MICROALGAE-BASED BIOREMEDIATION OF RADIOGRAPHIC X-RAY DEVELOPER SOLUTION

Thesis submitted in fulfillment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

IN

BIOTECHNOLOGY

BY

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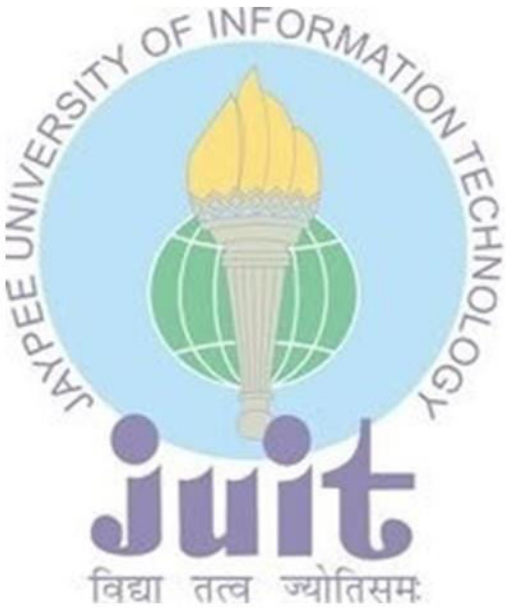
I hereby declare that the work reported in the Ph.D. thesis entitled **"Microalgae-based Bioremediation of Radiographic X-ray Developer Solution"** submitted at **Jaypee University of Information Technology, Waknaghat, India** is an authentic record of my work carried out under the supervision of **Dr. Garlapati Vijay Kumar**. I have not submitted this work elsewhere for any other degree or diploma. I am fully responsible for the contents of my Ph.D. thesis.



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SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled "**Microalgae-based Bioremediation of Radiographic X-ray Developer Solution**", submitted by **Swati Sharma** at **Jaypee University of Information Technology, Waknaghat, Solan (HP) India,** is a bonafide record of her original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

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(Swati Sharma)

ABSTRACT

The present research study focuses on the scrutiny of physico-chemical criterion and selection of microalgal toxicity range in X-ray developer (XD), and fixer effluent implicates towards the comprehensive studies of bioremediation. The research on characterization parameters reveals the existence of 0.01791±0.000 g/l of silver and proportions of 1.22±0.004 g/l and 27.29±0.230 g/l in biological and chemical oxygen demand, respectively in waste developer effluent. On the other hand, waste fixer solution characterization exhibited higher silver 3.49±0.01 g/l and BOD and COD values as 11.83±0.39 g/l and 506.15±0.20 g/l, respectively. The tolerance limits of microalgae, *Desmodesmus armatus* and *Scendesmus abundans* in diluted developer and fixer effluents discloses the significant growth of *D. armatus* in the diluted BBM and waste developer effluent in 3:1 proportion. The microalgal remediation had shown prominent values in the one-month cultivation period on the 19th day with relative percentages of 20.86% in BOD, 13.88% in COD, and 57.10% in phosphorus removal. The process of microalgal remediation also demonstrates the practical potential of relative reduction of silver 44.06% on the 19th day with concurrently 1.392 % of lipids formation. This prevailing research explains the probable approaches in phycoremediation to remove the contaminants from the waste developer solution and simultaneously production of lipids through green sustainable manner.

The valorization of food waste (FW) and agri-compost (ACM) as a nutritional medium for the growth of *D. armatus* is the ideal practice to utilize the waste in an eco-friendly manner reduces the cost of standard BBM (inorganic) medium towards valuable product formation. The *D. armatus* cultivated in various dilutions of FW and ACM medium with BBM medium separately, and promising results have been observed with the 3:1 (FW/ACM: BBM) dilution, which has been further compared with standard BBM media (inorganic) for one month. This diluted medium 3:1(FW/ACM: BBM) was

further studied for *D. armatus* growth kinetics in these diluted mediums and BBM medium, nitrogen, phosphorus removal, and macromolecules (lipids, carbohydrates, and proteins) production. The kinetic growth studies in "Fermentor" tool software revealed the different growth phases of *D. armatus* in diluted FW, ACM and standard BBM media and measured growth kinetic parameters. The highest lipid (9.925%), protein (112.5µg/ml), and carbohydrate (8.75µg/ml) contents have been observed with diluted ACM, BBM and diluted FW media, respectively.

This food waste and agri-compost medium were further employed in bioremediation studies of X-ray developer solution. The X-ray developer solution was diluted with FW and ACM in various dilutions and inoculated with *D. armatus*. The maximum growth of *D. armatus* was observed in 3:1 (FW and ACM: XD) dilution and was utilized for further phycoremediation studies of X-ray developer solution. The *D. armatus* exhibited more promising results on 19th day of cultivation in agri-compost diluted developer solution than the food waste. The relative % of BOD reduction-17.61%, COD reduction-11.11%, silver removal-43.69%, and simultaneously lipids production 1.42% observed with phycoremediation of X-ray developer solution using diluted ACM. This prevailing research explains the probable approaches in phycoremediation to remove the contaminants from the waste developer effluent and simultaneously produce lipids through green sustainable manner and utilizing the waste resources, i.e., food waste and agri-compost medium in cultivation and remediation purpose.

Keywords: *D. armatus* ; X-ray fixer solution; Bioremediation; Agri-compost media; Food waste media; Bioprocess dynamics; Silver removal; Lipid production.

LIST OF SYMBOLS & ABBREVIATIONS

°C	Degree Celsius
%	Percentage
ml	Milliliter
mg/ml	Milligrams per milliliter
NCIM	National Collection of Industrial Microorganism
µg/ml	Micrograms per milliliter
g/l	Grams per liter
h	Hours
mins	Minutes
nm	Nanometer
rpm	Revolutions per minute
$NaNO_3$	Sodium nitrate
EDTA	Ethylenediaminetetraacetic acid
$CaCl_{2.}\ 2H_2O$	Calcium chloride dihydrate
$MgSO_4.\ 7H_2O$	Magnesium sulfate heptahydrate
K_2HPO_4	Dipotassium phosphate
$KH_2\ PO_4$	Potassium dihydrogen phosphate
KOH	Potassium hydroxide
$FeSO_4.7H_2O$	Iron(II) sulfate heptahydrate
H_2SO_4	Sulfuric acid
H_3BO_3	Boric acid

$ZnSO_4.7H_2O$	Zinc Sulphate Heptahydrate
$MnCl_2.4H_2O$	Manganese(II) chloride tetrahydrate
$MO.O_3$	Molybdenum oxide
$CO(NO_3)_2.6H_2O$	Cobalt(II) Nitrate Hexahydrate
$CuSO_4.5H_2O$	Copper sulfate pentahydrate
HCl	Hydrochloric acid
NaOH	Sodium hydroxide
UV-VIS	Ultraviolet-visible
ICP-MS	Inductive coupled plasma-mass spectrophotometry
AAS	Atomic absorption spectrophotometer
O.D	Optical Density
BSA	Bovine Serum Albumin
FWM	Food Waste Medium
ACM	Agri-compost Medium
BBM	Bold Basal Medium
X.D	X-ray Developer Solution
X.F	X-ray Fixer Solution
dX.D	Diluted X-ray Developer Solution (3BBM:1X.D)

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CHAPTER 1

Introduction & Review of Literature

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1.1 Importance of taking care of medical effluents

The proliferation of bio-medical waste management in hospitals, medical labs, and other primary care has been a preeminent concern in the current years. Biomedical waste is categorized as solid infectious waste and liquid waste. The liquid waste comprises radioactive waste, chemicals, water from the floor washing, disinfectants, pathology waste, liquid from medical labs, and pharmaceutical waste [1]. The World Health Organization (WHO) evaluated that 5% of medical waste is considered as non-infectious, 10% is infectious, and the remaining 85% is non-toxic. However, it also consists of harmful chemicals such as formaldehyde and methyl chloride [2]. The total quantity of municipal waste is generated in an Indian city in which 1 to 1.5 % is biomedical waste, 10-15% recognized as infectious. The medical care units and hospitals produce 1-2 Kg per bed per day, which is more in the tertiary care hospitals in India. The dumping of medical effluents can be hazardous when mixed and disposed of with domestic waste in the open area or landfill sites near residential and slum areas. The inappropriate disposal of medical waste can lead to a higher risk of environmental deterioration and other deliberate health effects on human beings [3]. The legitimate management of biomedical waste can decrease the uncertainty towards the primary medical care services. Waste segregations, reusability, and toxicity are fundamental approaches to waste management [4].

The inadmissible way of biomedical waste management causes soil, air, and water pollution. The medical waste that damages the environment is divided into biological, chemical, and radioactive substances [5, 6]. The outbreak of infectious diseases such as pneumonia, tetanus, diarrheal diseases, whooping cough, tuberculosis, and other diseases is due to improper waste disposal in the environment [7, 8]. The biomedical waste disposal in the open area, near water supplies, and public dustbin cause the disease. Its treatment through incineration releases the harmful gases that are the origin of respiratory diseases [9]. Hospital waste is highly toxic compared with

household waste as it contains antibiotics, disinfectants, and radionuclide. The hospital waste can be considered as the origin and propagation of the pathogenic strains of bacteria. The waste derived from the hospitals has been a severe threat to the ecosystem and as well as public well-being [10–12]. If not conveniently disposed and handled, Biomedical waste is a source of infection to the medical staff and community through microorganisms spread when released into the environment from the medical care facilities [13, 14]. The improper management of biomedical waste in clinics, hospitals, and other medical labs generates a considerable amount of waste that should be disposed of with proper care and rules. Appropriate waste management is a significant concern noticed by the government and private medical agencies. The adequate and secure management of biomedical waste is necessary for every individual in legal and social practices [15].

1.2 X-ray waste discharges: Sources and characteristics

The X-ray waste is inimitable biomedical waste generated in the radiology department to accomplish dental and medical services. Around 2 billion of the radiographs generated per year globally comprise CT scans, mammograms, and chest X-rays [16]. The radiographic effluents consist of developer and fixer, a high amount of glutaraldehyde and formaldehyde, amalgam, lead foils, wash water, lead cover, and X-ray films used in X-ray processing [14]. Indeed, there is so much up-gradation in radiographic processing techniques, but most dentists still prefer traditional methods to retrieve the images. This practice involved the exposure of X-rays to radiographic films, subsequently developing of image, washing, fixing, and drying films during its processing. This radiographic methodology produces waste materials that permit environmental threats. The waste materials are waste films, processing solutions, cleaner, water, and lead foils [17, 18]. The developer and fixer are primary processing solutions with organic reductants, alkaline buffer, sulfites in a developer solution, boric acid, aluminum sulfate, sodium sulfite, and ammonium thiosulphate fixer solution [19, 20].

Table 1.1: Composition of the developer and fixer solution in the radiographic processing [20–24].

Developer solution	grams	Fixer solution	grams
KOH	50-170	Ammonium thiosulphate	145-150
Acetic acid	3-66	Sodium sulfite	8-10
Glutaraldehyde	30-40	Boric acid	1-7
Sodium meta-bisulfite	5-10	Acetic acid	5-8
Ethylene glycol	10-15	Ammonium acetate	10-20
Diethylene glycol	1-35	Aluminium sulphate	7-10
Morpholinomethanedifosfonic acid	0-7.5	Sulfuric acid	3-10
5-Methylbenzotriazole	0-80	2-Phenoxyethanol	0-3
1-Phenyl-1-H-tetrazole-5-thiole	0-10	Water	1 litre
Boric acid	1-5		
Potassium carbonate	10-20		
Ethylenediaminotetraacetic acid.4 $Na.2H_2O$	1-5		
1-Phenyl-3-Pyrazolidone	0-7		
Hydroquinone	4-20		
NaBr	0.5-1		
Phosphoric acid	0-20		
Water	1 litre		

The radiographic solutions consist of a large amount of silver ranging from 8000-12000 mg/l in silver- thiosulphate complex form in fixer solution. The developer solution has less amount of silver (generally below the 5 mg/l) and has a high amount of thiosulphate, which makes it corrosive [25, 26]. The waste fixer solution is a vital source of elemental silver with a concentration of 2-7 g/l. During manual radiographic processing on light exposure, the silver

halide is either reduced to metallic silver and remains dissolved by the thiosulphate in waste solution amid the developing and fixing process. Silver halides get converted into silver-thiosulphate complexes when treated with ammonium or sodium thiosulphate during the fixing process. The radiographic wastewater posses' free silver ions that show toxicity against the aquatic organisms even in minimal concentration [27, 28]. The radiographic waste constitutes of inorganic and organic compound mixture of developer, fixer, and used films. The occurrence of thiosulphate, sulfite, and other trace elements in the radiographic solution makes the solution more acidic or alkaline. The hydroquinone present in developer solutions decomposes into the oxidation form has genotoxicity with time [23, 29]. The fixer solution includes sodium sulfite, potassium bromide, acetic acid, and thiosulphate complexes as prominent compounds when removing the silver halide after the development process. The waste developer solution is usually not recognized as hazardous, but is discarded in a public sewer without treatment will adversely affect the environment. The pH of the developer solution falls in the alkaline category with its high corrosive behavior. The presence of quinone, sodium sulfite, hydroquinone, sodium thiosulphate, methol, boric acid, sodium acetate, acetic acid, and a small amount of silver complex can damage the environment if not appropriately treated [18]. The fixer solution has a high amount of stable silver thiosulphate complexes, which have low dissociation constant. The acidic pH of the fixer solution and higher silver concentration (3000-8000 ppm) consider hazardous waste. Therefore, the fixer solution does not directly drain into the public sewer and other litter. The silver carry waste has its side effects on the human body as well as on the environment. The soluble and colloidal form of the silver has the competence to produce an adverse impact on the human body, such as permanent skin or eyes discoloration (argyria) or (argyrosis). Silver also gets concentrated in the muscles and brain. The low amount of silver intake cause the symptoms of liver and kidney fatty degeneration, change in blood cells. The developer cleaner system may dissolve silver and contains sodium dichromate, which makes it toxic and has hazardous health effect on a human being; its unexpected consumption cause vomit, diarrhea, nausea, burning sense, shock, abdominal pain, etc. [17, 30, 31].

The waste developer and fixer solution poured into the public sewer system in the metropolitan cities. The pH, color, high BOD and COD, chlorides, sulfates, and higher turbidity prohibit the treatment processes in the treatment plants. The expulsions of the waste radiographic solution above the allowed limits without any treatment encounter severe environmental pollution

problems [22, 32]. The chemical-based radiographic solution of solid and liquid form is harmful to the environment and humans if not appropriately disposed of through the treatment process. Before the disposal of radiographic waste into the municipal sewer system, appropriate treatment is implemented for the recovery of precious metal, neutralization and extermination of the toxic substances, and maintenance of pH and other environmental parameters. Most of the silver content in the waste radiographic is generally retrieved by oxidation-reduction, electrolysis, sulfide precipitation, ion exchange, photo-Fenton oxidation, nanofiltration, and reverse osmosis [33, 34].

1.3 Bioremediation: Principles and advantages over existing remediation technologies

The escalation towards industrialization proportionately increases the usage of chemicals, energy sources, heavy metals, pesticides, and other neoteric products that contribute to soil, water, and air pollution. Contaminants released from the hospitals, sewerage treatment plant, agriculture area, and industries incorporated in controlled or uncontrolled ratios to the environment [9, 35–37]. The generation of toxic contaminants such as inorganic, organic, non-metals, metals, and metalloids exploits the environment through acceleration in anthropogenic activities for a better future. The primary consideration regarding these contaminants is their toxicity and health hazards to human beings and the environment. Therefore it is mandatory to diminish the contaminants and prevent their dispersion and infuse with surface and groundwater [38, 39]. The growth of microorganisms, reduction in soil microbes and fertility, disruption in biogeochemical cycles, and diminishing in aquatic organisms are fundamental challenges the contaminated environment faces and have a pernicious effect on human health. It is widely perceived that polluted resources cause the poor health of humans and the ecosystem. Its continuous exploration led to a global endeavor to remediate the contaminated sources and build to reuse them. The current scenario demands introducing preventive measures to revitalize the primary driving force machinery on the planet [40, 41]. The elimination of the organic and inorganic pollutants from contaminated sources is an essential concern to commend continuous improvement in our community.

The physical, chemical and biological treatment is the fundamental treatment process to dispose and degrade the waste [42, 43]. The substantial amount of sludge released from the industries and their removal required universally endorsed techniques consist of composting, landfilling,

burning, sludge spread at ample space, and drying at high temperature. The extreme expense encountered by waste management. Thus most of the effluent is directly dumped into the open area and farming fields. The small area for land filling, high cost, and polluting the natural water source is the leading issue to redirect the attention towards eco-friendly and low charge sludge treatment process [44, 45]. Various physical and chemical methods are utilized for the effluent treatment generated from multiple industries, sewage treatment plants, and other resources. These techniques have adverse effects as complexity, absence of public acceptance, harm to the environment, uneconomical [46, 47]. The treatment of acid mine drainage is accomplished by separation methods such as adsorption, filtration, and precipitation reaction. The shortcoming of these methods includes high operational and capital cost, low disposal capability, high amount of sludge generation, and intolerance to organic material [48, 49]. The heavy metal remediation includes methods such as chemical precipitation, filtration, adsorption, reverse osmosis, acid leaching, membrane processing, landfill, electro reclamation, ion exchange, and thermal treatment are not applicable due to low productivity, high expenditure, high cost of chemicals, inadequate removal of metal ions and decrease in the soil fertility and properties [50, 51]. The chemical methods primarily used in wastewater remediation are precipitation using aluminum salt, flocculation has a higher cost, secondary pollutant generation, and an enormous amount of sludge [52, 53]. The conventional methods of aquaculture treatment by filtration, aeration, and anaerobic-anoxic-oxic techniques have their limitations of high energy utilization. A considerable investment enhances the industry's economic strain, sludge formation, and carbon, nitrogen, and phosphorus not recycled as resources [54, 55]. The effluents from the medical clinics and hospitals are not earned so much consideration, and it disposes of either by process of incineration or autoclaving even though it disposes of with household waste in some countries [56–58]. The primary remediation techniques, coagulation, adsorption, chemical precipitation, incineration, and chemical oxidation adopted for wastewater undergo economic and technical limitations [59]. Physico-chemical methods such as sorption, Fenton oxidation, electrolytic recovery, ozonation, solvent extraction, chemical precipitation, membrane filtration, osmosis, and evaporation are not used in treating effluents in industries gratifying. These techniques require high capital and operational cost, high chemical and energy absorption, less efficient for removal of trace amount of contaminants, toxic by-product formation, which imperil the ecosystem sustainability [52, 60, 61].

Among the other physico-chemical processes, bioremediation is one of the cumulative aspects that deteriorate the hazardous pollutants from the environment through the use of each biological system. The microbes help in the remediation of the contaminants as well as clean or revive the polluted ground. The microbial community tends to oxidize, immobilize, and contaminants transform to reinforce the environment [62, 63]. The biological methods for the treatment of pollutants are inventive techniques to resolve environmental deterioration. The process of bioremediation is an eco-friendly approach that contributes to eradicating contaminants via the natural-biological process. The usage of green plants, bacteria, enzymes, microorganisms, and fungi to degrades the pollutants and change the environment to its original form. The microbes accelerate the metabolic process by degenerating or revolutionizing the contaminants into microbial biomass, water, carbon dioxide, and other co-products that are less toxic than primary compounds [64, 65]. Bioremediation has diverse applications that include treatment of waste, cleaning up the lagoon, soil, sludge, and water, enhancing water portability, and diminishing the concentration of pollutants in contaminated spots [66]. Remediation aims to degenerate the contaminants organically and maintain their toxic limits below the level set by government agencies. The efficacy of bioremediation strongly depends on the environment's condition that enables the microorganism growth and its actions against the removal and degradation of contaminants at the highest rate [42, 67].

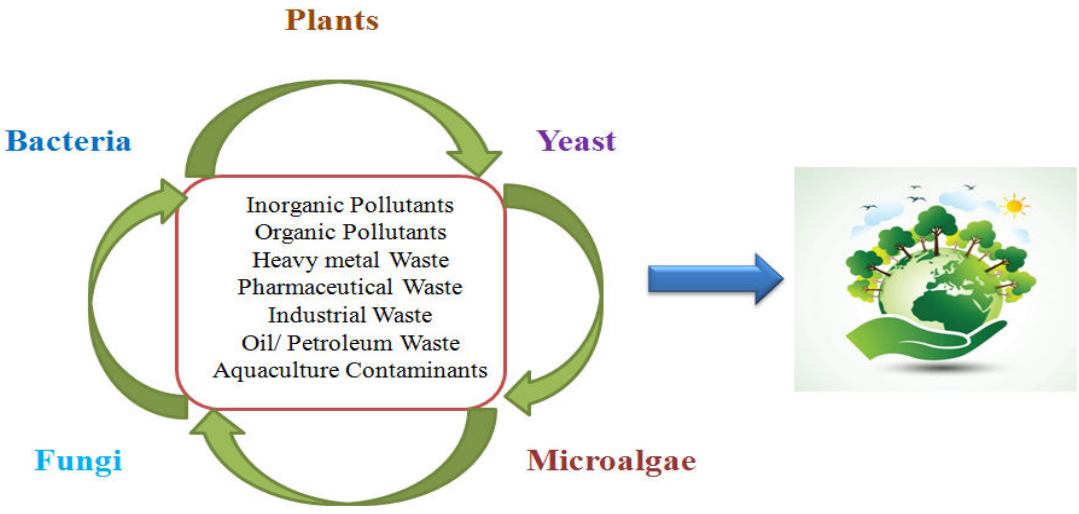

Figure 1.1: Process of bioremediation [68, 69]

The bioremediation process utilizes various microorganisms such as bacteria, fungi, algae, and plants to remove toxic effluent and retrieve unique valuable substances efficiently. The microorganism can easily isolate from any environmental conditions. The microbes are the trigger to remove the harmful organic contaminants to an ecologically safe level in water, soil, materials, sediments, and substances [68, 70, 71]. Environmental biotechnology is not strange to the research community; wastewater treatment and composting are extensively recognizable processes for clearing polluted areas and water. Over the last few decades, bioremediation techniques have been upgraded to fulfill the objective of degrading pollutants and reviving the polluted environment efficiently and economically in an eco-friendly way. Most research conducted on the bioremediation methods has been modeled and developed according to types of pollutant and source of pollution. There are no single techniques that exhibit the panacea for environment revitalization. The preference of bioremediation techniques relies upon its low cost and environment-friendly nature over other physical and chemical methods [72]. Bioremediation emerges as an inventive alternative to traditional methodologies to complement the environmental friendliness and cost feasibility. The vermicomposting, household and industrial wastewater can be managed through bioremediation [73, 74].

The bioremediation system is categorized in two forms on-site (in situ) and off-site (ex-situ) bioremediation through hybrid or pure microbial strain, plants according to their applicability, principal, and desirable outcomes. As their name illustrates, ex-situ techniques introduced the removal or treatment of the contaminants far from the original site of waste and in situ involve the no excavation and on-site removal of the pollutants through biological treatment [42, 75–77]. Example of in situ remediation includes organic solid phase treatment, composting, engineered remediation, soil piles, land farming, and bioreactor, whereas ex-situ involves bioaugmentation, biostimulation, biosparging, and biodegradation [36, 78, 79]. These methods entailed aerobic, anaerobic, and heterotrophic microorganisms for the bioconversion process. The potency of bioremediation relies on the environmental conditions that support microorganism growth and degeneration of pollutants at a rapid rate [80]. The primary element that influences the bioremediation process is pH, temperature, nutrients, nature of contaminants, electron donor and acceptor, soil type, oxygen, metabolites, microbial variety [42, 59, 81, 82].

The positive attributes of bioremediation are simple, no use of harmful chemicals, noninvasive, economically practical, treatment of solid and liquid waste, waive off transportation cost, no mining, engineer strain used for remediation purpose, minimum area requirement, controlled environment can be provided in a bioreactor for growth of microbes and removal of pollutants, use of acclimatized microorganism for better remediation, formation of a less toxic form of pollutants, secondary metabolites production (CO_2 and H_2O) [42, 83, 84]. Yet bioremediation cannot degrade the inorganic pollutant completely though it shifts the oxidation state, helping in uptake, accumulation, and adsorption. Adopting a strategic move to reduce contaminants implicates indigenous and engineered microorganisms to attain the best detoxification results [75].

