous Materials, 439, 129534.

Water, S., & World Health Organization. (2006). *Expert Consultation for 2nd Addendum to the 3rd Edition of the Guidelines for Drinking-water Quality: Geneva, 15-19, May 2006* (No. WHO/SDE/WSH/06.05). World Health Organization.

Water, U. N. (2008). Tackling a Global Crisis. International Year of Sanitation. http://www.wsscc.org/fileadmin/files/pdf/publication/IYS_2008.tackling_a_global_cr isis.pdf.

Willner, A. E., Huang, H., Yan, Y., Ren, Y., Ahmed, N., Xie, G., ... & Ashrafi, S. (2015). Optical communications using orbital angular momentum beams. *Advances in optics and photonics*, *7*(1), 66–106.

World Health Organization, 2006. WHO guidelines for the safe use of wastewater excreta and greywater (Vol. 1). World Health Organization.

World Health Organization. (2004). *Guidelines for drinking-water quality* (Vol. 1). World Health Organization.

Wu, W., Zhao, Y., Qi, J., Li, C., Fang, J., Xu, B., ... & Li, H. (2023). An amphiphilic flocculant with a lignin core for efficient separation of suspended solids. *Separation and Purification Technology*, *314*, 123640.

Xilei, G., & Xiaoli, H. (2010). Comparison of phencyclidine hydrochloride and atropine in the treatment of acute organophosphorus pesticide poisoning. In *2010 Peking Union International Summit for Emergency Medicine* (pp. 389–391).

Xu, Z., Qin, L., Cai, M., Hua, W., & Jin, M. (2018). Biodegradation of kraft lignin by newly isolated Klebsiella pneumoniae, Pseudomonas putida, and Ochrobactrum tritici strains. Environmental Science and Pollution Research, p. 25, 14171–14181.

Yadav, D., Singh, S., & Sinha, R. (2021). Microbial Degradation of Organic Contaminants in Water Bodies: Technological Advancements. *Pollutants and Water Management: Resources, Strategies and Scarcity*, pp. 172–209.

Yadav, V., Kumar, A., Bilal, M., Nguyen, T. A., & Iqbal, H. M. (2022). Lignin removal from pulp and paper industry waste streams and its application. In *Nanotechnology in Paper and Wood Engineering* (pp. 265-283). Elsevier.

Yaser, A. Z., Nurmin, B., & Rosalam, S. (2013). Coagulation/flocculation of anaerobically treated palm oil mill effluent (AnPOME): a review. *Developments in Sustainable Chemical and Bioprocess Technology*, 3-9.

Zhang, C., Chen, J. and Wen, Z., (2012). Alternative policy assessment for water pollution control in China's pulp and paper industry. Resources, Conservation and Recycling, 66, pp.15-26.

Zhang, L., Yan, C., Qi, R., & Yang, F. (2022). Quantifying the contribution rates of sulfonamide antibiotics removal mechanisms in constructed wetlands using multivariate statistical analysis. *Environmental Pollution*, *p. 292*, 118463.

Zhang, S., Xiao, J., Wang, G. and Chen, G., (2020). Enzymatic hydrolysis of lignin by ligninolytic enzymes and analysis of the hydrolyzed lignin products. Bioresource technology, 304, p.122975.

Zhou, P., Brown, G. M., & Gu, B. (2006). Membrane and other treatment technologies—pros and cons. In *Perchlorate: Environmental Occurrence, Interactions and Treatment* (pp. 389–404). Boston, MA: Springer US.