1.4 Phycoremediation: Fundamentals and positive attributes

The word phycoremediation as name defined “phyco” means “algae” and “remediation” means “to the eradication of pollutant.” Thus phycoremediation is stated as the use of algae to remediate the solid and liquid effluents. The deteriorated waste containing nutrients acts as a substrate for algae growth, which provides light-induced oxygenation of water and food for the next tropical food web level in the aquatic ecosystem [85]. The algae play an indispensable role in the supply of greater than 50% of photosynthetic activity by constituting the basic infrastructure for the food chain in the ecosystem [86]. These biological remediation practices achieved substantial consideration for the high tenacity of wastewater treatment and adequate removal of organic and inorganic pollutants and nutrients of wastewater [87]. The phycoremediation is the process of algae utilization to degrade and transform pollutants present in the air, water, and soil. Being autotrophic, an alga performs the process of photosynthesis and requires nitrogen, phosphorus, carbon dioxide, water, and trace elements to produce atmospheric O_2. The algae also utilized carbon in the presence of sunlight (mixotrophic) and the absence of the sun (heterotrophic). The algae represented the broad category of biodiversity, approximately 30,000 to 1,000,000 species [88]. This tremendous algal diversity consists of photosynthetic species ranging from micro to macroalgae and cyanobacteria. The microalgae are microscopic, unicellular, and prokaryotic or eukaryotic, while macroalgae are seen with naked eyes, multicellular and large size. Microalgae are cultivated in freshwater (ponds, rivers, and lakes), marine water (oceans), brackish water, and wastewater [89, 90].

The microalgae have attained appreciable importance by pervasive function in biopharmaceutical, bioenergy, and nutraceuticals industries. The microalgal species have been scrutinized for possibility as biofuels, carbohydrates, food additives, medicinal products, and formation of other bioactive compounds [91]. In recent times, microalgae have risen as one of the most crucial sources in bioremediation for its numerous applications. The microalgal species of phylum Chlorophyta, Charophyta, Rhodophyta, Phaeophyta, Diatoms, and Cyanophyta, can be utilized for the phycoremediation work. The utility of microalgae is recommendable on account of providing various aspects in bioconversion of hazardous contaminants, degradation of xenobiotics compounds, CO_2 and nutrients deportation from wastewater or air, and reuse of microalgal biomass for bioproducts formation [92, 93].

Microalgae can grow fast and have photosynthetic adaptability to bioproduct accumulation inside the microalgal cells and play raw material. The cultivation of microalgae does not need arable land; efficiently grown in any type of wastewater; higher biomass production; and no requirement of herbicides/pesticides for growth [91, 94, 95]. Microalgae utilize light energy, nutrients, and atmospheric carbon dioxide to generate biomass and other co-products. Microalgae in remediation form high biomass, remove nitrate, phosphate, sulfates over 70-90% efficiency and valuable product (lipids, proteins, carbohydrates, pigments, etc.) formation [96, 97]. The microalgal biomass primarily consists of lipids, carbohydrates, and proteins. The lipids formed inside the microalgal cells in the triacylglycerol molecule further produce biodiesel [98, 99]. The synchronic process of microalgae cultivation and phycoremediation is a convenient methodology to sustainably and cost-effectively. The cultivated microalgae have their benefits as a resource of fuel, food, bioenergy, stabilizers, manure production, biochar formation, carbon dioxide sequestration, and wastewater treatment [93, 100]. The wastewater discharge from the various industries and municipal corporations rich in organic, inorganic compounds are primarily phosphates, and nitrates cause eutrophication affirms the severe environmental threat. The growth of microalgae deals with this severe problem in wastewater as nutrient feeds have been regularly used globally. There are many strains of microalgae such as *Chlorella, Chlamydomonas, Scendesmus, Spirulina, Botryococcus* and *Phormidium* that have application in the treatment of household waste water, heavy metals and nutrients evacuation [95, 101, 102].

The microalgae have the potential to grow in mixotrophic conditions (in the presence or absence of solar energy). They can use organic acid, sugar, and glycerol as a carbon source from wastewater. The varied tendency of microalgae to grow in high to low temperatures facilitates the remediation process in discrete situations. The harvested microalgal biomass after wastewater treatment has numerous functions in biopolymer, pigments; Biofertilizer, aqua and animal feed, bioplastics; biofuels, and other co-products formation rely upon the urgency of the location where this technique is used [103, 104]. A microalga can transform from autotrophic to heterotrophic mode to assimilate the organic compound, depending upon the cultivation conditions [105]. The assimilation of carbon dioxide is ten times higher than other land plants photoautotrophically/ mixotrophically in microalgae, making them a satisfactory bidder for reducing greenhouse gases and biofuels generation [106].

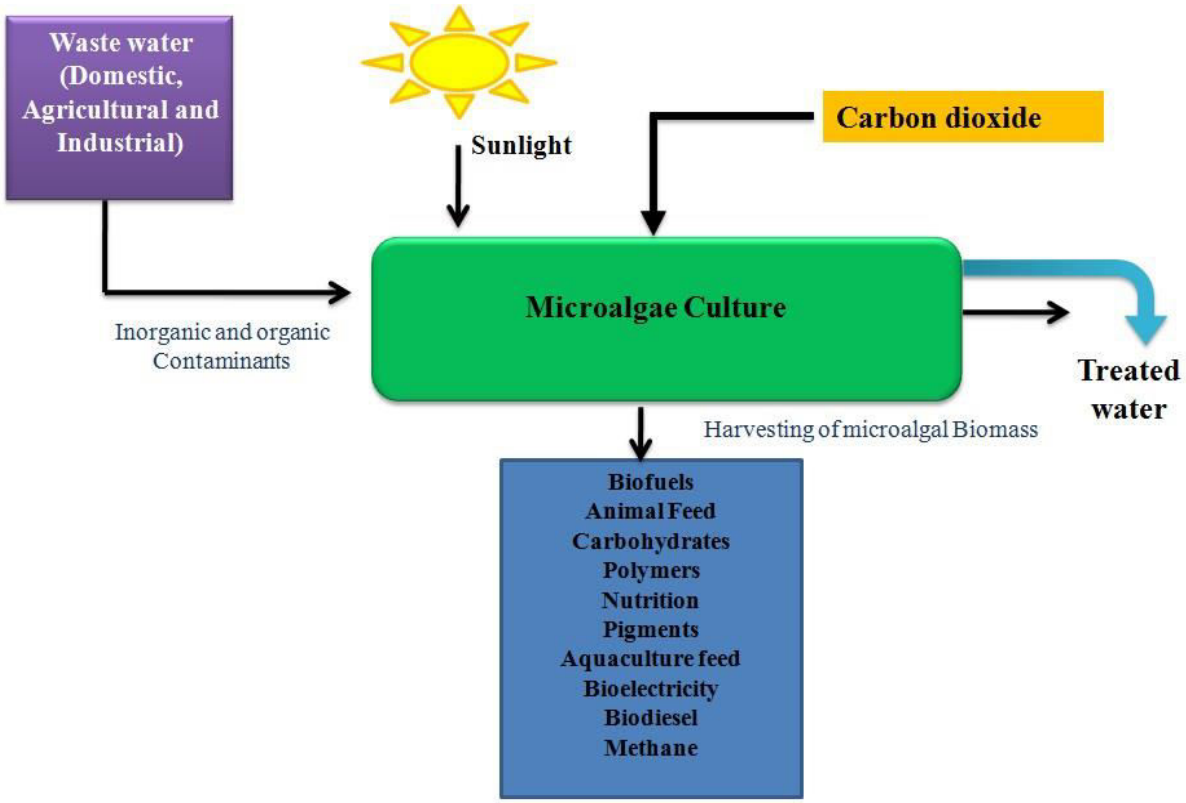

Figure 1.2: Schematic representation of microalgae in waste water treatment and applications of microalgal biomass

Currently, microalgae have become the uttermost approach in wastewater treatment, proposed easy, promising, and economical technology compared to other substitute biological methods

[107]. In the current period, the researcher examines the favorable application of phycoremediation to treat agriculture wastewater [108], distillery wastewater [109], pharmaceutical wastewater [110], municipal wastewater [111], textile wastewater [112], biodiesel wastewater [113], dairy wastewater [114], tannery wastewater [115], aquaculture wastewater [116], crude oil wastewater [117], effluent from coal gasification and bioethanol industry [118, 119] and piggery wastewater [120]. The microalgae constituents in the wastewater process are affected by different variables; these cause changes in richness, composition, and diversification in microalgal species. The structural changes in microalgae correlated to fluctuation in environmental conditions, wastewater components, photobioreactor structure, optimal conditions, and species interaction. These interactions and competition subsidize the increase in microalgal biomass, nutrients eradication, heavy metal removal [121, 122]. The incidental discharge of toxins from wastewater into the ecosystem originates the environmental and safety problems, health issues to the community. The microalgae are exploited for the detoxification of xenobiotics and heavy metals. Their potency regulates the preference of microalgal strain in wastewater treatment against wastewater and the adaptability of microalgae to grow in wastewater and take up the nutrients. The microalgae are *Chroococcus*, *Chlorella*, *Scenedesmus*, *Lyngbya*, *Gloeocapsa*, *Synechoccystis*, *Spirulina*, *Anabae na*, and *Oscillatoria* used in wastewater treatment [93, 123].

Microalgae cultivation in wastewater offers a couple of outputs such as treatment, greenhouse gas mitigation, and microalgal biomass reuses. The biomass is utilized as food and protein supplements (animal and plant), pharmaceuticals, bio ore for heavy metals, carbohydrates (bioethanol and sugar), cosmetics, and bioenergy (biofuels and biogas), and other product formation [124, 125]. The large surface area to volume ratio, heteropolysaccharides (carboxylic acid+ sulfate groups), proteins, and lipids in the cell wall provide multiple binding sites on the surface of a microalgal cell that assist the heavy metal sequestration in water. The mechanism of bioaccumulation and biosorption inside the microalgae endeavors to eliminate heavy metals from wastewater [126, 127].

The microalgal species consist of numerous strategies in favor of heavy metal toxicity, including extracellular degradation, accumulation, biosorption, and intracellular degradation. The cell wall of microalgae has multiple functional groups (phosphate, amino, thiol, carboxyl, sulfonate,

hydroxyl and imidazole) impart negative charge and attached with metal ions (anionic and cationic charged heavy metals) and contributed to biosorption [128–130]. The adsorption takes place at the microalgae cell wall with cation-charged metal ions and accumulation inside the cytoplasm of the microalgal cell. The metals ions actively transported into the cytoplasm with membrane proteins and transporters [131–133].

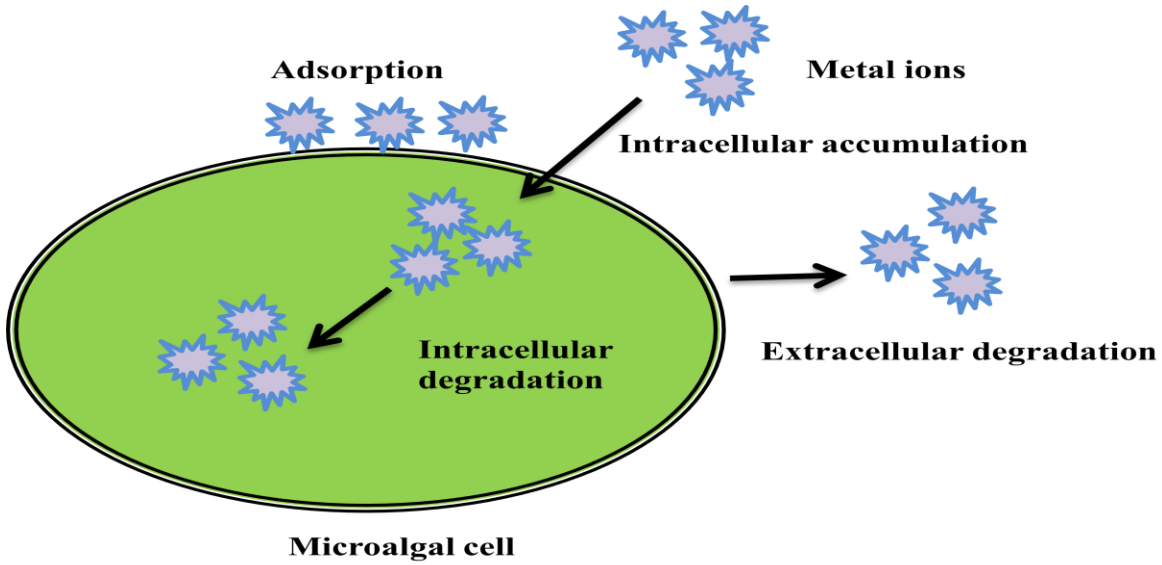

Figure 1.3: Mechanism uptakes by microalgae to remove heavy metals and other contaminants [131–133]

The phycoremediation process specifically draws attention by resolving many problems in a specific site. The process of phycoremediation has ample benefits over another process of bioremediation that build the most suitable technique and their advantages [97, 134–141] listed as

- Secure, economic, non-hazardous, and environment-friendly process of remediation of various types of wastewater.
- Expeditious growth of microalgae doubles the biomass in short generation time result in high content of lipids (20-50%) of its dry cell weight.
- Convert the sun energy and carbon dioxide emission from the power plant.
- The process of phycoremediation productively decreases the number of nutrients and further lowers the level of dissolved solids.
- Minimum operation and maintenance cost.

- Microalgae act as the non-pathogenic strain of microorganisms and do not form any lethal substances.
- The standard physico-chemical methods formed sludge as a consequence of treating the waste while phycoremediation detox, less odor formation, or permanently removing the sludge.
- The higher photosynthetic activity of microalgae than other plants enhances the dissolved oxygen level and reduces the chemical oxygen demand (COD) and biological oxygen demand (BOD).
- Microalgae have the ability for nutrients (N and P) and energy resumption, and their biomass is highly nutritive used as feed and biofertilizer.
- Treatment of wastewater with microalgae and simultaneously generation of bioelectricity and biofuels.
- Easily tolerate severe conditions and can reduce the greenhouse gases discharge.
- Microalgal-based biosensor discovers the hazardous compounds in wastewater and removes the heavy metals and degrades the xenobiotics compounds.
- Modulations in growth conditions of microalgae alter the biochemical composition result in a high yield of oil.
- The integrated function of microalgae in wastewater treatment, biohydrogen and biofuels production, and carbon dioxide fixation.

The microalgae culture system has typical features such as simple operational processes, proper light conditions, efficient material transfer, high area efficiency, less contamination rate, and a low-cost method. The great instrument and techniques have been upgraded in recent years to incline microalgae production [142]. The microalgae can be grown in the open, closed (photobioreactor), and composite (hybrid) conditions [143, 144]. The open pond involves (raceway pond), tanks, and closed controlled conditions includes different types of bioreactors. The fundamentals and design of the cultivation system are changed over time on their definite [145]. Numerous aspects influence the culturing of microalgae, such as pH, salinity, temperature, light, CO_2, oxygen, inorganic carbon, nutrients (nitrogen, phosphorus, potassium), and agitation for proper mixing [146].

The microalgae evolve as the most promising microorganism in wastewater treatment and further biomass utilization in producing other co-products. In recent times so much progress in

phycology implicates the advancement in microalgal species through the cooperation of genetic engineering and material science, which contributed to wastewater treatment and biofuels production from that waste. The microalgal characterization and its strains selection improvement exploit different techniques, proteomics, genomics, metabolic, and lipidomics to obtain desirable traits for wastewater treatment, lipid synthesis, high growth and tolerance in various environments, and formation of value-added products [97].

1.5 Utilization of wastes as a microalgae media and its growth dynamic studies

The growth of microalgae required various types of constituents comparable to another microorganism. The elementary composition of basic microalgae medium consists of nitrogen, phosphorus, calcium, potassium, vitamins, and trace elements [147]. There are immense numbers of mediums proclaimed for culturing microalgae. Biomass growth is regulated through the nature of cultivation media, light conditions, nutrient contents, temperature, and pH [148]. Selection of feasible nutrient medium relies upon the chemical constitution for growth and extension of microalgal products [149–151]. The medium composition strictly controls the microalgal growth and its cell concentration. The supply of enough nutrients in the cultivation medium helps enrich biomass and the development of microalgae. Carbon source is an essential factor in media provided in organic form as peptone, acetic acid, and inorganic from carbon dioxide. The nitrogen and phosphorus source is supplemented in nitrate and orthophosphate form, respectively. Other constituents include sodium, magnesium, calcium, selenium, potassium, iron and copper, boron, manganese, molybdenum, and zinc as trace elements. The de-ionized, distilled, filtered, and lately, household water can be used in the preparation of culturing media in small as well as in large-scale growth of microalgae [145, 152–155].

There is plenty of cultivation media for isolation, preservation, and biomass growth of microalgal species. For examples (1) modified Bold Basal Medium for freshwater microalgae (BBM) (modified from [156] (2) BG-11 Medium for cyanobacteria [157, 158] (3) Shihira-Ishikawa Kase (S-IK) Medium for heterotrophic cultivation of microalgae [159] (4) modified CFTRI Medium for *Spirulina* [160] (5) CHU Medium for *Cladophora, Coronastrum* and *Nostoc* mainly [161] (6) modified Hoagland's Medium [162] (7) modified Guillard's f/2 Medium for coastal marine microalgae [162] (8) modified Zarrouk Medium (MZM) for *platensis* algae [163] (9) Bristol Media for freshwater algal culture [162]. These media constituents are generally

retrieved from the inorganic fertilizers, which consider as unsustainable measures for cultivation. For large-scale production of microalgae and their biochemical components requires highly-priced nutrients and a large volume of freshwater. So, there is the urgency of substitute media which is economically feasible, presence of relatable nutrients for microalgae cultivation and biomass production [164–166]. Hence, plenty of research on microalgae cultivation in various types of wastewater, municipal waste, food waste, and agriculture waste [167–170].

The presence of carbon, nitrogen, phosphorus and other contents in agriculture waste is exploited to cultivate microalgae. Sugarcane bagasse, oil crop, rice straw, and sweet sorghum are used as a nutrient source for efficient microalgal biomass production in agri waste [171–174]. The soybean and refuse waste act as alternate cultivation mediums for microalgal growth and production of feed-grade protein [175]. The agriculture fertilizer medium can enhance microalgal growth and oil content in the open pond; microalgae can efficiently grow in any wastewater [167]. There is an enormous amount of food waste generation worldwide. The food wastes mainly consist of proteins, starch, and lipids from cereal, meat products, and fruits and vegetables. Recycling this wasted food as a cheap nutrient source is a prominent substrate for waste treatment approaches and, subsequently, microalgal biomass production for metabolites formations [169, 176]. The food waste serves as an equitable organic substrate for microalgae cultivation and lowers the overall cultivation cost. The food waste is disposed of by different methods such as landfill, anaerobic digestion, and incineration, reducing the resumption of nutrients and other compounds and generating environmental and health problems [177–179]. Therefore, depreciating the cost of microalgae production and developing a sustainable process could be mandatory to use biomass for co-metabolites further output. Food waste and agriculture compost can be the probable solution to fulfill the glitch of synthetic medium for microalgae cultivation. The organic content (N, P, K, and carbon) present in the food waste and agriculture compost supports the luxurious growth of microalgae and reduces the cost of the synthetic medium. These two organic wastes have been reserved as alternate nutritive mediums for microalgae production to a greater extent and simultaneously eliminate waste contaminates and form other valuable products. The capability of various types of algal species belongs to green, diatoms, cyanobacteria, and yellowish-green cultivated in the organic substrate in the absence of carbon dioxide in light and dark conditions [180–183].

The growth of microalgae mainly depends on the types of medium, the concentration of nutrients, and other factors such as light, temperature, and pH. The microalgae growth curve consists of three phases – (1) lag phase (induction), (2) log phase (exponential), (3) linear phase, (4) stationary phase, (5) decline phase (death) [184, 185]. Many mathematical models were proposed based on microbial growth studies to explain the dynamics of biomass with time. In most cases, J Monod's growth model and its variants serve as a basis that relies on specific growth dependence on a limiting substrate concentration [186]. The J Monod model suffers from the ambiguity of having a physical sense of the parameters mainly falls under logistic curves with the characterization of all microbial growth phases (substrate utilization-based). The generated model data won't have any broad practical applicability in biotechnological domains yet. Volterra (for growth associated) and Leudeking-Piret (for metabolite biosynthesis) models are some of the extensions for J Monod models, which were also not practiced well due to the negligence of factors influencing the cell growth, such as mass oxygen exchange [187].

The models of biomass growth are divided into either the structured one or the unstructured one. The structured models explain the fate of biomass growth with more than one substrate concerning the time. In contrast, the simpler unstructured models rely on the homogenous population with only one substrate. In both the structured and unstructured growth models, different equations will be drawn based on the consumption pattern of the substrate towards the incurred biomass by neglecting the influential factor of mass oxygen exchange which is primitive for the aerobic process [188]. Derbyshev et al. reported that the additional feeding arrangement with the balanced substrate medium concentrate helps in the situations of lack of substrates and growth inhibition due to high amount of substrates which facilitates a uniform oxygen mass exchange rate in the system, which in turn switches the system towards "zero" oxygen concentration [189]. The importance of dissolved oxygen concentration during GIP (growth inhibition phase) was also reported in Salmonella sp., where a constant specific rate was observed in the accumulation of proliferating cells, which seems to be equal to the presence of non-proliferating cells [190]. Hence, various structured models came into existence to explain the substrate consumption as a part of microbial growth based on the preliminary calculated parameters of designed unstructured models.

The proposed and presently utilizing structured model (Integrated Mathematical Model of Development, IMMD) by [191] assumes two groups of cells in the microbial growth population

with differed physiological conditions. Group I cells represent the young (newly generated, resting, zero-age) cells) and Group II cells represent the actively proliferating cells [186]. The group I cells are in G1 or the V phase as designated for prokaryotes/eukaryotes and exhibits minimal constant physiological functions [187]. The group I cells consume energy substrates as a part of the viability maintenance [190]. The proposed theory serves as a basis for the structured framing model for microbial growth dynamics by taking "mass oxygen exchange" as primary importance in the microbial population's energy consumption and cell viability [191]. The applicability of the proposed model was well acknowledged in explaining the microbial growth dynamics of obligate aerobes/anaerobes [192–194], and halobacterium [195].

1.6 Toxicity screening of microalgae towards phycoremediation

The intended and coincidental discharge of hazardous contaminants strictly intimidated the ecological community. These toxic contaminants influence and modify the aquatic microorganism balance that has an unfavorable impact on the action and structure of the entire biome. The small amount of toxicant probably leads to excellent interpretation in algae, which create microalgal toxicity test crucial for the environmental risk evaluation. This toxicity assessment determines the effects of various substances on microalgae growth [196, 197]. The regulated concentration of metal ions and their complexes have been buffered in media to examine the toxicity by checking the growth of algae [198]. The microalgae strains differ extensively in chemical contents, tolerance to toxic elements, nutrient restoration, and adaptation of climate variation. The abundance of microalgae has been assessed to treat wastewater, biofuels and other product formation, and carbon dioxide fixation [199]. The dark color and higher turbidity of wastewater (media) interrupted the light penetration and affected microalgae's growth. The higher concentration of nutrients in wastewater may also retain the risk of toxicity inhibition. Various studies estimated the practicability of microalgae cultivation utilizing the toxic character of sewage. The origin and concentration of wastewater have been predominant factors for the growth of every microalgae species. The distinctive strains of microalgae counter disparately to the exact nature of wastewater. The selection of favorable species of microalgae is essential for that category of wastewater. The cultivation mode of microalgae such as batch and fed-batch can be efficient to intensify the tolerance for huge wastewater concentrations [200–203].

The toxicity screening of two microalgal species has been evaluated in diluted wastewater with a standard medium to assess the suitable microalgal species by taking its cell count for growth studies. As much higher the cell counts of the algal cell, it forms the higher microalgal biomass. This biomass efficiently removes the waste contaminants present in the wastewater and simultaneously produces the other products that have application in scale-up studies. The purpose of toxicity assessment is to select the best microalgal strain from many species and its growth rate in a particular medium (any wastewater containing heavy metals, pharmaceuticals, and other contaminants diluted with fresh/marine water or standard growth medium).

The microalgal species and growth conditions affect the growth rate and microalgal production and simultaneously removal of heavy metals from the wastewater. The microalgal species are commonly exposed to the immense concentration of heavy metals for a long duration in contaminated water. In the remediation process, the survival of microalgae in a toxic environment defined the capability of algal species. To enhancement the tolerance level of microalgae in different types of heavy metals and magnify the capability of microalgae in bioremediation, it is essential to know the behavior of microalgae towards the heavy metals [204, 205]. The screening of microalgal strain from different habitats and its cultivation medium composition and condition play a significant aspect in biomass production and further applications in lipids formation that can be exploited for biodiesel production [206]. Various microalgae species screened to check the potential of cadmium removal in cultivation medium consist of different cadmium concentrations. The microalgae can grow in a cadmium stress medium and uptake those cadmium ions on its surface or intracellular accumulation [207]. The outdoor cultivation of microalgae added a new feature in the wastewater treatment and tolerance of microalgae in wastewater conditions and efficient removal of nutrients and growth of microalgae in it [208].

The screening procedure of different microalgae is an essential step to check the growth of microalgal sp. in various wastewaters. The primary screening concludes that this suitable microalga grows best in this dilution of wastewater, and it has the ability for bioremediation [112]. The growth of microalgae in a mixture of standard medium and industrial wastewater was assessed for lipids content and biomass production. The microalgal strain grows in dilution of both (synthetic media and industrial wastewater) in various ratios to analyze the suitable ratio for

its growth and efficient removal of nutrients from wastewater [209]. The co-culturing of suitable microalgae in different concentrations of wastewater and synthetic medium has an application to scrutinize the ability of microalgal strain [210]. The toxicity assessment of heavy metals has a significant aspect in that a high concentration of zinc in swine wastewater might hinder microalgae growth.

As a consequence, it influences nutrient removal in wastewater. So it becomes crucial to determine the toxicity evaluation of various zinc concentrations upon microalgal growth [211]. The toxic test sensitivity relies upon the initial microalgal cell inoculum; higher the initial algal density encompasses more surface metal ligands feasible leads to less toxic response on the microalgal cell through metal. Further experimental information illustrates that toxicity actively depends on the free metal ions instead of overall metal concentration [95, 212–214].

1.7 Phycoremediation of industrial effluents

The acceleration in population, urbanization, and industrialization discharge a tremendous amount of hazardous waste into the water bodies. These organic wastes are added to the environment through agriculture, industrial waste release, and other waste discharge. The configuration of wastewater reflects lifestyle and techniques proceeding in the modern community [215, 216]. The discharge of an enormous amount of effluent to water streams substantially negatively impacts the natural water source and ecosystem. The inorganic and organic contaminant from industrial, agriculture, and domestic wastewater leads to environmental pollution [126, 217]. Microalgae propose an efficient and economical way to remove the waste contaminates from different sources and produce valuable products. Microalgae recognized as green cell factories are favorable in eradicating hazardous waste but also implicate in carbon dioxide sequestration and aerate the atmosphere, thereby making them excellent competitors among the bioremediation process [218–220].

Microalgae have broad applications, from easy cultivation to nutrients and metals removal and biomass utilization for various product formations. Large volumes of wastewater are generated from textile, oil mill, food, steel and iron, nuclear, brewery, paper and pulp, dairy, and poultry industries. Every industry has its method of effluents treatment to reduce the chemical oxygen demand and other parameters to its permissible limits set by authorities [221–226]. The industrial

process generates a large variety of the waste that is unacceptable beyond its environmental limits. Microalgae acclimate easily and grow autotrophically, heterotrophically, and mixotrophically in any environment. The microalgae effectively deteriorate and accumulate pollutants from hydrocarbons, phenolics, heavy metals, biphenyls, and pesticides from the waste [227]. Various types of green microalgae such as Scenedesmus sp. or Desmodesmus sp. [105, 159,228–230], Chlorella sp. [231], *B.braunii* [232], Chlamydomonas sp. [233] and cyanobacteria have applicability to treat the wastewater and couples with biofuels production. These microalgae tolerate the toxic environment, have a higher growth rate, accumulate lipids and starch, and efficiently remove phosphate, nitrogen, and COD [234–236]. The synthetic dyes such as malachite green, methylene blue, acid orange 7, disperse blue 2BLN, acid black 210 dye used in paper-pulp, food, cosmetics, pharmaceutical, and textile industries. Few dyes have a probability of carcinogenicity, mutagenicity, and toxicity to living organisms in the ecosystem. Among all the promising methods, the microalgae Desmodesmus sp., *Chlorella sorokiniana*, *Spirulina platensis*, and *Chlorella Vulgaris* gained appreciable significance in degrading decolorize the synthetic dye effluent [237–244].

The green microalgae *Scenedesmus dimorphus*, Chlorella sp., and *Scenedesmus obliquus* eliminate the toxic contaminants from the urban, brewery, poultry, cattle breeding, swine, and dairy wastewater. They effectively evacuate the organic carbon, nitrogen, and phosphorus and significant microalgae growth [245–249]. Microalgae cultivation through raceway pond and photobioreactor allowed an appropriate amount of light penetration and carbon dioxide sparging in wastewater treatment and growth of microalgae [250, 251]. Most microalgae species conformed to flourish in industrial wastewater efficiently. Reduction in the overall cultivation cost of microalgae by considering wastewater to the culture of nutrient-rich microalgae [252, 253]. Industrial wastewater consists of organic chemicals contaminating hydrocarbons, biocide, surfactants, and heavy metals such as chromium, copper, zinc, and other metal ions [254]. Discharge hazardous heavy metal ions from textile, tannery, distillery, electroplating, leather, metal processing units, and other industries. The tolerable amount of metals ions, nitrogen, and phosphorus in industrial wastewater supports microalgae's luxuriant growth and its applicability toward accumulating the metal ions [215, 249]. The wastewater discharge from various industries has a higher amount of total suspended solids (TSS), biological oxygen demand (BOD), and chemical oxygen demand (COD). The selection of efficient

microalgal strain for remediation depends on the type of wastewater and its composition. The microalgal species adequately grow in untreated industrial wastewater, such as *Chlorella saccharophila*, *Pleurochrysiscarterae* and *B. braunii* [231, 235, 249] Household waste consists of a mixture of toxicants from personal care products, endocrine disrupting compounds, pharmaceuticals, nutrients, and heavy metals, and its treatment through the conventional process has limited. The freshwater microalgae *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, *Chlorella pyrenoidosa,* and *Scenedesmus obliquus* have been employed for the removal of organic contaminants, nitrogen, phosphorus, and metals and further its biomass use for biohydrogen and other products formation [139, 255–257]. The pharmaceutical waste consists of active pharmaceutical ingredients (API), catalysts, intermediates, solvents, and other raw materials. These have destructive effects such as carcinogenic, mutagenic, and adversely affect the water and area where they dispose of these materials [258, 259]. The pharmaceutical traces are also detected in the wastewater treatment plants, and these cause detrimental effects to be aquatic and human life [260, 261]. The mechanism of removal of pharmaceutical contaminants by microalgae is well reported in the literature. Microalgae have tremendous approach to degrading the effluents from pharmaceutical industries [262, 263]. The pharmaceutical contaminants constitute Cefradine, ibuprofen, 17a-Ethynylestradiol, Estradiol, ampicillin, paracetamol, norgestrel, trimethoprim, diclofenac, levofloxacin, ciprofloxacin, gentamycin, sulfamethoxazole, and others [132, 264, 265]. Various strains of microalgae *Chlorella pyrenoidosa*, *Scendesmus obliquus*, *Chlamydomonas mexicana*, *Desmodesmus subspicatus*, diatom, Navicula sp., *Chlorella vulgaris*, *Dunaliella salina*, Nannochloris sp., *Pseudokirchneriella subcapitata*, *Selenastrum capricornutum* utilizes for the elimination of pharmaceutical contaminants [132, 233, 255, 266–269].

The microalgae bioremediation evolved as one of the most appealing technology for remediation of waste, carbon capturing techniques, and its utilization for biomass and valuable output. The acid mine drainage is highly acidic, consists of many heavy metals, and creates hurdles to the environment due to its leaching property. Microalgae species *Scendesmus, Chlorella* sp., *Oscillatoria, Phaeodactylum tricornutum,* Spirulina sp., *Anabaena* and *Cladophora* exhibited the excellent removal efficiency of heavy metals from acid mine drainage [270]. Industrial activities such as mining, petroleum refining, agricultural and chemical production originate the pollution with hydrocarbons, heavy metals, particulates, salts, etc. Crude oil extraction and transportation

from the sea affect marine life, and its complex hydrocarbons compounds lead to severe environmental trouble. The polycyclic aromatic hydrocarbons (PAHs) are a cluster of chemicals constituted with angular or cluster arrangement and fused aromatic rings. PAHs are imperative components of petroleum as their stable and recalcitrant nature form them as carcinogenic and threatening to the environment. Microalgal strains *Scenedesmus quadricauda*, *Anabaena oryzae*, *Chlorella vulgaris*, *Raphidocolis captricornutum*, *Scenedesmus platydiscus*, *Chlorella kessleri*, and *Selenastrum capricornutum* have been tested for PAHs and oil spillage removal in autotrophic and mixotrophic cultivations [271–274].

The agro-based industries involve diary, tannery, piggery, cassava mill, edible oil refinery, meat-processing, slaughterhouse, distillery, and food processing industry releases high strength wastewater which has, higher chemical oxygen demand, pH, suspended solids, color, turbidity, and nutrients (N and P) [200, 275–278]. The usages of industrial wastewater for microalgae cultivation reduce the water and nutrients problems and further biomass utilization for biofuels and biodiesel formation. Microalgae species *Chlorella vulgaris, Chlorella pyrenoidosa, Desmodesmus subspicatus,* Scendesmus sp., *Chlamydomonas reinhardtii*, *Neochloris oleoabundans*, *Chlorella sorokiniana*, *Scendesmus obliquus*, *Scendesmus abundans*, *Arthrospira maxima*, Desmodesmus sp. have been used for the treatment of raw and anaerobic digested industrial wastewater and sustainable biomass generation [93, 200, 275, 279–286].

The wastewater released from different water bodies such as municipal, mining waste, metal and ore processing, industrial waste, and solid waste has small to high concentrations of heavy metals [287, 288]. The existence of these toxic heavy metals affects the environment and is hazardous for human and aquatic life. Microalgae have established a considerable mechanism (intracellular and extracellular) to endure heavy metal toxicity. Heavy metals such as zinc, aluminum, copper, cadmium, and iron accumulated inside the *Chlorella vulgaris*, *Euglena acus*, *Phacus curvicauda*, *Oscillatoria bornettia* [289], *Scenedesmus acuminatus*, *Chlorella sorokiniana* [204]. In the past few years, there has been lots of research conducted on the application of microalgae, macroalgae, and cyanobacteria to remove heavy metals and other metal ions from various categories of wastewater. The removal of heavy metals K, Mg, Al, Ni, Fe, Ca, Se, As, Sr, Ag, V, Zn, Co, Hg, Mn, Cu, Pb, Mo, and Cd through various strains of microalgae such as *Chlorococcum spp.*, *Chlorella sorokiniana*, Desmodesmus sp., *Cyclotella cryptica*, *C.*

vulgaris, *Spirogyra spp.*, *Stigeoclonium tenue*, *Chlorella miniata*, *Chlorella pyrenoidosa*, *Desmodesmus pleiomorphus*, *Pseudokirchneriella subcapitata*, *Chlamydomonas reinhardtii*, *Coccomyxa actinabiotis*, *Stichococcus bacillaris*, Dunaliella sp., *Lyngbya taylorii*, *Scenedesmus obliquus*, *Scenedesmus abundans*, *Phaeodactylum tricornutum*, *Anabaena subcylindrica*, *Nostoc muscorum*, *Scenedesmus subspicatus*, *Porphyridium purpureum*, *Spirulina platensis*, *Anabaena* and *Scenedesmus quadricauda* [198, 290–299].

The microalgal-based treatment was established as more productive for contaminants removal and uptake nutrients and heavy metals from wastewater than other physico-chemical treatments. The significant pilot-scale analysis fills the void between lab scales to commercial-scale applications [300]. In the current time, there is statistically more research conducted on the industrial wastewater treatment by microalgae such as textile effluent, fish-farm wastewater, sugar mill wastewater, carpet mill effluent, paper-pulp industry wastewater, electroplating wastewater, petroleum wastewater, coal-fired metal-contaminated effluent, and pharmaceutical effluent. Photo bioreactors, High rated algal ponds, and oxidation ponds engaged as cultivation of microalgae in industrial and municipal wastewater and simultaneously biomass production [235, 244, 301]. Even though there are innumerable findings on the remediation of industrial effluents with microalgae, the microalgae *D. armatus* and *S. abundans* showed promising results in bioremediation studies with tolerance capability of effluent chemical constituents [302-306]. Although, many remediation strategies were in practice for industrial effluents, the microalgae-based phycoremediation emerged as an efficient and economical remediation approach with the concomitant biocommedities production.

Objectives

- Analysis of X-ray waste effluents and screening of microalgal strain towards bioremediation
- Food Waste and agriculture compost as media for *D. armatus*: Bioprocess dynamic study.
- Bioremediation studies of X-ray developer solution by *D, armatus* using BBM.
- Bioremediation studies of X-ray developer solution by *D. armatus* using food waste and agri-compost media.

CHAPTER 2

Materials and Methods

CHAPTER 2

Materials and Methods

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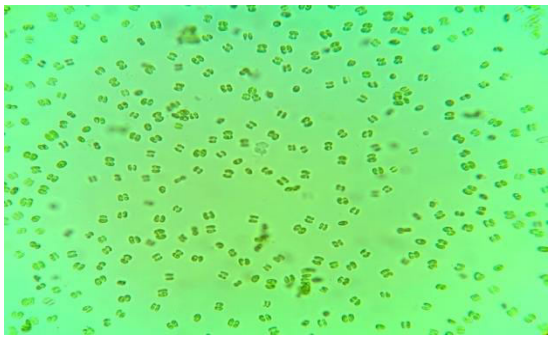
2.1 Microalgae collection and classification

The fresh water microalgae *Desmodesmus armatus* and *Scendesmus abundans* were obtained from the National Collection of Industrial Microorganism (NCIM) Pune. The agar slant was stored at 4 °C till sub-culturing of microalgae.



(a) *Desmodesmus armatus*

(b) *Scendesmus abundans*

Figure 2.1: Microscopic visualization of (a) *Desmodesmus armatus* and (b) *Scendesmus abundans* under light microscope

Taxonomic Classification:

Empire- Eukaryota
Kingdom- Plantae
Subkingdom- Viridiplantae
Phylum- Chlorophyta
Class- Chlorophyceae
Order- Sphaeropleales
Family- Scenedesmaceae
Genus- *Desmodesmus*
Species- *armatus*

Empire- Eukaryota
Kingdom- Plantae
Subkingdom- Viridiplantae
Phylum- Chlorophyta
Class- Chlorophyceae
Order- Sphaeropleales
Family- Scenedesmaceae
Genus- *Scendesmus*
Species- *abundans*

2.2 Bold Basal Medium (BBM) Composition [1]

Table 2.1: Components of bold basal medium (BBM)

Components (BBM media)	**(Stock solutions) grams (g) for 100 ml of distilled water**	**Working Solutions (In** DW)
$NaNO_3$	2.5g	10ml of stock solution/l (DW)
$CaCl_2.2H_2O$	0.25g	10ml/l
$MgSO_4.7H_2O$	0.75g	
NaCl	0.25g	
K_2HPO_4	0.75g	10ml/l
KH_2PO_4	1.75g	
EDTA	5g	1ml/l
KOH	3.1g	
$FeSO_4.7H_2O$	0.498g	1ml/l
H_2SO_4	0.1ml	
H_3BO_3	1.142g	1ml/l
Micronutrients		
$ZnSO_4.7H_2O$	0.882g	1ml/l
$MnCl_2.4H_2O$	0.144g	
$MO.O_3$	0.071g	
$CuSO_4.5H_2O$	0.157g	
$CO(NO_3)_2.6H_2O$	0.049g	

A working solution of the basal medium was added to the respective volume of distilled water and adjusted its pH at 6.6 through 0.1 N HCL and 0.1 N NaOH solutions by pH meter after that solution was sterilized in an autoclave at 121 °C , 15 psi for 20 mins.

2.3 Chemical reagents

All the chemicals and other reagents (AR grade) used in this study were procured from Sigma-Aldrich/Merck, HI media, and SRL. The other analytical standards were purchased from Sigma-USA for the experimentation.

2.4 Sub-culturing of microalgae and its growth conditions

The microalgae *D. armatus* and *S. abundans* are sub-cultured in 100 ml of bold's basal medium (BBM) [1] in 250 ml Erlenmeyer flasks. After sub-culturing, the flasks were incubated at 25±1 ^{o}C temperature and 100 rpm shaking conditions in an orbital shaker. The photosynthetic light of 1400-1500 lux intensity (12h dark: 12 h light) was also subjected to an orbital shaker for one-month microalgae growth.

Figure 2.2: Sub-culturing of microalgae in BBM and other cultivation conditions

2.5 Determination of microalgae cell growth

2.5.1 Optical density at 680nm

The microalgae cell growth of *D. armatus* and *S. abundans* was determined by measuring optical density at wavelength 680 nm in UV-VIS spectrophotometer for one month.

2.5.2 Cell count

The cell count of microalgae *D. armatus* and *S. abundans* was observed in the Neubauer hemocytometer chamber under a light microscope. The cell count was calculated in ($\times 10^4$/ml) for one month of cultivation.

2.5.3 Dry cell weight (DCW)

The DCW of microalgae culture was measured by weighing the empty Eppendorf in a weighing balance. After that, 1 ml of microalgae was introduced in pre-weighed Eppendorf and then centrifuged at 5000 rpm for 15 mins. Pellet was subjected to drying in a hot air oven, and cleared supernatant was removed. The dried pellet is again weighed in a weighing balance and calculates DCW in mg/ml.

2.6 Waste radiographic solutions collection and storage

The waste radiographic solutions (X-ray developer and X-ray fixer solution) were collected in plastic cans from the private X-ray lab, Shimla. The samples were stored at 4 ^{o}C in the refrigerator for further use.

(a) Waste X-ray developer solution **(b) Waste X-ray fixer solution**

Figure 2.3: Collected radiographic developer and fixer solutions

2.7 Characterization of waste radiographic solutions

The waste X-ray developer and fixer solutions were characterized for study its physicochemical parameters such as pH, Total solids (TS), Total suspended solids (TSS), Total dissolved solids (TDS), Total volatile solids (TVS), Total fixed solids (TFS) done according to APHA, 2005 standard methods [2]. The Density and Conductivity were estimated through density meter and conductometer, respectively.

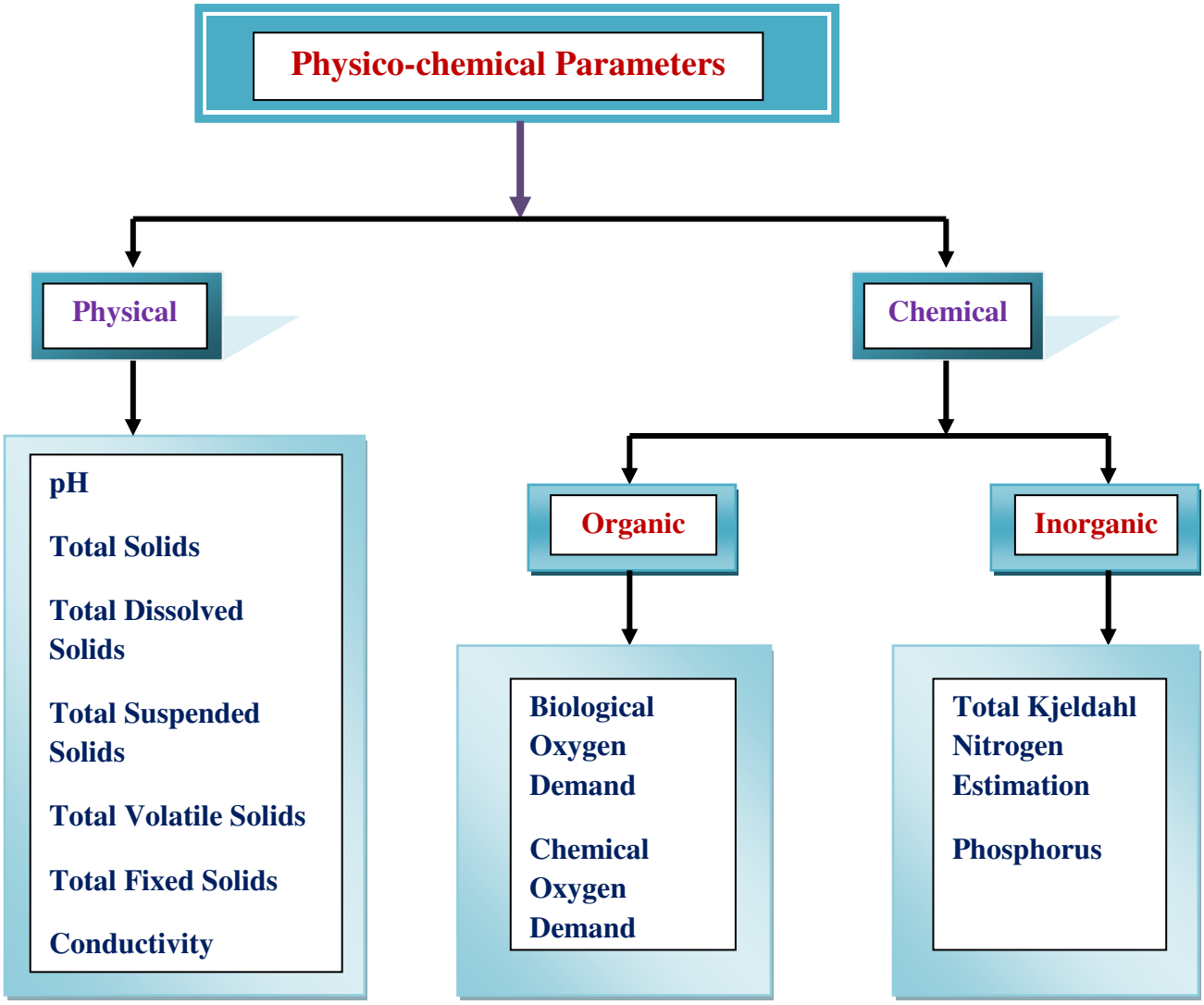


Figure 2.4: Simplified depiction of characterization of waste radiographic solutions parameters

The inorganic content of waste radiographic solutions Totals Kjeldahl nitrogen (TKN) and Phosphorus estimated through methods given by Jackson, 1958 [3] and Fiske and Subbarow, 1925 [4], respectively. The organic content Biological oxygen demand (BOD) and Chemical oxygen demand (COD) are determined according to APHA, 2005 standard protocols [2]. All the experimentation was done in triplicates.

2.7.1 pH determination

The pH of waste X-ray developer and fixer solutions was measured through a digital pH meter.

2.7.2 Total solids (TS)

Weighed the empty porcelain dish on the weighing balance and filled it with a known amount of both waste radiographic solutions in separate dishes; after that, heated the sample for more than 1-2 h at 105 ^{o}C in a hot air oven. After heating cooled the dishes in a desiccator and weighed the residues. The process was repeated until getting the constant weight of residues in porcelain dishes.

2.7.3 Total dissolved solids (TDS)

The known amount of waste X-ray developer and fixer solutions passed separately through the filter paper to the different pre-weighed porcelain dishes. These samples were heated in a hot air oven in >1 to 2 h at 105 ^{o}C temperature. Dried residues were cooled down in the desiccator and weighed. The procedure was repeated till obtained constant weight.

2.7.4 Total suspended solids (TSS)

The empty filter papers weighed on the analytical balance. Poured known amount of both waste radiographic solutions through the separate filter papers into dishes. These filter papers with residues are heated at 103 ^{o}C- 105 ^{o}C for 1 h in a hot air oven. Heated filter papers were cooled in a desiccator and then weighed. Repeat this process till getting the constant weight of filter papers [2].

2.7.5 Total volatile solids (TVS) and fixed solids (TFS)

Both waste radiographic solutions known amount added to the separate pre-weighed dishes; after this, these dishes were heated in a muffle furnace at 550 ^{o}C ± 50 ^{o}C temperature for 3 h. The solids that ignite at this temperature are volatile, and the remaining one is fixed solids. After that, heated dishes cooled in a desiccator and weighed in analytical balance again. The process repeated till attaining the constant weight of residues.

2.7.6 Biological oxygen demand (BOD) and chemical oxygen demand (COD)

The biological oxygen demand of both waste radiographic solutions was determined by Dissolved Oxygen (DO) Meter ((Model: HQ30D Portable, Hach, USA) in initial and after 5 d (DO5) of incubation at 20 ^{o}C [2].

The chemical oxygen demand of waste radiographic solutions was analyzed by adding a strong oxidant under acidic conditions. For the COD testing, 10 ml of 0.25N potassium dichromate ($K_2Cr_2O_7$) was added to the 20 ml of waste sample and also have 30 ml of silver sulfate (Ag_2SO_4) in sulfuric acid (H_2SO_4) as a catalyst and mercuric sulfate ($HgSO_4$) in reaction bottles. The reaction bottles were introduced into the pre-heated (120 ^{o}C) digested blocks for 2 h. After that, cooled the content of reaction bottles and relocated them into a 200 ml flask. The 80 ml of distilled water was added to the flask, and excess potassium dichromate was titrated through standard ferrous ammonium sulfate (FAS) with the help of 4-5 drops of ferroin indicator. The content color changed from yellow to red-brownish in the flask, and titration value was noted from the burette for calculations [2].

2.7.6.1 Preparation of Reagents

0.25 N $K_2Cr_2O_7$- 12.259 g of $K_2Cr_2O_7$ dissolved in 1000 ml of distilled water.

Sulfuric acid reagents**-** 10.12 g of silver sulfate dissolved per liter of H_2SO_4.

0.1 N FAS- 40 g of FAS dissolved in 40 ml of H_2SO_4 and 200 ml of distilled water initially, then diluted with distilled water to make final volume 1000 ml and mix properly.

2.7.7 Total Kjeldahl nitrogen (TKN) determination

The 10 ml sample (X-ray developer and fixer solution) were taken separately in a different micro-Kjeldahl flask and added total 3g mixture of copper sulfate ($CuSO_4$) and potassium sulfate (K_2SO_4) in 1:5 ratio and 10 ml sulphuric acid (H_2SO_4) in each flask. Digested the flask contents for 2 h at 100 ^oC till its color changed either light green or colorless in the fuming hood chamber. After digestion, the flask was transferred to Kjeldahl distillation equipment and titrated with 40% sodium hydroxide (NaOH) until its color changed to light brown. The sample was distilled for 5-10 mins and collected in 25 ml 4% boric acid in the flask. After that, 5-10 drops of Bromocresol green and Methyl red (1:1) followed by titration with 0.1N HCl from burette till its color becomes light pink. Note the concordant readings [3].

2.7.7.1 Preparation of Reagents

40% NaOH- 40 g of NaOH dissolved in 100 ml of distilled water.

4% Boric acid- 4 g of boric acid dissolved in 100 ml of distilled water.

2.7.8 Phosphorus estimation

The phosphorus was estimated in both waste radiographic solutions according to Fiske and Subbarow, 1925 [4]. The 1 ml of freshly prepared coloring reagent and 1 ml of waste samples were mixed, and OD (optical density) was taken at 750 nm. The coloring reagent has ferrous sulfate ($FeSO_4$), ammonium molybdate ($(NH_4)_2MoO_4$), sulphuric acid (H_2SO_4), and distilled water. The unknown phosphorus concentration was calculated in waste solutions through the standard curve of the known concentration of K_2HPO_4.

2.7.8.1 Preparation of coloring reagent

2.7 % ferrous sulphate (w/v), 1.5% ammonium molybdate (w/v), 5.5% sulphuric acid (v/v) was added in to distilled water.

2.7.9 Silver estimation

For silver quantification, primarily, both waste radiographic solutions (X-ray developer and fixer solutions) were diluted with distilled water separately in different beakers. The diluted waste

solutions were filtered through Whatman filter papers and followed by 0.2 µm filter paper. Both diluted samples were analyzed in atomic absorption spectrophotometer (AAS) (Perkin Elmer Analyst 400) and Inductive coupled plasma-mass spectrophotometry (ICP-MS) (Thermo Fisher Scientific, USA) and 1 % HNO_3 taken as blank for this estimation.

2.8 Screening of microalgae *D. armatus* and *S. abundans* in different concentrations of waste radiographic solutions diluted with standard BBM

Both freshwater microalgae screened for their toxicity in the various concentrations of X-ray developer and fixer solutions. The developer and fixer solutions were diluted separately with the standard basal medium in the ratio of 1:3, 2:2, 3:1, whereas BBM and developer and fixer solutions as control. The log phased *D. armatus* (15-day culture) inoculated in both diluted developer and fixer solution ratios concerning BBM and same as *S. abundans* inoculated in both solutions. The inoculated waste samples were kept in the orbital shaker with photosynthetic light 1400-1500 lux intensity of dark and light period (12h: 12h) at temperature 25±1 ^{o}C and 100 rpm shaking conditions for one month. The toxicity screening of microalgae *D. armatus* and *S. abundans* were checked in these waste radiographic samples by taking cell count ($\times 10^4$/ml) in Neubauer chamber after every three days of cultivation for one month. The best-screened microalgae and the waste radiographic solution (X-ray developer and fixer solutions) in which it grows adequately were used for further bioremediation experimentation.

2.9 Food waste and agriculture compost as alternative nutrient medium for *D. armatus* cultivation

2.9.1 Collection of food waste (FW) and agriculture compost (AC)

The mixed food waste (3 Kg) was collected from the mess and agri-compost (3 Kg) from Agriculture development Block (Sunder Nagar HP, India). The majority of the literature reported the normal concentration of nitrogen: 0-5% and phosphorus: 0-2% in mixed food waste. On the other side, the agri-compost medium has nitrogen: 0-2% and phosphorus: 0.5-1 % reported in the literature [5–9].

2.9.2 Preparation of food waste medium (FWM) and agriculture compost medium (ACM)

The collected food waste was kept for drying at 60 ^{o}C for one week. After that, food waste and agri-compost were powdered through mortar and pestle to coarse materials and sieve through the aid of sieve no. 25. The 50 g of food waste and agriculture compost were weighed separately and dissolved in 1000 ml of distilled water. Both waste media (FWM and ACM) were kept in the incubator shaker at room temperature and 150 rpm for at least 24 h. After that, filtering both mediums by Whatman filter paper to separate the large particles and autoclaved to remove other microbial contaminants [7]. Both food waste and agriculture compost medium used for the cultivation of microalgae. The different dilutions (2:2, 3:1, 1:3) of waste medium (FW / ACM) and BBM were utilized for microalgal cultivation by taking the individual media (FW, ACM, BBM) as controls. The growth of *D. armatus* in both diluted and control mediums were calculated by taking optical density at 680nm. The best growing ratio of both (FWM and ACM) media was further selected for the upcoming analysis of biochemical constituents.

2.9.3 *D. armatus* growth estimation

The growth of *D. armatus* in both media (FWM and ACM) and standard BBM and their various ratios was computed in UV-VIS spectrophotometer by taking optical density at 680nm. The dry cell weight assessment of *D. armatus* in FWM and ACM and standard BBM was done by weighing the empty Eppendorf in analytical balance. After that, one ml of microalgae *D. armatus* was introduced in previously weighed Eppendorf, and then centrifugation was done at 5000 rpm for 15 mins. Pellet was subjected to drying in a hot air oven, and cleared supernatant was removed. The dried pellet is again weighed in a weighing balance and calculates weight in mg/ml. The cell count of *D. armatus* in FWM, ACM and standard BBM was observed in the Neubauer hemocytometer chamber under the light microscope. The sample was collected separately from these mediums every 3-4 d, and cell count was calculated ($\times 10^4$/ml) for one month.

2.9.4 Biofabrication of microalgal growth kinetic study through "biofermentor" tool software

The growth dynamics study of *D. armatus* in different media (BBM, Food waste media and Agri-compost media) was performed through the "Fermentor" tool software (https://www.fermentertool.com/en). The input data for the software is the biomass concentration with time (d) by taking the minimum 8 biomass concentration data points (~4 data points from the exponential phase of the growth curve and ~4 data points from the slow phase of the growth curve) from the S-shaped growth of microalgae in different media [10].

The microalgal growth dynamics put forth the information about the initial, final and maximum specific growth rates of biomass and information about the growth logarithmic and growth inhibition phases appearance along with the information about the ratio of dividing and non-dividing cells in different growth media (BBM, FWM, ACM) [10]. The growth dynamics give information about the structured models for LGP, GIP and Non-dividing (stable) cells for GIP phases of microalgal growth kinetic parameters in different media, along with the determination of different growth kinetic parameters such as the maximum algal biomass specific growth rate, stable cells concentrations at the end of the logarithmic growth phase and dividing and non-dividing cells ratio [11].

2.9.5 Total Kjeldahl Nitrogen (TKN) and phosphorus in FW, ACM and BBM media at initial and final day of *D. armatus* cultivation

The Total Kjeldahl Nitrogen (TKN) and phosphorus were estimated according to the methods recommended by Jackson, 1958 [3] and Fiske and Subbarow, 1925 [4]. The *D. armatus* was cultivated in the diluted ratio of 3:1 (FWM/ACM: BBM) for one month. After that, TKN and phosphorus values were evaluated at the start and final days of the cultivation in the respective medium.

2.9.6 Determination of biochemical concentration in selected diluted ratio (3:1) of FWM, ACM and BBM

2.9.6.1 Microalgal lipid estimation

The *D. armatus* biomass collected from the selected ratio of the FWM/ACM and BBM flasks separately and analyzed for the lipids content, method specified by Bligh and Dyer (modified method) with use of solvents such as methanol (CH_3OH), chloroform ($CHCl_3$) and water (H_2O). The microalgal biomass was first centrifuged at 5000 rpm for 5 min so that the biomass settled down, and then biomass was kept for drying. The drawn biomass and appropriate volume of solvents mixed in the proportion of 1:2:0.8 ($CHCl_3$:CH_3OH: H_2O) followed by sonication in an ultrasonic water bath for 5 min. Again the solvent mixture chloroform: distilled water ($CHCl_3$:H_2O) added in the equal proportion (2v:2v) and sonicated for 5 min. Afterward, this mixture vortexes and centrifuge for 5 min at 3000 rpm. The bottom layer of chloroform was removed and transferred into the pre-weighed fresh vial. The vial was subjected to the hot air oven and, after that, desiccated in a desiccator at room temperature and weighed again. The lipid content in terms of weight % was calculated [12].

2.9.6.2 Carbohydrate estimation

The carbohydrates in the microalgal biomass were determined through the protocols given by Pleissner et al. 2013 [13] with a few variations. The microalgal biomass was taken out from the FWM, ACM and BBM flasks and centrifuged at 5000 rpm for 5 min. After centrifugation, the pellet was kept back and dried for further use, and the supernatant was discarded. The addition of 0.5 ml of sulphuric acid into the biomass for the initiation process of acid hydrolysis and the reaction time was set to 30 min. After that, 4.5 ml of distilled H_2O was added to this mixture in the falcon tubes and placed at 90 $^{\circ}$C for incubation in a water bath for 90 min. The falcon tubes were removed from the water bath and subjected to centrifugation for 10 min at 5000 rpm. The pellet was discarded, and the supernatant solution was analyzed for carbohydrate estimation (Phenol- sulphuric method) given by Dubois et al. 1951 [14]. The 5 ml of sulphuric acid was put into the supernatant containing test tubes and heated up for 10 min at 95 $^{\circ}$C. Then phenol was added to the test tubes containing the mixture, and vortexing was done for 5 min. Then take the optical density in UV-VIS Spectroscopy at 490 nm and known concentrations of the starch

standard graph were utilized to know the unknown carbohydrate concentration in the sample by correlating the absorbance values.

2.9.6.3 Protein estimation

The proteins estimated *D. armatus* biomass cultivated in FWM, ACM and BBM. The microalgal biomass is subjected to sonication for proper cells disruption [15]. The *D. armatus* biomass was initially centrifuged for 5 min at 5000 rpm, and the pellet was set aside for drying. Then, water (10ml) was added with potassium hydroxide (0.5 N) into the microalgae pellet, followed by vortexing for one min for proper mixing. Then the mixture was ultasonicated at a 25 pulse rate for 10 min in a sonicator. After the ultrasonication, the mixture was centrifuged at 7000 rpm for 5 min. The pellet was removed, and the supernatant was analyzed for protein. The protein was evaluated using the Bradford method specified in the formed pellet [16]. The standard graph of BSA was prepared to know the unknown protein concentration in the sample by taking the optical density at 595nm.

2.10 Experimental layout for bioremediation studies of waste X- ray developer solution (X.D) by *D. armatus* using BBM

The selected concentration (3 BBM: 1X.D.) from the screening procedure (as mentioned under section 2.8) was further analyzed to study the bioremediation parameters by utilizing BBM media. The 10% of *D. armatus* (15 d old) was inoculated in the 100 ml of developer solution and BBM media in 1:3 ratio. After inoculation, the flasks were incubated in the 1400-1500 lux intensity of photosynthetic light, 25±1 ^{o}C, and 100 rpm agitation speed in the incubator shaker for one month. The aliquot of samples was taken out after 3-4 d to analyze the characterization parameters before and after *D. armatus* treatment. The pH, TS, TDS, TSS, TVS, TFS, conductivity, density, BOD, and COD were calculated according to the protocols given by APHA, standard [2]. The TKN and phosphorus estimation was performed according to the Jackson, 1958 [3] and Fiske and Subbarow methods [4]. The silver was also determined in the control (no *D. armatus* treatment) and treated samples for one month through the ICP-MS technique. The detailed protocols of physico-chemical parameters mentioned in above column (2.7). The bioremediation potential of *D. armatus* was calculated of each physico-chemical parameter, comparative percentage estimated in the microalgal-based remediated sample

concerning control (without microalgae treatment). The calculation of bioremediation potential (%) was measured by using the subsequent formula:

$$Bioremediation\ Potential\ (\%) = \frac{Value\ of\ Bioremediated\ Sample}{Value\ of\ Control\ (without\ algal\ treatment)} \times 100$$

2.10.1 Measurement of microalgae growth

The *D. armatus* growth in the X.D solution diluted with BBM medium (3 BBM:1 X.D.) was measured through cell count (× 10^4/ml) and dry cell weight (g/l) as mentioned protocols in the above column (2.5) for one month of bioremediation studies.

2.10.2 Lipids estimation

The *D. armatus* biomass was taken out from the treated samples of developer solution in dilution with BBM medium after 3-4 d to determine the lipids content. The lipids determination was done through the Bligh and Dyer protocol [12] as protocols mentioned under section 2.9.6.1 and calculated in (% wt).

2.11 Toxicity screening of the *D. armatus* in the various concentrations of X.D. utilizing FWM and ACM

The FWM and ACM were initially diluted with BBM in 3:1 ratio to enrich the media for microalgal growth. The *D. armatus* grow fine in this selected concentration (3:1) in previous studies, as mentioned under section 2.9.2. Various concentrations of FW and ACM were selected to screen the *D. armatus* growth in the X.D. The microalgae *D. armatus* inoculated in 3 FWM /ACM: 1 X.D., 2 FWM/ACM: 2 X.D., 1 FWM /ACM: 3 X.D. and using 100 % X.D, FWM, ACM as a control media. The microalgae cultivated in photosynthetic light 1400-1500 lux intensity of light and dark periods (12:12 h). The temperature was maintained at 25 ±1 ^oC and 100 rpm shaking conditions for one month. The cell count (×10^4/ml) in the Neubauer chamber was taken after every 3 d till one month of cultivation. The selected dilution from this screening process was further analyzed for the bioremediation parameters in future experiments.

2.12 Experimental outline for remediation of X.D. by *D. armatus* using FWM and ACM

The 3FW/ACM: 1BBM dilution was utilized for bioremediation studies of the X-ray developer solution. The 10% of *D. armatus* inoculum was transferred in the 100 ml of diluted developer solution diluted with food waste and agri-compost medium in separated flasks. After that, these flasks were subjected to the 1400-1500 lux intensity of photosynthetic light, 25±1 ^{o}C, and 100 rpm agitation speed in the incubator shaker for one month. The aliquot of samples was taken out after 3-4 d to analyze the characterization parameters before and after *D. armatus* treatment in both media (FWM and ACM). The pH, TS, TDS, TSS, TVS, TFS, conductivity, density, BOD, and COD were done according to the protocols given by APHA, standard [2]. The TKN and phosphorus estimation was performed according to the Jackson, 1958 [3] and Fiske and Subbarow methods [4]. The silver was also determined in the control (no *D. armatus* treatment) and treated samples for one month of treatment through the ICP-MS technique. The bioremediation potential of *D. armatus* was calculated from each parameter's relative bioremediation potential (%). The samples without *D. armatus* treatment were taken to control bioremediation potential (%) [17]. The calculation of bioremediation potential (%) was measured by using the subsequent formula:

$$Bioremediation\ Potential\ (\%) = \frac{Value\ of\ Bioremediated\ Sample}{Value\ of\ Control\ (without\ algal\ treatment)} \times 100$$

2.12.1 Measurement of microalgae growth

The *D. armatus* growth in the developer solution diluted with food waste and agri-compost medium (3 FWM/ACM: 1 X.D.) was measured through cell count (× 10^4/ml) and dry cell weight (g/l) (as mentioned under section 2.5) for one month of phycoremediation studies.

2.12.2 Lipids estimation

The *D. armatus* biomass takes out from the treated samples of X.D in dilution with FWM and ACM after 3-4 d to determine the lipids content. The lipids determination was done through the Bligh and Dyer protocol [12] as mentioned under section 2.9.6.1 and calculated in (% wt).

CHAPTER 3

Analysis of X-ray Waste Effluents & Screening of Microalgal Strain towards Bioremediation

CHAPTER 3

Analysis of X-ray Waste Effluents & Screening of Microalgal Strain towards Bioremediation

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3.1 Introduction

The radiographic waste discharged from the numerous medical laboratories, clinics, and health care centers have several additional chemicals, films, developer, fixer solutions, lead foils, and coats. The waste radiographic solutions fall in the category of hazardous waste because these waste solutions also consist of minute to vast amounts of silver and other heavy metals [1]. Among other waste radiographic solutions, the X-ray fixer was contemplated as more pernicious due to its higher silver content (more than 3 g/l). The developer solution has less silver range than the fixer solution. In radiograph processing, developer and fixer solutions and other compounds such as thiosulfate, heavy metal traces, and sulfites [2, 3]. The existence of organic/inorganic contaminants in fixer composition has more substantial BOD and COD levels [4]. The hospital and other medical clinics discharge have a higher volume of silver, and other inorganic components disposed into the municipal sewerage system. These solutions have enormous pH values, chlorides, sulfates, color, turbidity, COD, and total dissolved solids, above the permissible limits prescribed by authorities. Typically, the waste radiographic solutions with <5 ppm silver content can be released from the medical centers into the public sewer setup. Although the X-ray fixer solution contains a tremendous quantity of silver 3-8 g/l, that is higher than the permissible limits. So, there is the urgency of applicable practices for silver elimination in radiographic solutions and their release into the sewer [5]. Adopting Ag recovery prevailing techniques (precipitation, enzymatic process, adsorption, cation/anion-exchange, electrolysis, and metallic replacement) experienced huge expenditure and use of the hazardous chemicals makes the ecosystem vulnerable and causes financial problems [6]. The usage of microalgae came in the scenario from the previous few years and emerges as a substantial mechanism for pollutant elimination from diversified squander [7]. The supplemented basal medium with salts, essential elements traces, and phosphorus, nitrogen is the main nutrients supply, and it's proficiently used for the luxurious growth of microalgae [8].

The various advantages favour the microalgal-based remediation over the existing approaches which include faster growth of microalgae, utilization abundantly available light and CO_2 as inputs, able to use the inorganic components of waste effluents, existing metal ions uptake mechanisms, eco-friendly process and can be integrated with the desirable co-valued products [9–11]. The best lenient microalgae sp. for bioremediation studies include *Chlamydomonas*, *Chlorella*, *Oscillatoria*, *Scendesmus*, *Euglena*, *Stigeoclonium*, *Nitzschia* and *Navicula* [12]. The success of microalgal-based bioremediation process is mainly depends on the metal toxicity (of industrial effluent) tolerance of the respective microalgal sp. [13]. The freshwater microalgae Desmodesmus sp. and Scendesmus sp., possessing application of rapid growth, efficient cultivation, and removal of heavy metals, and production of biofuels, has been taken as significant microalgae in the current analysis [14–16].

In the current investigation, the characterization of waste developer and fixer solutions and its evaluation in screening microalgae to check the toxicity limits is a lesser-explored area. Thus, the current investigation demonstrated the characterization of radiographic developer and fixer solutions and *D. armatus* and *S. abundans* toxicity forbearance limits in various concentrations of developer and fixer solutions, supporting the feasible microalgal-based remediation.

3.2. Results and Discussion

3.2.1 Physico-chemical parameters analysis of radiographic solutions (X-ray developer solution (X.D) and X-ray fixer solutions (X.F))

3.2.1.1 Description of characterization parameters of X.D

There is essential to characterize the physico-chemical parameters of X.D before discharge into the water bodies. The apposite disposal of waste is indispensable to surmount the health consequences of humans and flora and fauna. Hence, guidelines are specified by the administrative section for waste evaluation before dumping into the environment and are based on the physico-chemical parameter analysis. Various physical and chemical parameters related to radiographic developer solution characterization are listed in Table 3.1. The pH of X.D was found to be 8.80±0.005 (Table 3.1). The alkalinity of radiographic developer solution due to the existence of chemical constituents EDTA, potassium hydroxide, hydroquinone, acetic acid, sodium metabisulfite, sodium bromide, etc. The wastewater discharge from developing and fixing methods in radiographic practices treated with the biological method has a pH equal to

8.2-9.3 [17]. The density and conductivity values in X.D are approximately 1039.56±0.057 g/l and 49.13±0.057 mS, respectively (Table 3.1).

The radiographic developer solution is typically acquitted from the health centers and medical labs disposed of into the public drainage output [18]. The measured quantities of total solids were 62.66±0.577 g/l and total dissolved solids 51.83±0.737 g/l (Table 3.1) in X.D. After filtering the waste developer solution, the suspended solids persisted is incredibly minute in amount and range up to 10.83±0.208 g/l (Table 3.1) when correlated with dissolved solids. The dissolved solids in various recovery methods were estimated before and after a resurgence of the silver. Moreover, the amount of suspended solids was also calculated in the radiographic fixer solution [19].

The X.D carries a great degree of biological and chemical oxygen demand levels, compared to the limits prescribed by the Indian authorities to release this waste in the sewage unit. The biological oxygen demand (BOD) and chemical oxygen demand (COD) exhibited values 1.22±0.004 g/l in BOD and 27.29±0.230 g/l in COD (Table 3.1). In hospital solutions, inorganic and organic composite is measured through BOD and COD estimations. The control has estimated 278 mg/l of BOD_5 and 622 mg/l amount of COD [20] when compared with bacterial treatment, the values of BOD and COD were elevated. One more study reports that hospice waste has 129.3 mg/l amount of BOD_5 and COD with 662.9 mg/l values, respectively [21]. The vast COD of hospital waste is the consequence of various salts and phenolic compounds that should be remediated before discharge into the water reservoirs [22]. From Table 3.1, the radiographic developer solution was evaluated and had approximate total kjeldhal nitrogen (TKN) of 0.22±0.004 % and total phosphorus (TP) of 123±24.51µg/ml. The wastewater from the developing solution of radiographic processing has a 9200 g/m^3 amount of total nitrogen [17]. The estimated Ag amount in the X.D is around 0.01791±0.000 g/l (Table 3.1), which is already above the regular limits set for wastewater discharge into the environment. Generally, the X.D consists of less silver (below 5 mg/l) and has contaminated traces, making it hazardous. Most medical labs and healthcare centers openly release radiographic solutions into the public sewerage system. Silver metal is not toxic; its toxicity mainly relies upon the abundance of metal present in the atmosphere. Silver is present in the varied form in the atmosphere, but Ag^+ is more

hazardous compared to other silver forms, and its tendencies are significantly less to exist in this form.

The pH, turbidity, redox potential, solids substances, and another form of silver metal are the main factors related to the toxicity in the environment. The most challenging aspect is recognizing the accurate concentration of silver in the water streams because free silver (Ag^{+}) can attach with another anionic void [23]. During the progression of the radiography method, the KODAK GBX fixer or replenisher and CRONEX HSF/M spent radiographic fixer solution has biological oxygen demand value 7140-19400 mg/l, respectively. Before silver recovery in the fixer solution, the KODAK GBX fixer or replenisher and CRONEX HSF/M have chemical oxygen demand of 46900-60900 mg/l correspondingly [19].

Table 3.1: Physico-chemical parameters analysis of X-ray developer solution. Values are showed as ± standard deviation of triplicates

Parameter	Results	Units
pH	8.80±0.005	-----
Total solids	62.66±0.577	(g/l)
Total dissolved solids	51.83±0.737	(g/l)
Total suspended solids	10.83±0.208	(g/l)
Total volatile solids	8.83±0.577	(g/l)
Total fixed solids	53.5±0	(g/l)
Conductivity	49.13±0.057	(mS)
Density	1039.56±0.057	(g/l)
Biological oxygen demand	1.22±0.004	(g/l)
Chemical oxygen demand	27.29±0.230	(g/l)
BOD/COD ratio	0.044	-----
Total kjeldahl Nitrogen	0.22±0.004	(%)
Total Phosphorus	123±24.51	(µg/ml)
Silver	0.01791±0.000	(g/l)

The silver quantity in X.F is 15 g/m^3 and whereas 10 g/m^3 for X.D [17]. The X-ray processing solution from the hospital has an immense amount of approximately 13336 ppm of BOD. The COD level depicted around 33337 ppm, which is in elevated quantity compared to standard limits. The silver quantity 0.2354 ppm was estimated in waste solution before the plasma treatment [23]. The outcomes of physical and chemical parameters of radiographic developer solution have been tabulated in Table 3.1.

3.2.1.2 Physico-chemical examination of X.F

There is no worldwide acceptance to discharge the waste X.F and other hazardous materials straight into the solution treatment plant and other sewage systems. There is a prerequisite to knowing waste water's properties, toxicity assessment, and influence on the surrounding habitat. The alkalinity and acidity of the waste X.F rely upon the inorganic and organic components of the solution. The calculated pH of the X.F is 10.13±0.005 and values of total dissolved solids (TDS) 128.75±0.31 g/l and total suspended solids (TSS) 10.98±0.34 g/l in the solution (Table 3.2). The density and conductivity of the X.F are 1092.76±0.04 g/l and 68.56±0.05 mS, respectively (Table 3.2). During the fixing procedure, numerous chemicals, hardeners, buffers, and preservatives were used to make the solution more toxic. The BOD and COD of the squander X.F were evaluated as 11.83±0.39 g/l and 506.15±0.20 g/l (Table 3.2). The waste X.F contains various organic and inorganic compounds such as acetate, thiosulfate, EDTA, ammonium sulfate, metabisulfites, and silver thiosulfate which account for the high values of COD in the fixer solution. The estimated BOD and COD values are more than the stated regular standards. The TKN and TP contents of X.F were found to be 10.67±0.69 % and 37.33±3.05 µg/ml, respectively (Table 3.2). There is the generation of a high quantity of silver in complex form (silver thiosulphates) during radiography in X.F. The estimated silver amount in the waste X.F is 3.49±0.01 g/l (Table 3.2). With the 3000-8000 ppm of silver content, the waste fixer solution negatively affected the environment. The removal, management, and restoration of silver in waste fixer solutions are essential to environment security [5]. Every adverse outcome of X.F approaches towards environmental deterioration is mainly due to an abundance of Ag amount (2000-6000 mg/l) [24]. The investigation studies related to the physico-chemical parameters of waste fixer solution explain the hazardous nature and its toxicity extent to the surroundings (Table 3.2). The radiographic fixer solution generally surpasses the highest admissible limits to discharge the radiographic solution specified by the Indian ordinances for feasible bioremediation access.

Table 3.2: Physical and chemical parameters of the X.F. Values are showed as ± standard deviation of triplicates

Parameters	Results	Units
pH	10.13±0.005	-----
Total solids (TS)	139.73±0.02	g/l
Total dissolved solids (TDS)	128.75±0.31	g/l
Total suspended solids (TSS)	10.98±0.34	g/l
Total volatile solids (TVS)	36.9±0.285	g/l
Total fixed solids (TFS)	102.83±0.30	g/l
Conductivity	68.56±0.05	mS
Density	1092.76±0.04	g/l
BOD	11.83±0.39	g/l
COD	506.15±0.20	g/l
BOD/COD ratio	0.023	-----
TKN	10.67±0.69	%
TP	37.33±3.05	µg/ml
Silver	3.49±0.01	g/l

3.2.2 *D. armatus* and *S. abundans* growth studies in BBM

The cultivation of microalgae is accomplishing the new goals in biofuels production, pharmaceuticals industries, food, feeding industries, elimination of solutions, etc. [25, 26]. The optical density (OD) and growth state (d) of various microalgae deviated with a variety of species [27]. The current analysis depicted the *D. armatus* and *S. abundans* growth in the bold basal medium (BBM) for one month, as represented in Figures 3.1 and 3. 2.

The microalgae *D. armatus* show the highest cell count, 1433×10^4/ml, on the 24th day of cultivation compared to *S. abundans* which exhibited the maximum cell count of just about 1284×10^4/ml on the same day of cultivation. The optical density of *D. armatus* and *S. abundans* was calculated around 0.454±0.01 and 0.438±0.03 at the end (31 d) of the microalgae

growth periods. Cell count provides the best estimate of cell growth. But OD measured the turbidity of culture. It does not measure the cell number and does not provide any relevant information about the other debris in the culture. So this debris interfered with OD's values and showed an increase in OD after the 24th day. But cell count depicted the appropriate estimate regarding the growth of microalgae.The microalgal growth phase studies (cell count and OD) revealed that *D. armatus* and *S. abundans* have a logarithmic phase at 6-24 d in one month of cultivation in BBM medium (Figures 3.1 and 3.2).

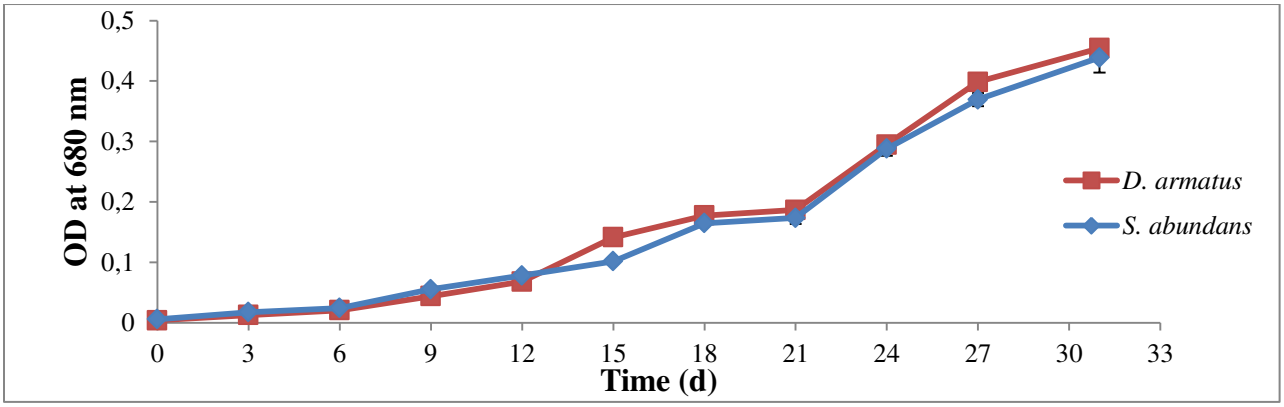


Figure 3.1: *D. armatus* and *S. abundans* growth studies through OD at 680 nm in bold basal medium (BBM). All values are represented as ± s.d of triplicate experiments.

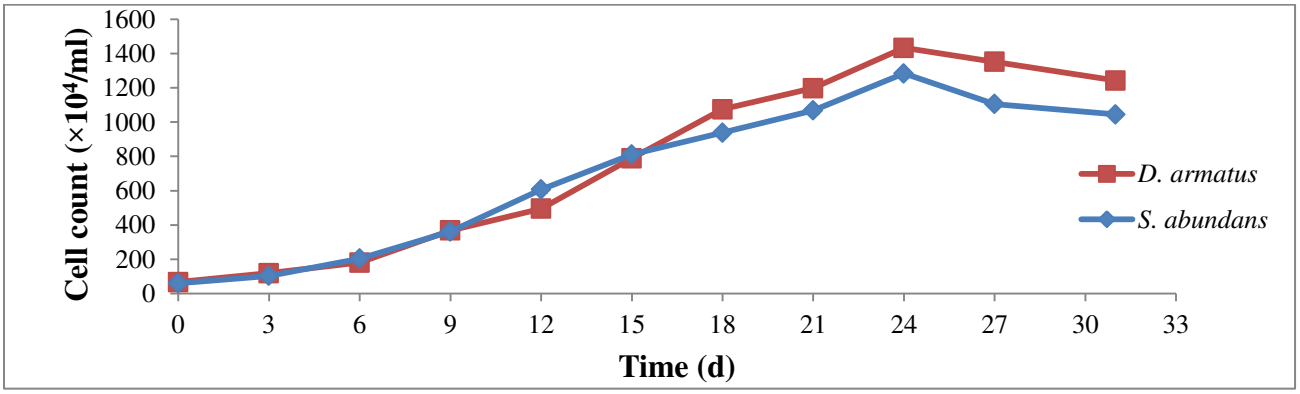
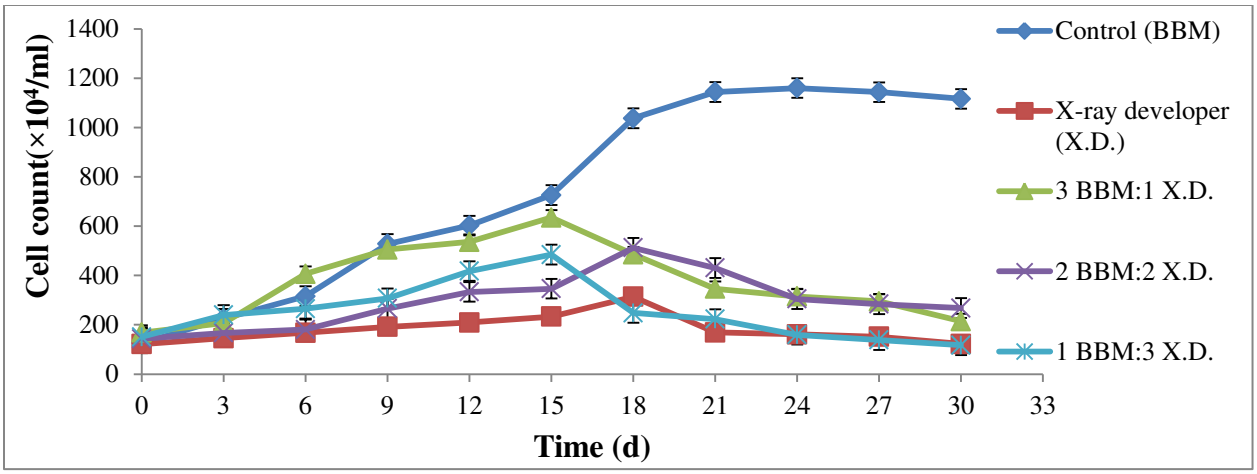


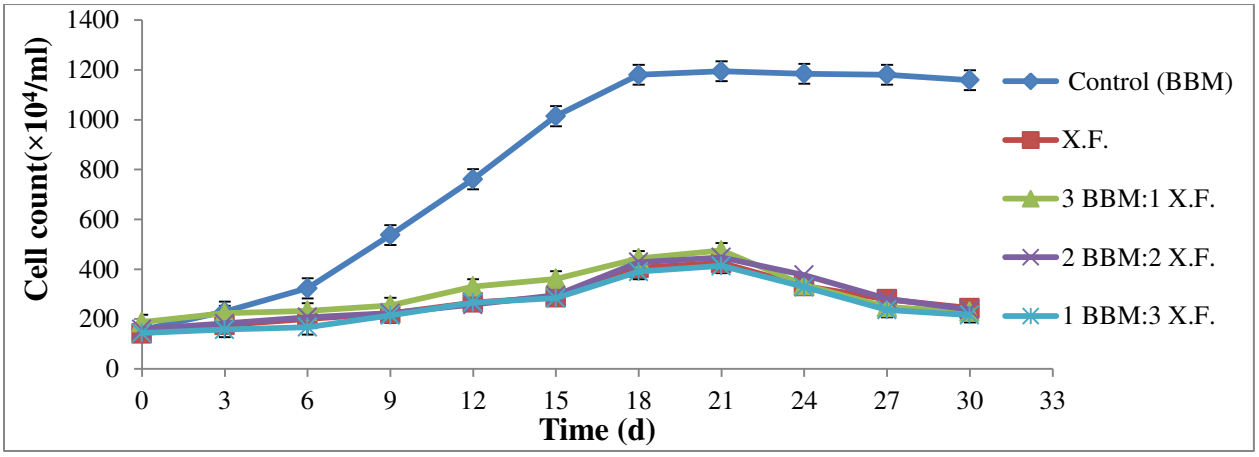
Figure 3.2: *D. armatus* and *S. abundans* growth study in bold basal medium (BBM) represented in terms of cell count (×10^4/ml). All values are represented as ± s.d of triplicate experiments.

3.2.3 Toxicity evaluation of microalgae *D. armatus* in various concentrations of X.D /X.F and BBM

The feasible accomplishment of microalgal-based remediation profoundly relies on limits of tolerance followed up by microalgae in the respective industrial waste discharge [28, 29]. The microalgae *D. armatus* has been candidly grown for one month in various concentrations of X.D /X.F and BBM. The cell count measurements of diluted X.D with BBM (3:1) shown promising growth results (Figure 3.3 (a). The presence of low-content of nutrients/higher toxicity in the other dilutions of X.F and X.D terminated microalgal growth. The microalgal growth was ascertained to be satisfactory throughout the complete cell count up and microscopic studies, which signify that *D. armatus* can cultivate in the X.D (Figure 3.3 (a) (b) by utilizing the microalgal growth in BBM as a control for the toxicity tolerance exploration. The promising microalgal growth was observed during 15^{th} – 19^{th} d of cultivation in both the diluted X.D and X.F. Microscopic analysis shows that the microalgal cells appears to be viable with the different dilutions of X.D with BBM rather than in different dilutions of X.F with BBM. The X.F consists of a higher amount of inorganic/organic contaminants and silver, which prohibited the growth of *D. armatus* in various diluted ratios and the control medium (fixer solution) (Figure 3.3 (b). The satisfactory microalgal growth was observed in the diluted X.D because of sufficient nutrient stock in the respective dilution of waste solution. The utilized intact X.F (control) exhibited the lesser microalgae growth. The X.F possesses higher BOD and COD values which hails the growth of microalgal cells (Table 3.1 and 3.2). The integration of X.F with the BBM accomplishes the nutrient accessibility rooting for microalgae cultivation. The nutrition insufficiency is fulfilled by the presence of N and P with waste streams of X.D for microalgal growth and subsequent removal of N and P from the X.D [14]. The Desmodesmus sp. usually tolerates maximum COD and acidic conditions and can be promptly cultivated in the diluted oil refinery wastewater [30]. The *Chlorella minutissima* and *Spirulina platensis* cultivated in the regular medium with 10 % dilution of cassava processing squander considered finer dilution for microalgae growth through overcoming the inhibition response prevailing chemicals in the cassava waste [31]. Li et al. 2011 [32] experiments also signify the importance of dilution for toxicity tolerance in Scendesmus sp. *and* Chlorella sp. cultivation featured for the effective removal of industrial waste through microalgae treatment.



(a)

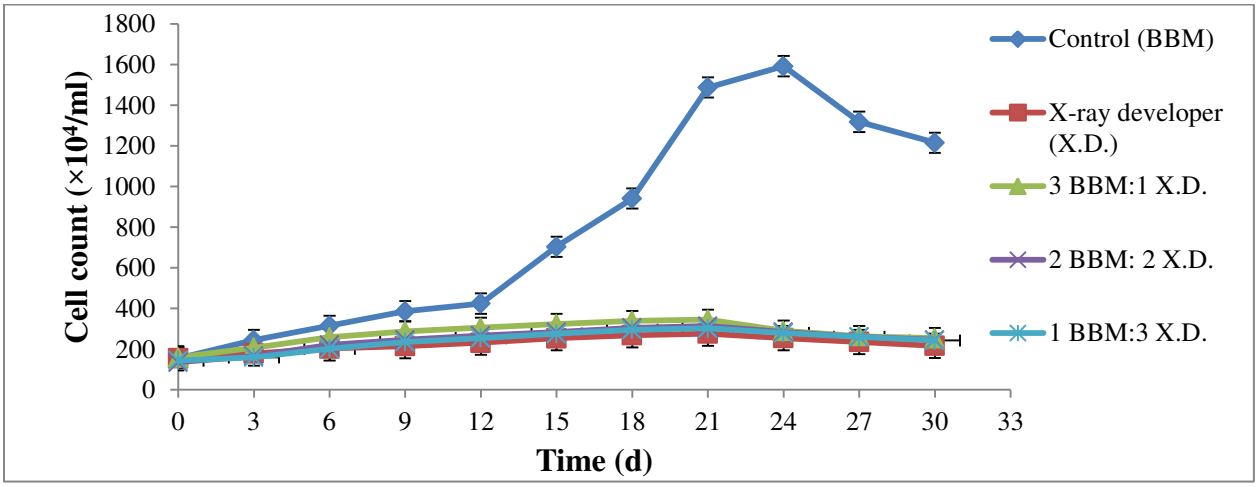


(b)

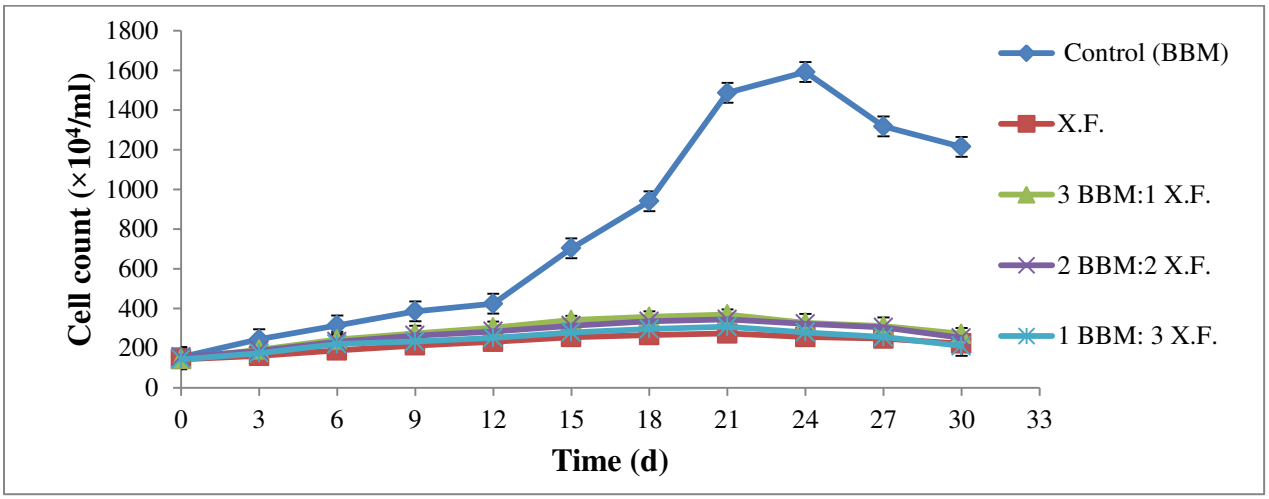
Figure 3.3: Microalgae *D. armatus* toxicity illustrations in various ratios of radiographic solutions in dilution with bold basal medium (BBM) (a) Developer solution (b) Fixer solution. All values are represented as ± s.d of triplicate experiments.

The diluting combinations of the F/2 medium and industrial devastate water found as a suitable culturing medium for the *Tetraselmis chuii* and *Nannochloropsis oculata* . The dilution combinations showcased the expected outcomes with 75:25 dilution ratios of industrial wastewater and F/2 synthetic medium [13, 33]. In the report by Ding et al. 2015 [34], the microalgae culturing in diluting medium (20% dairy farm solution+ distilled H_2O) manifested as the preeminent mixture to accomplish the remediation experiments along with appropriate growth of the microalgal cell.

3.2.4 Toxicity evaluation of microalgae *S. abundans* in various combinations of X.D /X.F and BBM



(a)



(b)

Figure 3.4: *S. abundans* toxicity evaluation by taking cell count (×10^4/ml) in various diluted concentration of radiographic solutions and basal medium (a) Developer solution (b) Fixer solution. All values are represented as ± s.d of triplicate experiments**.**

The X.D and X.F possessed high values of characterization parameters (Table 3.1 and 3.2), and it's essential to eliminate the waste contaminants before discharging them into the surrounding environment. The use of microalgae in waste solutions remediation is a prominent sustainable methodology that uptake the metals (e.g., silver) and employ phosphorus and nitrogen for

microalgae growth and utilize the possible biological and other constituents for various by-products formation [35]. The growth of *S. abundans* in the different proportions of diluted X.D/X.F with BBM exhibited disparate growth patterns. The *S. abundans* growth in X.D and X.F is not much rapid at the start of days, with increased in the cultivation period (d), the cell count of microalgae enhanced till 21st d of cultivation (Figures 3.4 (a) (b)).

From Figure 3.4, among all these diluted X.D and X.F, a maximum cell count 344×10^4/ml was observed in 3 X.D: 1 BBM dilution compared to the 3 X.F :1 BBM ratio (cell count of 370×10^4/ml). The microalgae have tolerance limits in different culturing mediums such as Scendesmus sp. ISTGA1 cultivated in the BG-11 medium and whereas *N. oculata* grown in F/2 medium with various diluted ratios of industrial waste appeared as a promising mechanism before the complete analysis of microalgal bioremediation [13, 15, 36]. The present study showed the significance of radiographic solution (X.D and X.F) characterization and optimal ratio of 1:3 (X.D: BBM) of *D. armatus* tolerance limits along with favorable growth in it. Furthermore, this ratio and microalgae were selected to precede the research on microalgal-based bioremediation towards removing silver and eradicating the environment-threatening properties of the solutions.

3.3 Conclusion

The X.D and X.F have higher quantity of contaminants (organic and inorganic) which contributed to the higher values of physical and chemical parameters. The X.F consisted huge amount (3.49 g/l) of silver than the X.D (0.01791 g/l), which released during the process of radiography and considered as more hazardous. The determination of characterizing parameters of X.F yields the extensive amount of BOD and COD values too, i.e., 11.83 g/l and 506.15 g/l, respectively. In comparison with *S. abundans* growth in X.D, *D. armatus* showed higher growth in diluted X.D with BBM (3:1) than the diluted X.F with BBM (3:1). The *D. armatus* selected as suitable candidate for the consecutive bioremediation studies of X.D.

CHAPTER 4

Food Waste & Agriculture Compost as Media for *D. armatus*: Bioprocess Dynamic Study

CHAPTER 4

Food Waste & Agriculture Compost as Media for *D. armatus*: Bioprocess Dynamic Study

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4.1 Introduction

The vast amount of organic wastes originating from municipal and rural regions needs an enlarged requirement of industrial and farming production globally [1]. Natural fertilizer production accomplishes the 4-81 Kg CO_2 release for each ton of foodstuff and farming waste [2]. The enlarged production of food squanders comes out as one of the foremost challenges in most countries and its treatment pattern and disposable methodology in landfill areas [3]. The nitrogen and phosphorus as organic fertilizer formed during the various foodstuffs composting and further employed in agriculture sustenance. The food waste consists of a high quantity of lipids, proteins, and carbohydrates, which supply sufficient nutrients and proceed as feedstock to cultivate microorganisms [4]. The continuous escalation of microorganisms is ensured by utilizing wasted food compost over again as a culturing medium. The reusing of wasted food as nutrient media eventually reduces the environmental contaminants originated through fertilizer and increases the potential of nutrient usage. The microalgae have plenty of distinctiveness, such as photosynthetic character and candidly growth in composted waste with nutrients absorption and biomass production. CO_2 and sunlight are the primary conditions for microalgae growth and grow well in a heterotrophic environment. The basic elements abundantly present in the microalgal biomass are proteins, carbohydrates, amino acids, lipids, vitamins, and additional biological compounds [5–7].

The microalgae culturing requisite relies on fresh H_2O and fertilized land availability [8]. The essential conditions such as microalgal species, culturing medium, growth environment, and biochemical constituents required to accomplish the maximum amount of biomass for valuable products and further biofuels formations [9]. The C, P, N, and supplementary nutrients are the

main growth constituent for microalgae growth, and it adjoin the extra cost in microalgae production. For cost minimization, instead of using the synthetic medium, microalgae can be grown in waste solution. The microalgae culturing in waste medium utilizes its available nutrients, treats the waste solution, subordinates the BOD and COD level, and makes it clean water [10]. Agriculture waste and food remain the low-priced and renewable sources that can utilize for microalgae cultivation [11]. The mixotrophic cultivation of *Chlorella* in hydrolyzed food waste is utilized for lipids production and an economical approach to use waste as a cheap nutrient source [12].

The growth of microalgae firmly relies upon the nutrient's concentration and affects the microalgae's biochemical constitution. The nutrients contents and culturing medium preference endorses the microalgal biomass fabrication [13, 14]. The diverse quantities of livestock waste manure are utilized as the culturing medium for microalgae growth, and it enhances the biomass and lipids amount [15]. There are many troubles, including hazardous compounds' presence, an abundance of various nutrients, and immense quantities of pollutants associated with the exploitation of wastewater as a nutritive medium. Eventually, it arrests microalgal growth. The H_2O and nutrients abundant requirement for biomass formation is not possibly cost-effective techniques. For the adequate growth of microalgae, there is a prerequisite for a low-priced and highly nutritive medium. The food waste also comes into sight as an alternate culturing medium for biomass formation and is furthermore utilized for value-added product formation [16, 17]. The microalgae served as constant support to energy revival and sustainable, cost-effective processes and were employed as an alternate resource in cultivation and fermentation in food waste [18].

The various microalgae biomass adaptation and transformation approaches have application in the formation of biofuels, biogas, bioethanol, biodiesel, and other refinery products [19, 20]. The wasted food hydrosylates consist of phosphate, glucose, and FAN, which can supply vital nutrients for the microalgae formation in heterotrophic mode [21]. The micro pollutants like phosphate and ammonium, which are profusely present in the food waste, are used to grow microalgae and reduce the quantity of these pollutants. The mixture of medium, i.e., wasted food compost and inorganic (usual synthetic medium) utilized as substrate for growth and cultivation of microalgae and further has benefit in the industrial level scale up for the microalgae [5]. The

biomass from microalgae has been documented as a resource of proteins, lipids, and carbohydrates. The consideration of microalgal biomass came out as the nourishing constituents because of its enormous function in forming biofuels, biodiesel, and bio feed in aquaculture. The lipids from the microalgal biomass usually contain PUFA, ALA and DHA, and other fatty acids that provide significant health assets [22, 23]. The utilization of different sources of medium (waste compost) reduced the cost of culturing medium by providing all the valuable nutrients for the continual growth of microalgae and evaluated for diminishing the contagion extent.

Thus, pre-eminent conditions for microalgae growth and the most critical factor directed towards the lesser growth should be evaluated carefully to attain maximum biomass production. The kinetic studies calculated the potential growth of microalgae in the respective nutritive medium, which involves lag, log, stationary, and death phase [24, 25]. Biomass dynamics provide information about the involvement of divided and undivided cells' role in the production of the metabolites and the kinetic constants of the production. The dynamics facilitate the understanding of the physiology of cells to adjust with various process conditions, which helps in taking the corrective measures of biological output [26, 27].

This study has a major objective to investigate the food waste and agriculture compost as a nutritive medium for the *D. armatus* cultivation and its dynamic bioprocess studies. The method of composting alters the organic waste into creditable biological products through the sustainable green methodology. The exploitation of N, P, and other nutrient contents of wasted food and agriculture compost formulate the appropriate medium for microalgae growth. The substitution of standard BBM with the Food Waste Media (FWM) and Agri-compost Media (ACM) in various diluted concentrations was assessed for the *D. armatus* growth. The best suitable FWM/ACM and BBM concentration for *D. armatus* growth was chosen for further dynamic growth studies and estimation of carbohydrates, proteins, and lipids content.

4.2 Results and Discussion

4.2.1 *D. armatus* growth studies in the FWM

The various dilutions (3:1, 1:3, and 2:2) of FWM and BBM were used as a growth media for *D. armatus* cultivation. The obtained growth results were compared with the results of *D.armatus* grown in intact BBM and FWM (as control). The FWM comprise a variety of micronutrients that

augment the microalgae growth. The food waste composting decomposes the organic matter and proceeds as a resource of microalgae growth [28].

The additional nutrients present in the waste combinations persuade the growth of microalgae. The microalgal biomass growth enhances at the suitable dilution of FWM and BBM. Figure 4.1 showed the growth of *D. armatus* in various dilutions of FWM with BBM, and diluted ratio of 3 FWM:1BBM shown promising biomass growth compared to other dilutions after the 6th day of the *D. armatus* cultivation. In this experiment, the BBM was used as a nutrient medium for the *D. armatus* growth. There was no considerable escalation of microalgal biomass until the 12th day, and after that, *D. armatus* grew efficiently. The diluted ratios 2:2 and 1:3 of FWM: BBM exhibited the almost equivalent *D. armatus* growth patterns till the declining phase. The intact FWM consists of a considerable amount of organic material. There is no rise in biomass growth in the initial 15 d of cultivation. After that, the *D. armatus* biomass increased, and microalgae entered the exponential phase. The overall outcome of this growth study in diluted FWM with BBM showed the amplified growth of *D. armatus* in contrast with usual BBM solely. The FWM makes available all necessary ingredients (nutritional elements) for enough microalgae growth and production of biological products. The mixing of food waste with the usual cultivated medium up to the level of 50% has the advantage for the adequate growth of microalgae [5].

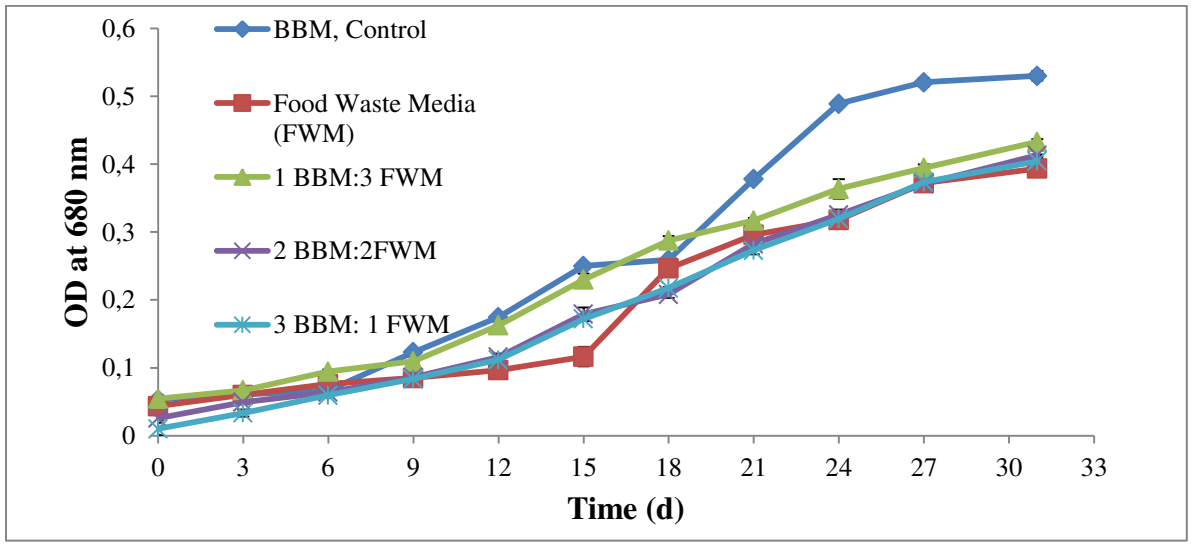


Figure 4.1: Cultivation of *D. armatus* in different concentration of organic (food) and inorganic medium (BBM). All values are represented as ± s. d of triplicate experiments.

4.2.2 *D. armatus* growth studies in the agri-compost medium (ACM)

The manure of agriculture rotted organic matter is the sort of agriculture waste and utilized as a nutrient medium for microalgae cultivation. The ACM diluted with BBM in varied ratios similar to FWM were employed for the culturing of *D. armatus*. Figure 4.2 depicted the growth of *D. armatus* in the assorted ratio of diluted ACM with BBM medium and prominent growth seen in the dilution ratio of 3:1 (ACM: BBM) with an increase in biomass after the 9th day up to the exponential phase. The ACM (no dilution with BBM) exhibit the rise in the biomass growth from the initiation of log-phase same as ACM [diluted with BBM in the ratio (3:1)] and after that, ACM (with no dilution) has lesser biomass growth when reaching near to the static phase of cultivation.

The *D. armatus* growth in the 3:1 (ACM: BBM) showed a sudden increase at the end of the log phase, whereas BBM medium (control) and ACM (no dilution) showed a fairly similar trend at the static phase of cultivation. Based on the drawn conclusion from Figure 4.2, the 3:1 (ACM: BBM) showed fine growth of *D. armatus* compared to other dilutions 2:2, 1:3 (ACM: BBM) ratio and control media (intact ACM and intact BBM medium). The best appropriate dilution of ACM was utilized for further *D. armatus* dynamic growth and biocommodities production.

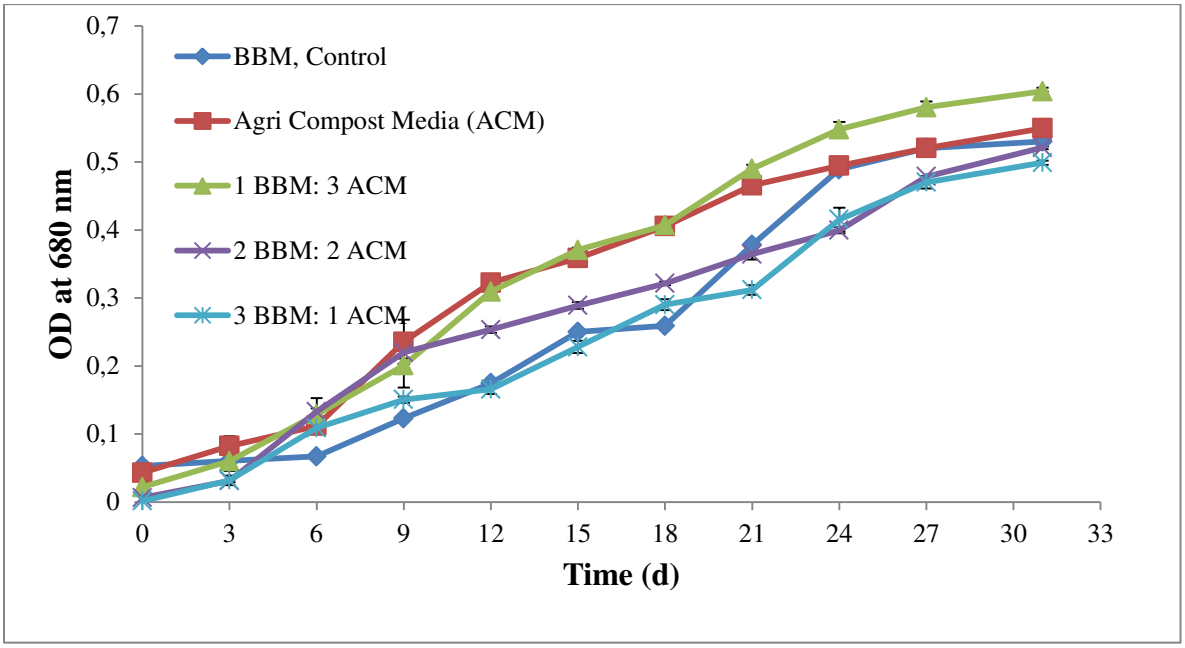
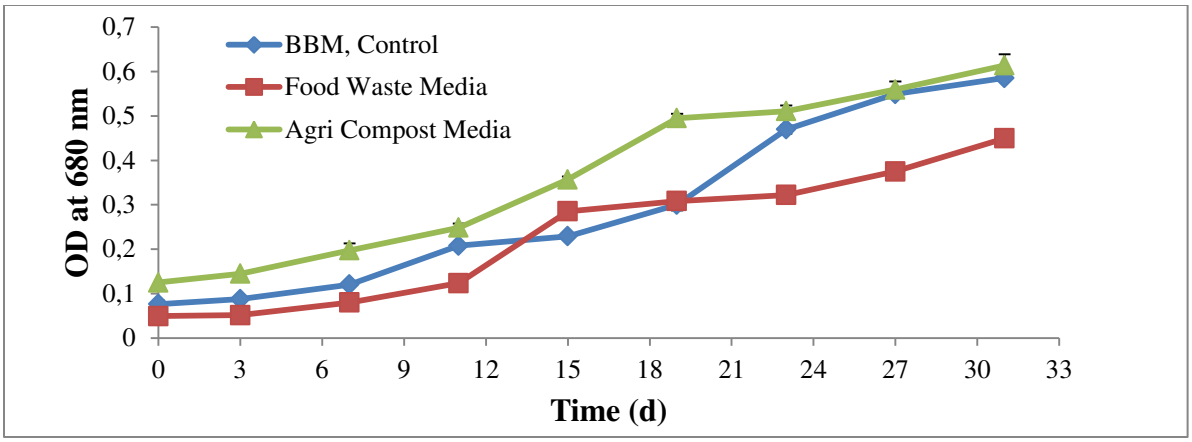


Figure 4.2: Cultivation of *D. armatus* in different concentration of organic (agricultural) and inorganic medium (BBM). All values are represented as ± s. d of triplicate experiments.

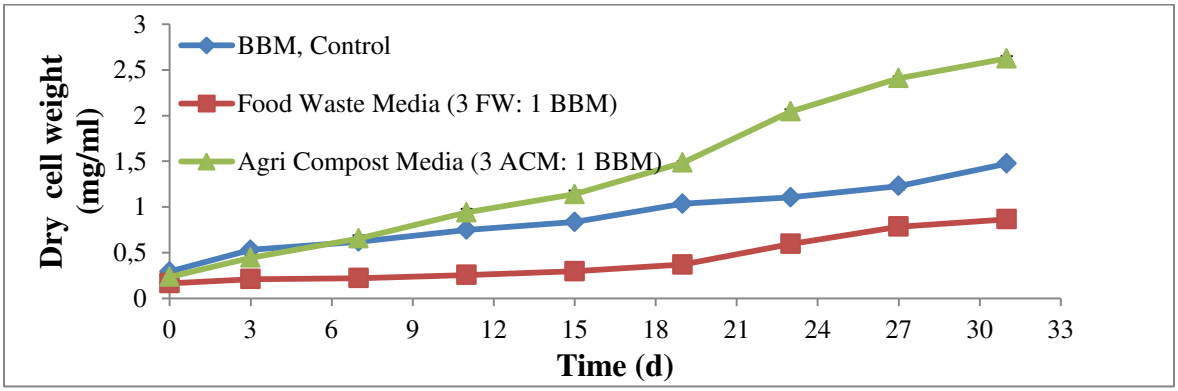
4.2.3 *D. armatus* cultivation in 3FWM/ACM: 1BBM ratio

The *D. armatus* growth is depicted in Figure 4.3 in the selected ratio of 3:1 (FWM/ACM: BBM) and intact BBM. Figure 4.3 (a) showed the growth of *D. armatus* through optical density at 680 nm in the selected concentration (3 FWM/ACM: 1 BBM). The *D. armatus* growth in the FWM did not show that much biomass enhancement in the first week of cultivation and after that end of a second-week sudden rise in the biomass growth. The cultivation in the BBM exhibited the speeding up in the growth when coming into the exponential phase. After 12 d, there is a considerable boost in the growth till the stationary phase. The *D. armatus* culturing in the ACM showed maximum biomass growth compared to the other two media (FWM and BBM) growths. The dry cell weight of *D. armatus* was evaluated every 3 d for one month same as optical density measurement. The initial weight of the *D. armatus* in BBM came up to 0.292 mg/ml, and with an increase in the cultivation perioid (d), its growth reached 1.47 mg /ml towards the end of the growth cycle.

The cultivation in the FWM illustrates lesser growth of *D. armatus* in co-relation with the ACM and BBM. The cell weight of dried *D. armatus* in the ACM (Figure 4.3 (b)) showed an increase in the biomass at the beginning of the cultivation. The dried cell weight measured around 0.235 mg/ml on the initial day and 2.625 mg/ml on the end days of the growth phase in the ACM. The cell count studies of *D. armatus* in the three media showed approximately 74×10^4/ml, 72×10^4/ml, and 73×10^4/ml cell count at the start of the growth cycle in BBM, FWM, and ACM, respectively. With the increase in the culturing days, the cell count also increased concerning the cultivation medium and maximum cell count examined in the ACM having 1285×10^4/ml cell count and 19-23 d of *D. armatus* cultivation. The FWM and BBM have lesser cell counts of *D. armatus* 1146×10^4/ml and 1180×10^4/ml, respectively. Later the *D. armatus* came into the static phase, and lesser growth of microalgal cells was observed after the 23rd d of the cultivation.



(a)



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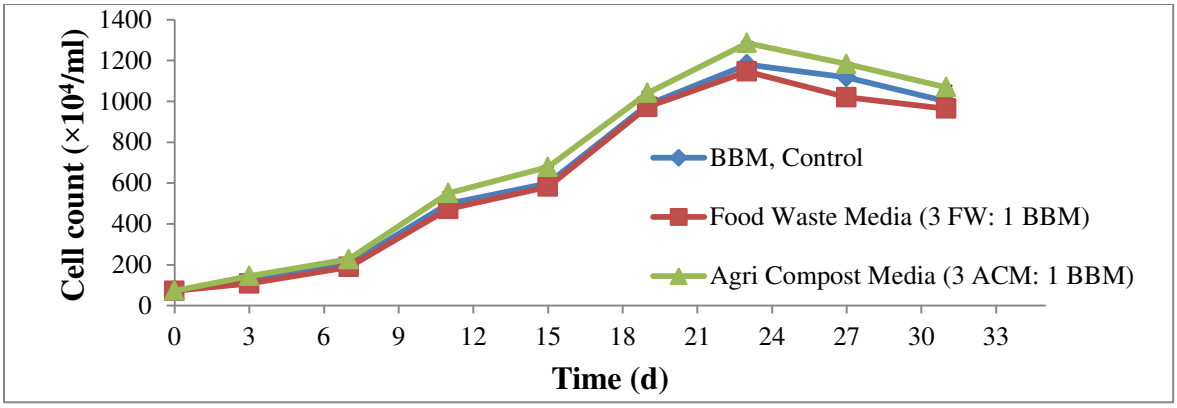


(c)

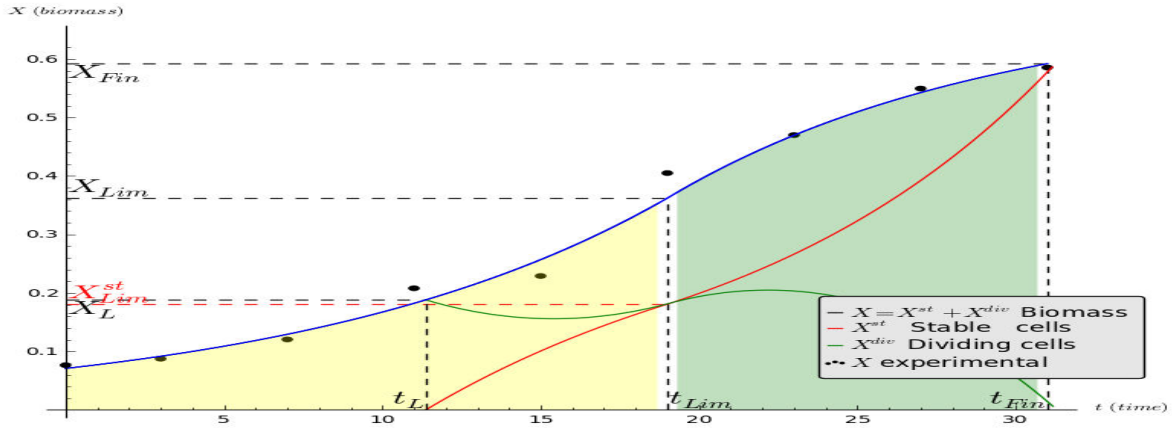
Figure 4.3: *D. armatus* cultivation in the selected concentration (3:1) of food waste and agri-compost medium and BBM as control (a) OD at 680 nm (b) Dry cell weight (c) Cell count. All values are represented as ± s.d of triplicate experiments.

Compared with all three media (FWM, ACM, BBM), the *D. armatus* showed slighter higher growth in the ACM. The ACM diluted with BBM possibly fulfilled microalgae's growth nutrients requirements and showed maximum biomass in the exponential phase. The compost (soy-bean extract and refuse extract) cultivates *C. pyrenoidosa* and *S. quadricauda* and maximum biomass formation [29]. The vermiwash (leachate of vermicomposting) abundant source of nutrients utilized as an economical, alternate source of microalgae cultivation. The Cyanophyceae and Chlorophyceae microalgae effortlessly grow in the vermi-wash different concentrations (25-100%) [30]. The *C. vulgaris* efficiently grow in the different concentration of peat moss and animal-based compost in dilution with water [31].

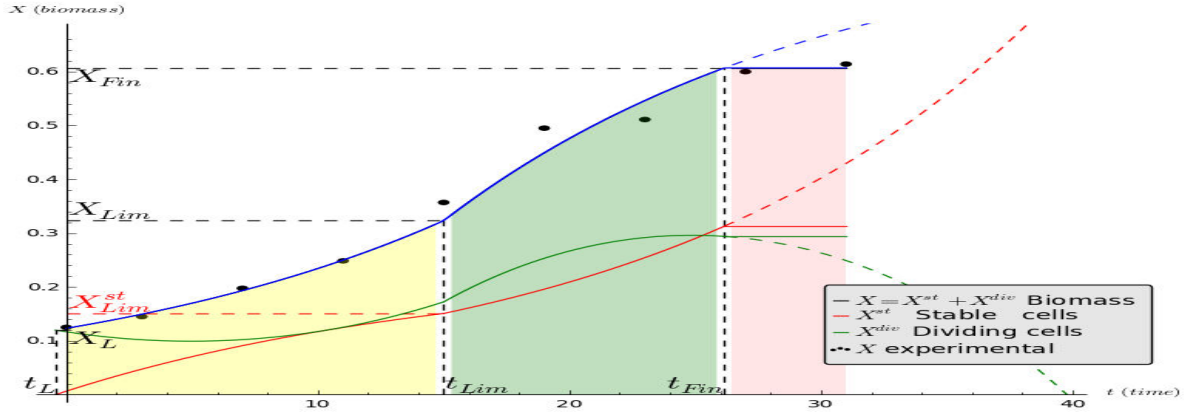
4.2.4 Biofabrication of *D. armatus* growth kinetics through "biofermentor" tool

The *D. armatus* bio fabrication studies in diluted ACM (dACM)/ FWM (dFWM), and BBM are depicted in Figure 4.4. It can be seen in Figure 4.4 (a) and (c), i.e., BBM and dFWM variants have a value of R_{Final} as R_{Final} = 1 or close to 1 at the end of the process, in contrast to (b) dACM variant at R_{Final} ~0.5. This calculation suggested that the fermentation process in (b) dACM is not completed. Additional optimization in this variant can be done, and a higher yield of algae biomass can be obtained (Figure 4.4). dACM as a cultivation media may formulate the fermentation process lengthens by approximately 10 h. Still, the result on algae biomass yield will be about 25% higher as it cleared from Figure 4.4 by way of a comparing of the biomass yields, which is around 0.6 units for BBM and dFWM (Figure 4.4 (a) and (c) and the expected biomass yield of ~ 0.75 units for (b) dACM option with a final time of 40 h of growth.

As illustrated in Table 4.1 equations and Figure 4.4 (a), (b), and (c), the end time of the processes, t_{Final}, in variants (a) and (c) occurs earlier than for option (b). The transition time between LGP and GIP, t_{Lim} (termination time of exponential phase) is more optimal in dACM (b) and dFWM (c) variants. The stable cell concentrations, X_{Lim}st has the highest values for BBM (a) variant. This suggests that the initial conditions for cell growth were more preferable than in the other two variants. But, these conditions quickly ended and the general feature of the process BBM (a) is slightly lower than for options dACM (b) and dFWM (c) (Table 4.1).



(a)



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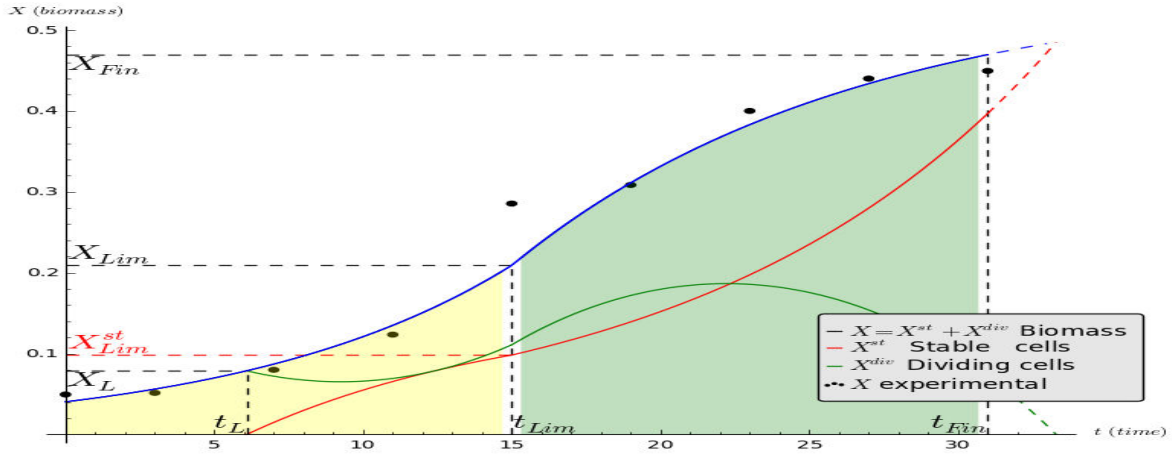


(c)

Figure 4.4: Growth kinetics studies of *D. armatus* through biofermentor tool fabrication grown in (a) BBM (b) Agri-compost media (3 ACM: 1 BBM) (c) Food Waste media (3 FW: 1 BBM)

Table 4.1: Description of different growth phases of algae in BBM, dACM and dFWM.

Growth curve phases information	BBM (Control)	dACM(3 ACM: 1 BBM)	dFWM (3 FWM: 1 BBM)
LGP (Logarithmic Growth Phase)	Total theoretical biomass for the LGP: (0-19 d) X=0.0709*EXP[0.0858 *t]	Total theoretical biomass for the LGP: (0-15 d) X=0.123*EXP[0.0643*t]	Total theoretical biomass for the LGP: (0-15 d): X=0.0404*EXP[0.11*t]
GIP (Growth inhibition phase)	Total theoretical biomass for the GIP(19-31 d): X=0.697-(0.697-0.362)*EXP[-0.0968*(t-19)]	Total theoretical biomass for the GIP(15-31 d): X=0.871-(0.871-0.323*EXP[-0.0654*(t-15)]	Total theoretical biomass for the GIP(15-31 d): X=0.555-(0.555-0.209)*EXP[-0.0873*(t-15)].
Non-dividing (stable) cells for GIP	Non-dividing (stable) cells for GIP (19-31 d): X(st)=0.181*EXP[0.0968*(t-19)].	Non-dividing (stable) cells for GIP (15-31 d): X(st)=0.151*EXP[0.00564*(t-15)].	Non-dividing (stable) cells for GIP (15-31 d): X(st)=0.0982*EXP[0.00873*(t-15)].

The results of Table 4.2 are in complete concurrence with Table 4.1 and Figures 4.4 (a) (b) and (c) above. The rapid growth of *D. armatus* in BBM (a) stops rapidly. The dACM (b) attained a low R_{Final} value. The *D. armatus* cultivation in dFWM (c) gives a lower yield of biomass. The dACM (b) can get higher *D. armatus* biomass yields by feeding the nutrient concentrates of the same compositions to allow cells to complete the division. The BBM (a) and dFWM (c) can also be considered as promising alternative media due to the short growth processes. However, the *D. armatus* higher growth is not simply attained through simple nutrient balancing but can improve by using some physical factors (mixing) that could help increase efficiency.

Table 4.2: Growth kinetic parameters of microalgae grown in BBM, dACM and dFWM

Growth Kinetic parameter	**Growth Media**		
Growth parameter	**BBM**	**dACM (3 ACM: 1 BBM)**	**dFWM (3 FW: 1 BBM)**
Initial biomass concentration (X_0) (g/l)	0.0709	0.123	0.0404
Maximum specific growth rate of a biomass, X (μ_{max})	0.0858	0.0643	0.11
Termination time of exponential growth phase (t_{Lim}) (d)	19	15	15
The end of a Logarithmic growth phase, LGP and beginning of growth inhibition phase, GIP (X_{Lim})	0.362	0.323	0.209
Concentration of stable cells at the end of exponential growth phase (X_{Lim}^{st})	0.181	0.151	0.0982
Ratio of energy consumed for growth of biomass to total expenses of energy (A)	0.0968	0.0654	0.0873
Theoretical maximum biomass concentration (X_p) (g/l)	0.697	0.871	0.555
Time moment for a final biomass concentration, X_{Fin} (t_{Fin}) (d)	31	26.2	31
Ratio between dividing and non-dividing cells for a t_{Fin} (R_{Fin})	0.974	0.534	0.856

4.2.5 Nitrogen (TKN) and phosphorus content assessment in dFWM, dACM and BBM inoculated with *D. armatus*

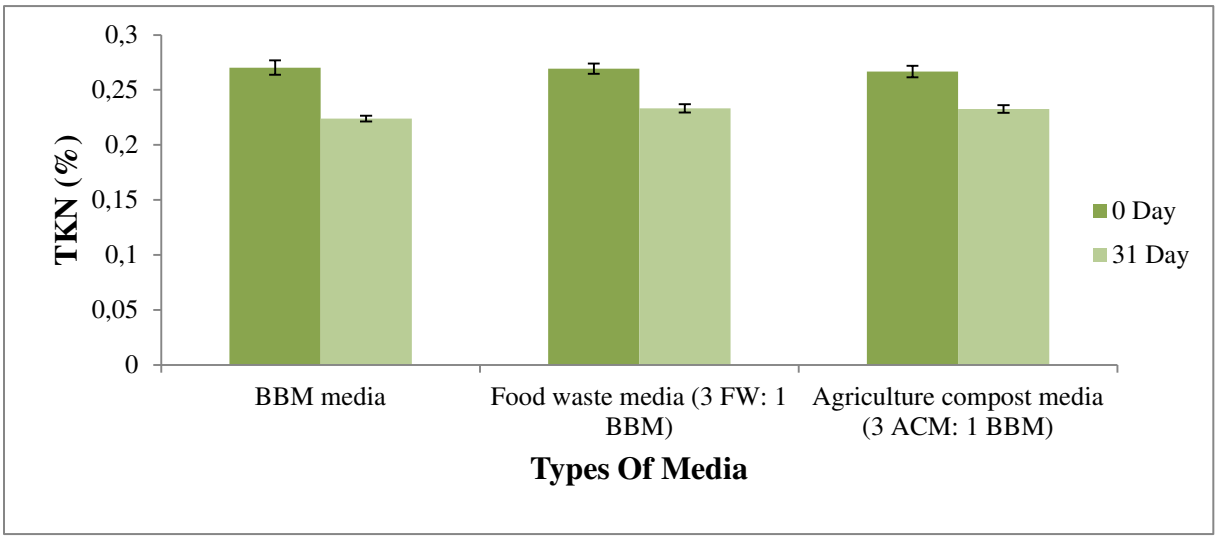
The dFWM and dACM serves as a prominent growth media for *D. armatus* was further assessed for the nitrogen and phosphorus content at the start and end of the cultivation days. Figure 4.5 (a) depicted the nitrogen estimation in the dFWM, dACM and compared with the standard BBM.

The results showcases the reduction in the TKN content in dFWM, dACM and BBM at the end days of *D. armatus* cultivation (31 d). The TKN removal estimated around 0.232% (31 day) from the initial values 0.266% (0 day) in dACM whereas in dFWM, its removal was 0.233% (31 d) from 0.269% values (initial) (Figure 4.5 (a). The relative % of TKN removal in the dACM was around 87.21% observed concerning the initial days of *D. armatus* cultivation (Table 4.3). There is less nitrogen removal in dFWM, dACM, and BBM at the end of *D. armatus* cultivation. The removal of nitrogen depends on forms (nitrate, nitrite, ammonia, organic nitrogen, ammonium ions) and nitrogen concentration in different cultivation mediums.

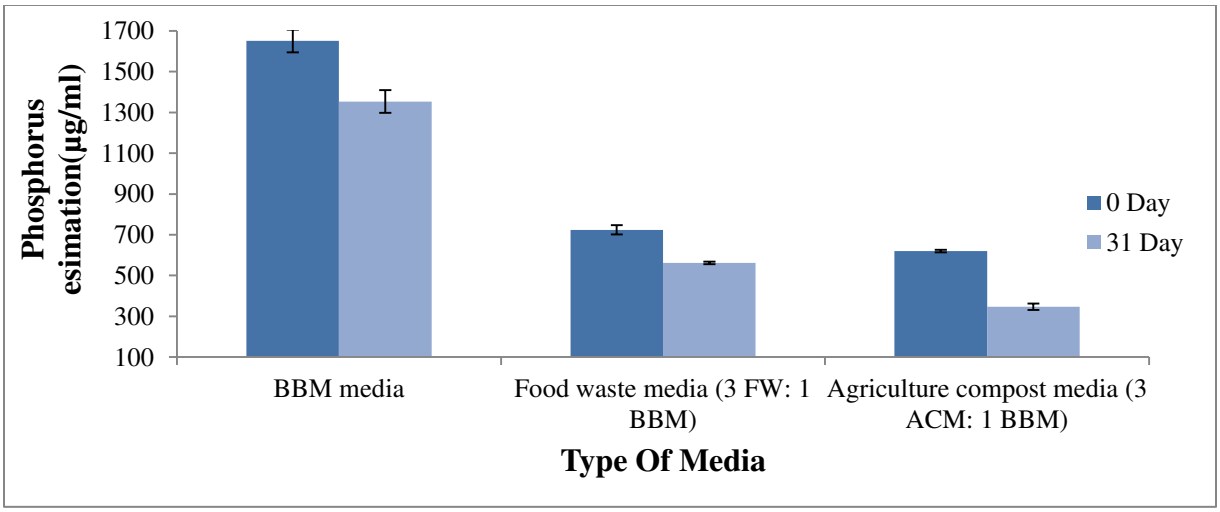
The efficiency of nitrogen reduction is significantly impacted by numerous factors such as color, density, and ambiguousness of the cultivation medium. These factors make the medium turbid and dark, resulting in less light diffusion inside the microalgae cell and small nitrate uptake in the cultivated medium [32]. The insufficient quantity of carbon in the growth medium for cultivation of *Auxenochlorella protothecoides* UMN280 affects the ammonium removal (lesser removal). The opaqueness of the cells perturbs the light intrusion inside the microalgal cells [33]. The different microalgae strains utilize the various forms of nitrogen, i.e., nitrate, ammonium, nitrite, and urea. The microalgae *Chlorella vulgaris* and *Scendesmus obliquus* sort of microalgal strain uptake the more significant ammonium ions in the wastewater compared to the other form of nitrogen [34–36]. The microalgae growth firmly depends on the N and P elements present in the cultivation medium and these nutrients utilized by microalgae for its growth [37]. The BBM consists of a higher amount of phosphorus when analyzed on the first day of *D. armatus* cultivation compared with dFWM and dACM. The dACM exhibited a more amount of phosphorus uptake in comparison with the usual BBM [38].

As shown in Figure 4.5 (b), with each transitory day of *D. armatus* cultivation, there is a reduction in the phosphorus content in the respective medium and a higher reduction observed in the dACM, approximately 347.333 µg/ml at the final day of cultivation. The relative % of phosphorus removal attained around 56.02% in the dACM concerning initial day value (Table 4.3). The microalgae have the potential to utilize enough phosphorus content for biomass production and increase its growth. The microalgae also tend to store the phosphorus within the microalgal cells and utilize it while there is a shortage of phosphorus in the medium. Some microalgae, like *chlorella vulgaris* culturing in the food waste medium, use a minor quantity of

phosphorus for its growth than the composted medium [16]. The microalgae cultivation in increased compost concentration enhanced the overall phosphorus removal up to 50% [5].



(a)



(b)

Figure 4.5: (a) Total Kjeldahl nitrogen, TKN (%) (b) Phosphorus concentration (µg/ml) in BBM, dFWM and dACM at initial and final day of *D. armatus* cultivation. All values are represented as ± s.d of triplicate experiments.

Table 4.3: Relative (%) of nitrogen and phosphorus in dFWM and dACM

dFWM (3 FWM: 1 BBM)			
Element	**Initial amount**	**Final amount**	**Relative %**
Nitrogen (%)	0.26±0.004	0.23±0.003	86.61%
Phosphorus (µg/ml)	724.33±22.81	562.33±6.11	77.63%
dACM (3 ACM: 1 BBM)			
Element	**Initial amount**	**Final amount**	**Relative %**
Nitrogen (%)	0.26±0.005	0.23±0.003	87.21%
Phosphorus (µg/ml)	620±7	347.33±15.30	56.02%

4.2.6 Biochemical contents (lipids, carbohydrates and proteins) determination in dFWM, dACM and BBM

In further experiments, the *D. armatus* culturing in the selected ratio 3:1(FW/ACM: BBM) of food waste, agri-compost and standard BBM medium was also scrutinized to estimate carbohydrates, lipids, and proteins.

4.2.6.1 Lipid content estimation in the dFWM, dACM and BBM variants

The lipid estimated in the dFWM and dACM and calculated its relative % concerning the standard BBM in terms of weight %. The *D. armatus* growth in dACM exhibited higher lipid content, 9.925 wt% approximately in the end day of its cultivation compared to the other two media (dFWM and BBM). The lipid content profiling in dACM grown algal biomass illustration was depicted in Fig. 4.6. The organic substances abundance in the medium increases the lipids reserve inside the microalgae [39]. From Figure 4.6, the BBM medium has 7.035 wt% lipids content which is higher than microalgae cultivated in the dFWM and less than the dACM. The

relative % of lipids of 141.08% was observed with *D. armatus* biomass grown in dACM concerning BBM as a control medium for relative % estimation (Table 4.4). The minute nutrients' existence in the medium straightly escalates the biochemical constituents and lipids formations in the microalgae. The dilution of FWM / ACM with the BBM (in 3:1 ratio) media serves as a better culturing media for lipid production. The lipid content came around 215 mg/g-219.7 mg/g when microalgae were cultivated in the waste food compost [5].

As depicted in Fig. 4.6, the lipid content of *D. armatus* biomass grown in dFWM was found to be 5.815 wt% (31 d). The variance in lipid content (wt %) with different media is mainly attributed to the color and density of the utilized growth media, although provided the similar growth facilities for same cultivation time. Light intensity is one of the aspects for proper microalgae growth. The color and medium opaqueness influence the light dispersion inside the medium and halter the growth of microalgae and eventually on the lipid formation [5,40]. The lipids formation from the microalgal biomass consists of 37.6 kJ/g lipids and is further employed to produce biodiesel [41].

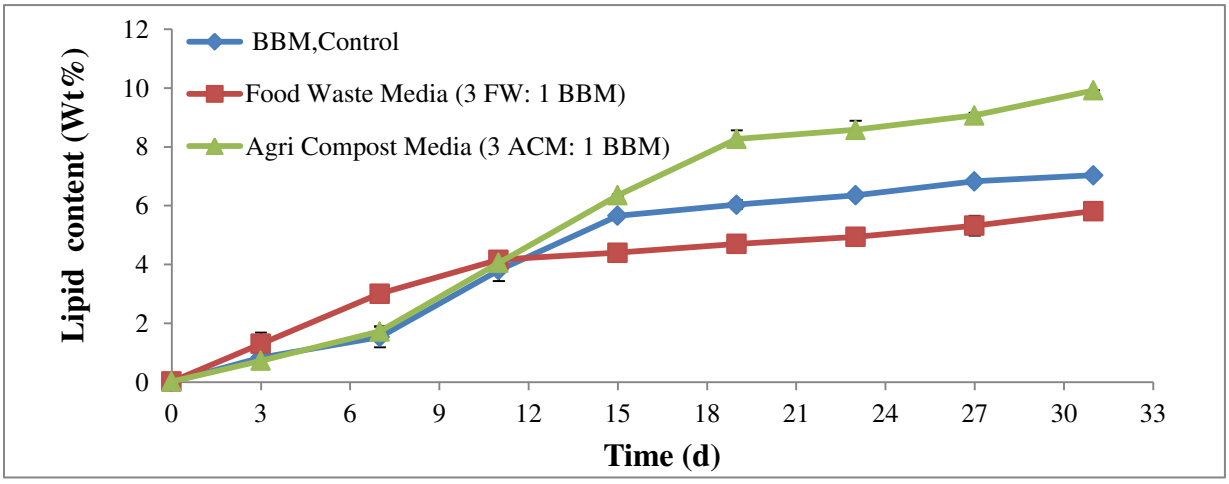


Figure 4.6: Lipid content (wt %) profiling with the *D.armatus* biomass grown in dFWM, dACM and BBM. All values are represented as ± s.d of triplicate experiments.

4.2.6.2 Carbohydrates content estimation in the dFWM, dACM and BBM variants

The microalgal cell wall has cellulose and plastids, and other polysaccharides that reserve starch inside the cell as a carbohydrates resource. The microalgae can store approximately 50% of cellulose and starch inside the cell wall when grown in a suitable culturing environment [42].

The carbohydrates amount and production also strictly rely on the medium type and conditions for the microalgae cultivations. The maximum carbohydrate content of 8.75µg/ml was estimated in the *D. armatus* grown in the dFWM compared with other media (dACM and BBM) (Fig. 4.7). As shown in Fig. 4.7, there is no such enhancement in the carbohydrate content up to the 12th d in the dFWM, and after that, it starts increasing. In co-relation with the other two media, i.e., dACM and BBM, exhibited the same tendency of carbohydrates formation 3.6µg/ml and 3.95µg/ml, respectively, which was lesser in amount than the dFWM variant at the end day (31st d) of *D. armatus* cultivation. The relative % of carbohydrate content of *D. armatus* biomass grown in dFWM was around 221.51% (maximum) compared with dACM variant and the relative % of carbohydrate content was found to be 91.13% while compared with the BBM variant (Table 4.4).

The amount of carbohydrates content rigorously relies upon the microalgal sp. The *Spirogyra* consists of 33-64% of carbohydrate content [43] and whereas *Chlorella vulgaris* has 21% of carbohydrate content [21]. Another research showed that when mixed with the usual inorganic medium, composted food consists of a higher amount of carbohydrate content. The mechanism is paired with carbon absorption and further carbohydrate accumulation in the microalgae cells. Higher organic carbon content in the food waste supported more carbon assimilation in the microalgae cells and ultimately enhanced the carbohydrates accumulation in the microalgae [44]. The carbohydrate content 197.2 mg/g-346.5 mg/g is estimated in *C. vulgaris* cultivated in various concentrations of food waste medium [5].

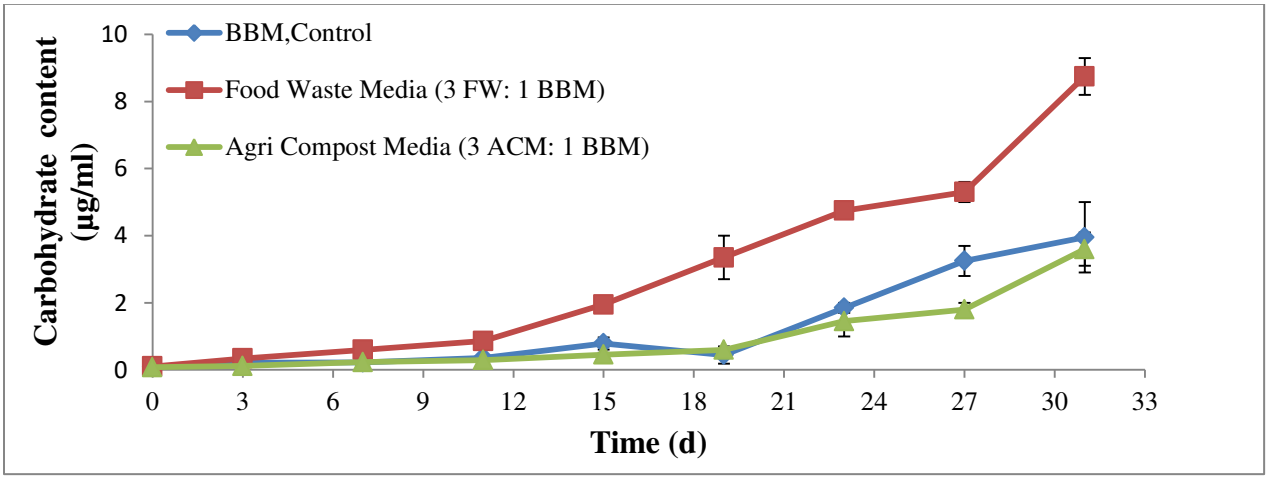


Figure 4.7: Carbohydrate content (µg/ml) profiling with the *D.armatus* biomass grown in dFWM, dACM and BBM. All values are represented as ± s.d of triplicate experiments.

4.2.6.3 Protein content estimation in the dFWM, dACM and BBM variants

The protein content with the microalgal biomass varies with the sp. and culturing conditions of microalgae. Usually, microalgae consist of 40- 50% protein content [45]. Fig. 4.8 illustrates the protein content of *D.armatus* biomass grown in the dFWM, dACM and BBM. In Figure 4.8, the *D. armatus* cultivated in the dACM did not show the much protein content till the 15^{th} d, and after that, it was enhanced to106 µg/ml on the 31^{st} d of cultivation. The maximum protein content was analyzed at approximately 112.5µg/ml in the BBM compared to dFWM and dACM. The *D. armatus* cultivation in dFWM and dACM has nearly similar protein content (Fig. 4.8). The relative % of protein content was 94.22% calculated in *D. armatus* biomass cultivated in dACM compared to BBM as a control media (Table 4.4). The protein content in microalgae depends on the nitrogen abundance in the culturing medium and enhances the overall proteins synthesis inside microalgal cells [46]. The microalgae cultivated in kitchen digested waste have lesser protein content than the BG-11 medium; BG-11 consists of a higher total nitrogen concentration than other diluted mediums [28]. In most research investigations, the food waste has a higher quantity of proteins, around 60-100 mg/g [47], which serve as the foremost nutrient resource for the microalgal growth [16]. The highest protein content of *D. armatus* biomass grown in BBM, dFWM and dACM was found to be equal to the 112.5µg/ml, 103.5µg/ml and 106µg/ml, respectively. The *C. vulgaris* shown a maximum protein content of around 70.3 mg/g-128.4 mg/g in the various diluted concentration of food waste medium [5]. These protein evaluation studies showed that the combination of FWM, ACM with BBM has showed similar protein content to the usual BBM.

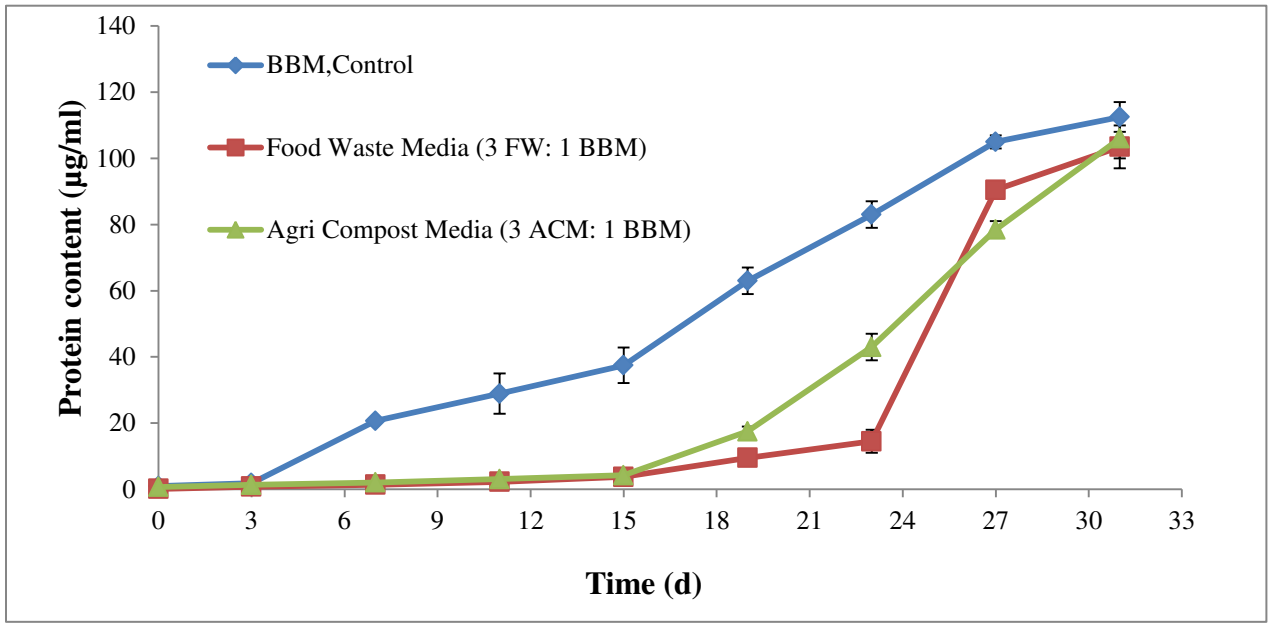


Figure 4.8: Protein content (µg/ml) profiling with the *D.armatus* biomass grown in dFWM, dACM and BBM. All values are represented as ± s.d of triplicate experiments.

Table 4.4: Relativity (%) of lipids, carbohydrates and protein contents of algal biomass grown in dFWM/dACM Vs BBM (31st d).

Compound	BBM (100%)	dACM (Max. Amount, % relativity with BBM variant)	dFWM (Max. Amount, % relativity with BBM variant)
Lipids (%)	(7.03±0.02, 100%)	(9.92±0.01, 141.08%)	(5.81±0.02, 82.65%)
Carbohydrates (µg/ml)	(3.95±1.05, 100%)	(3.6±0.5, 91.13%)	(8.75±0.55, 221.51%)
Proteins (µg/ml)	(112.5±4.5, 100%)	(106±6, 94.22%)	(103.5±6.5, 92%)

4.3 Conclusion

The microalgae with the diverse industrial applications can be easily cultivated in the waste, and its biomass can be used to produce co-added products. The present study proposed the usage of food waste and agriculture compost as an alternative source for cultivating *D. armatus*. The microalgae are grown in the different dilutions of FWM, ACM, and standard BBM. The *D. armatus* shown good growth in diluted ratio of 3 FWM/ACM:1BBM compared with standard BBM for 31st d of cultivation. The growth kinetic study through "fermentor" tool software notified the dACM could be considered as an excellent alternative medium for the *D. armatus* growth compared with other media (dFWM and BBM). The *D.armatus* biomass grown in dFWM, dACM and BBM were further analyzed for lipid, carbohydrate and lipid contents. The higher lipid content (9.925%), protein content (112.5µg/ml) and carbohydrate content (8.75µg/ml) was found with the *D.armatus* biomass grown in dACM, BBM and dFWM, respectively. Overall, the present study reveals the use of the food waste and agri-compost regarded as low-priced and very nutritive sources for *D. armatus* cultivation and the formation of value-added products.

CHAPTER 5

Bioremediation Studies of X-Ray Developer Solution by *D. armatus* using BBM

CHAPTER 5

Bioremediation Studies of X-Ray Developer Solution by *D. armatus* using BBM

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5.1 Introduction

The radiography derive as essential segment towards progress in X- ray facilities, mammograms and CT scan to recognize the health related issues in medical labs and hospitals [1, 2]. During the radiographic film methodology, the steps involve developing, fixing and washing which forms enormous quantity of solids and liquid pollutants. The X-ray processing chemicals from the medical wards, nursing homes/ hospitals has huge values of physical and chemical parameters such as turbidity, total solids, pH, biological and chemical oxygen demands limits [3, 4]. In radiographic processing, the silver metal retains an important place in the development of X-ray images. The formation of dissolvable Ag from the X-ray labs is typically solid waste and their erroneous approaches of dumping into environment have precarious effect [2]. The inadmissible regulation of the radiographic squander which has huge quantity of inorganic and organic contaminants and silver element affects the water resources, fisheries and marine life. Therefore the incomplete information of radiographic solution management and risk associated with inappropriate disposal of solution have to be look out in an eco-friendly way for eradicating the radiographic solution from non toxic category [5]. The researchers utilizes varied methodology such as electrolysis, metallic replacement and precipitation to retrieve the Ag from radiographic solution and majority of these techniques failed because of their harmful byproducts, higher cost to collect the pure silver from crude and huge operational cost [6]. Thus, there is the prerequisite of the competent technique that effectively eliminate the heavy metals in a sustainable mode [7, 8]. In comparison with other assorted biological methods, microalgae seems to be more efficient in waste water treatment and have prominent attributes such as photosynthetic nature, higher growth rate, environment friendly and easy cultivation [9, 10]. The microalgae have property to metal ions uptake and disinfect the different waste water [11] and formation of various bio products [12–14]. The microalgae decomposed the harmful compound present in the landfill sites and microalgal biomass will form various macromolecules, functioning as substrate in

biofuels, pharma and food segments [15, 16]. The waste categorization helps in the handling and accessibility of nutrients in waste, promotes the growth of microalgae [17]. Microalgae have potential to bear the massive heavy metal concentration for extensive period, therefore evaluating the tolerance limits of waste solutions is indispensable for fruitful outcomes in microalgae remediation investigation [18, 19]. The characterization and biological remediation of radiographic developer solution is a lesser explored research area due to existence of silver and precarious chemical composition of inorganic/organic contaminants. The green microalgae Desmodesmus sp. has substantial applications as remediation of heavy metals and production of biofuels in economical manner [20]. The microalgal based bioremediation and simultaneously production of lipids from microalgal biomass [21, 22] supports feasible feedstock for biodiesel formation by the process of transesterification [23, 24]. From various radiography labs, the extravagant contaminates and toxic silver presence in waste radiographic developer solution requires eco friendly green techniques to remove these contaminates and silver content. The present studies primarily focused on feasible green method utilization towards the microalgal remediation of unexplored waste radiographic developer solution and simultaneously with lipids production.

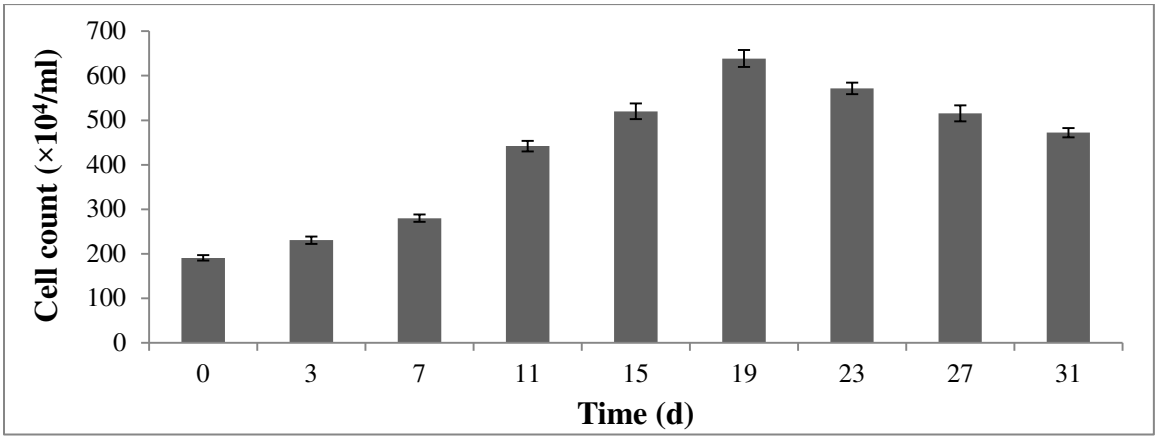
5.2 Results and discussion

5.2.1 Microalgal remediation of X.D with *D. armatus*

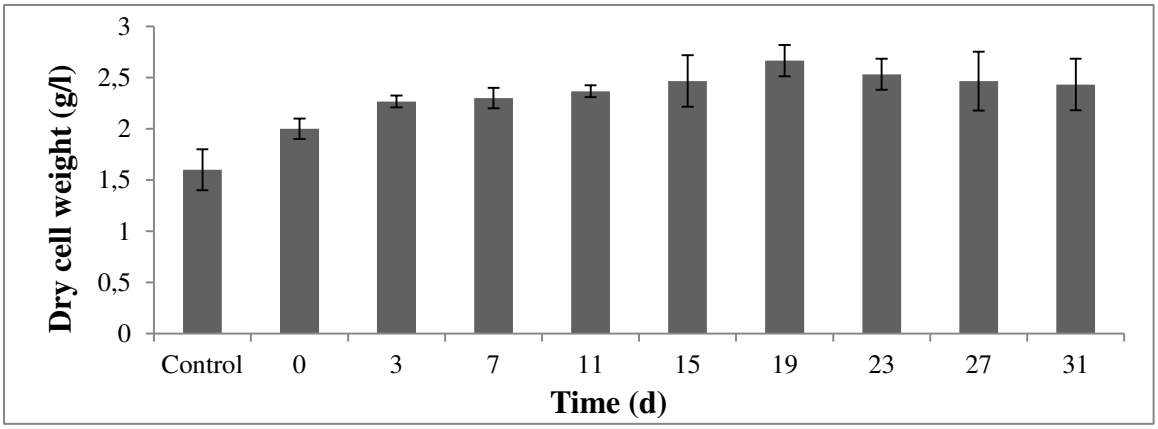
The preliminary experimentation persisted on evaluation of *D. armatus* toxicity in various dilutions of X.D with BBM found that 3 BBM:1 X.D ratio is suitable for bioremediation studies. The aquatic *D. armatus* has distinctive features such as photosynthetic ability coupled rapid growth in waste solutions, suitable for the progress towards phycoremediation investigation. The diluted X.D (dX.D, 3 BBM:1 X.D) will serve as a cultivation medium for microalgae-based remediation and dX.D (without microalgae) serves as a control for the phycoremediation analysis. The characterizing limits of waste X.D were measured for one month period in both manners; before and afterward treatment with microalgae.

5.2.1.1 Growth kinetics of *D. armatus* in dX.D

The exploration of *D. armatus* growth in d X.D depicted in the Figure 5.1 (a) and (b). In the cell count and dry cell weight computation, the higher cell count and dry cell weight of *D. armatus* was found on the 19th d and growth of microalgal cell escalating with cultivation period.



(a)



(b)

Figure 5.1: Depiction of *D. armatus* growth in dX.D (3 BBM:1 X.D.) (a) Cell count (×10^4/ml) (b) Dry cell weight (g/l). All values are represented as ± s.d of triplicate experiments.

After attaining the higher growth on 19th day the *D. armatus* cells acquired a consistent state (stable/stationary phase) in dX.D. Usually, microalgal growth relies on a number of cultivation factors including temperature, culturing medium, light and cultivation period. The medium opaqueness created the obstacles in passing of light within the microalgal cell; as a consequence

microalgal growth was inhibited [25]. Microalgae *D. armatus* readily cultured in mixotrophic solution conditions, and BBM displayed more prominent expansion and phycoremediation efficacy [26]. The nature of cultivation medium is one of the considerable parameter and microalgae *D. communis* exhibited the appreciable growth with change in the cultivation medium [27]. The *D. armatus* dry cell weight enhanced in light period from 36 to180 pg in strain B1-76 and 30 to 228 pg in strain 276-4d [28].

5.2.1.2 pH profiling with phycoremediation

The outline of changed pH during the *D. armatus* remediation in waste dX.D is represented in the Figure 5.2. A decreased pH was observed in the *D. armatus* treated waste dX.D in comparison with control medium (No *D. armatus* treatment). Maximum decrease in the pH level noticed on the 19th day of *D. armatus* cultivation period and pH decreased ranges from the 8.63±0.02 to 8.09±0.01 (Figure 5.2). The alkaline nature of radiographic developer solution discharged from the health centers [29], and this solution used as growth medium for microalgae culturing. The reduction in pH during the phycoremediation process occurred, because of H^+ ions release from the existed ammonium content in waste developer solution. The microalgae *Chlorella salina* and *Chlorella vulgaris* have potential to decrease the pH level while cultivated in various types of waste water. The microalgae have advantageous attributes to reduce the physicochemical parameters of the waste matter throughout the remediation process of waste water [30].

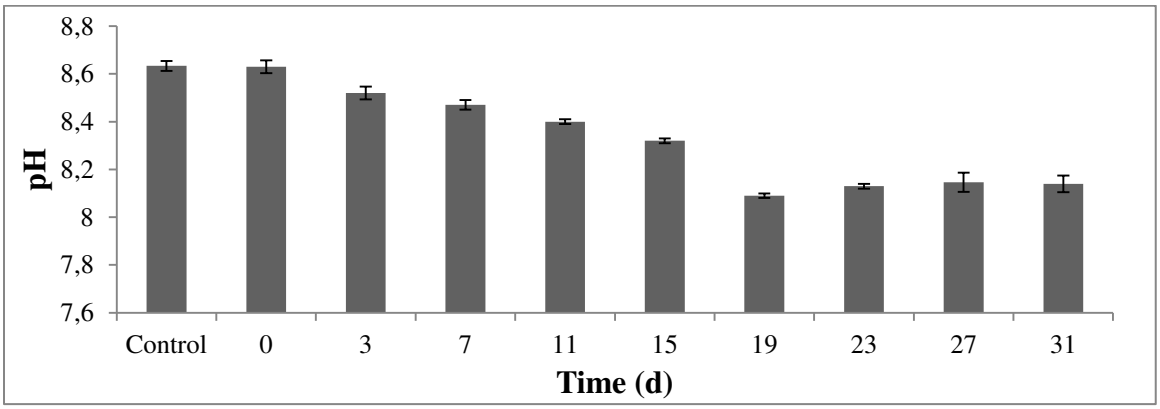


Figure 5.2: Depiction of pH in the remediated samples of dX.D. All values are represented as ± s.d of triplicate experiments.

5.2.1.3 Total solids (TS) profiling with phycoremediation

The amount of total solids represented in Figure 5.3 of control medium (dX.D without microalgae treatment) is approximately 28.08±0.104 g/l. The microalgal treatment analysis of in dX.D featured a remarkable decline in total solids values around 23.78±0.076 g/l in the log period. The foremost cause of decrease in total solids substance is utilization of pollutant like sodium, sulfates and potassium for the utility of microalgae nutrition, which ultimately decreases the preliminary total solids matter in waste developer solution. The effective depletion of total solids matter as well observed in the carton box waste solution through the treatment of microalgae Scendesmus sp. and Chlorella sp. [31].

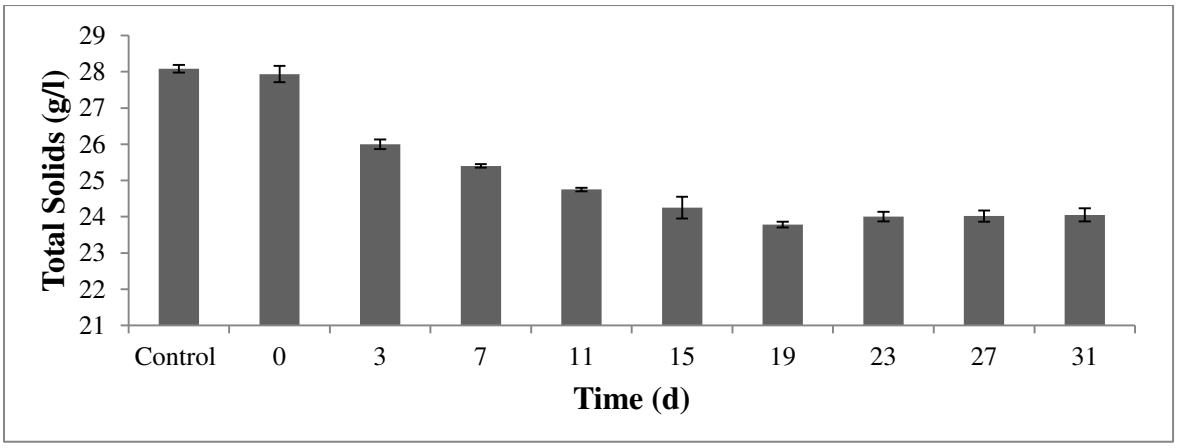


Figure 5.3: Depiction of total solids in the remediated samples of dX.D. All values are represented as ± s. d of triplicate experiments.

5.2.1.4 Total dissolved solids (TDS) profiling with phycoremediation

The values of dissolved solids interpreted with the *D. armatus* treatment to dX.D and as well without treatment of *D. armatus* microalgae (control) as shown in Figure 5.4. From the estimated results, the maximum reduction in total dissolved solids appeared to be 18.41±0.057 g/l on *D. armatus* treatment and control medium have TDS value 21.41±0.028 g/l. The complete dissolving feature of TDS in waste water makes them unable for consumption in industrial and agricultural purposes, drinking and high level of TDS carrying waste solution requires more O_2 demand. The total dissolve solids consist of potassium, magnesium, sodium, calcium, sulfates, inorganic and organic salts and as well metals. The microalgae Scendesmus sp. and Chlorella sp.

- based remediation of solutions also decrease the dissolve solids matter [31]. In mixotrophic growth conditions, the microalgae *D. armatus* decreased the dissolve solids content approximate to the 83% in cassava waste solution. The reduction in dissolve solids reported differ in hetero (intial-1960.00 ± 0.06 mg/l final-548.80± 0.03 mg/l) and mixotrophic conditions (initial-1960.00 ± 0.04 mg/l final-352.80 ± 0.01 mg/l [26].

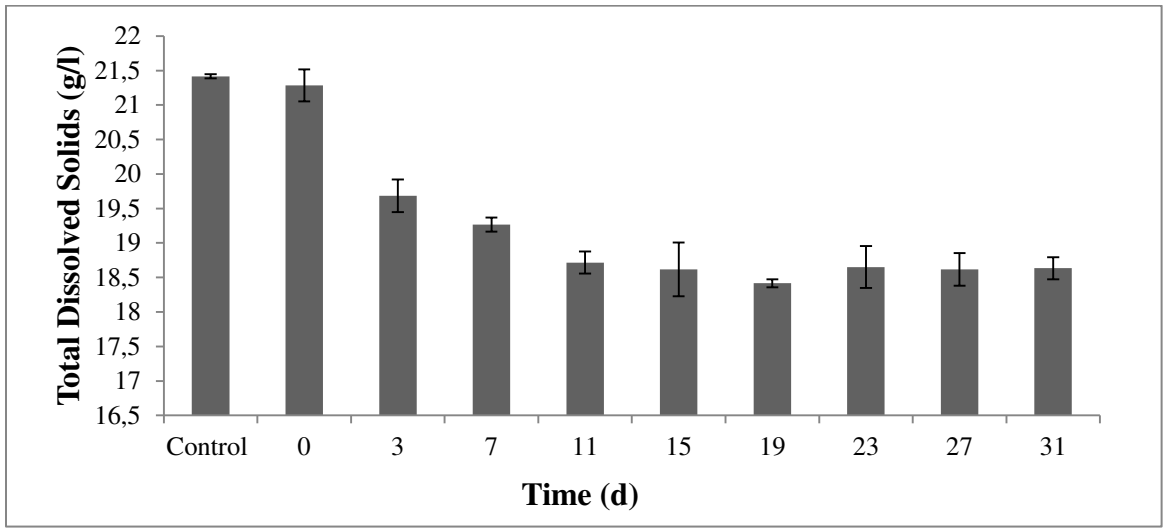


Figure 5.4: Depiction of total dissolved solids in the remediated samples of dX.D. All values are represented as ± s.d of triplicate experiments.

5.2.1.5 Total suspended solids (TSS) profiling with phycoremediation

The suspended solids typically found in floating form and very small in appearance and obstructs the light dispersion in the medium, which ultimately terminates the photosynthetic efficacy of the microalgae. The microalgae have applicability to decrease the level of TSS in remediation process and eventually it diminishes the turbidity of waste water and as well expedites the light diffusion. The Figure 5.5 showed the reduction in the TSS values in *D. armatus* treated dX.D to the 5.36±0.104 g/l from its preliminary value 6.66±0.115 g/l in control sample (devoid of *D. armatus* treatment).

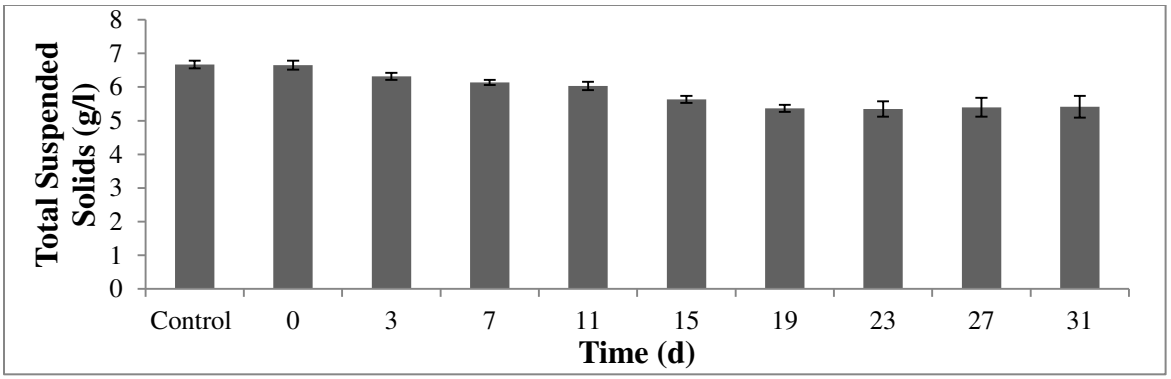
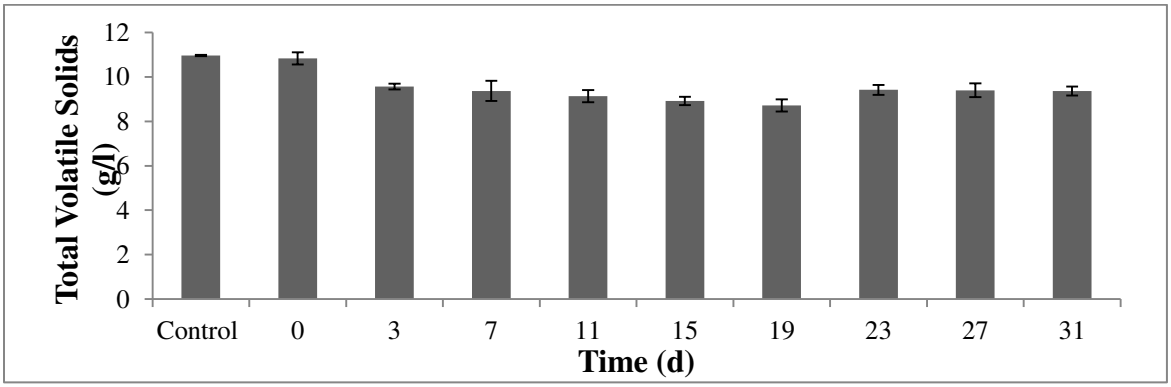


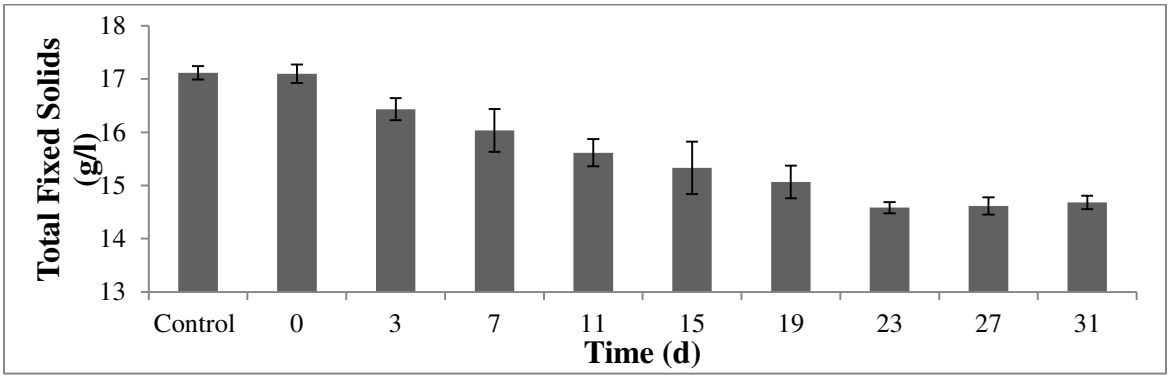
Figure 5.5: Depiction of total suspended solids in the remediated samples of dX.D. All values are represented as ± s.d of triplicate experiments.

5.2.1.6 TVS and TFS profiling with phycoremediation

The total volatile (TVS) and total fixed (TFS) trends in dX.D with microalgae remediation represented in Figure 5.6 (a) and (b) respectively.



(a)



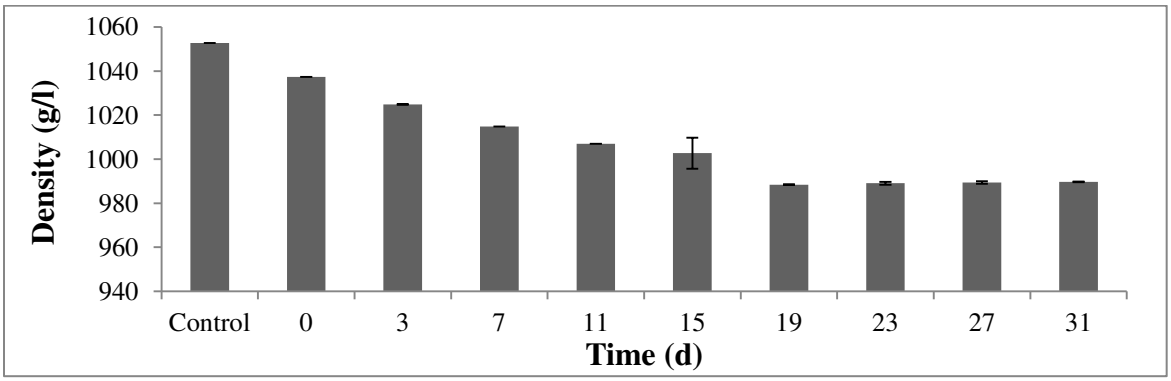
(b)

Figure 5.6: Depiction of (a) TVS (b) TFS in the remediated samples of dX.D. All values are represented as ± s.d of triplicate experiments.

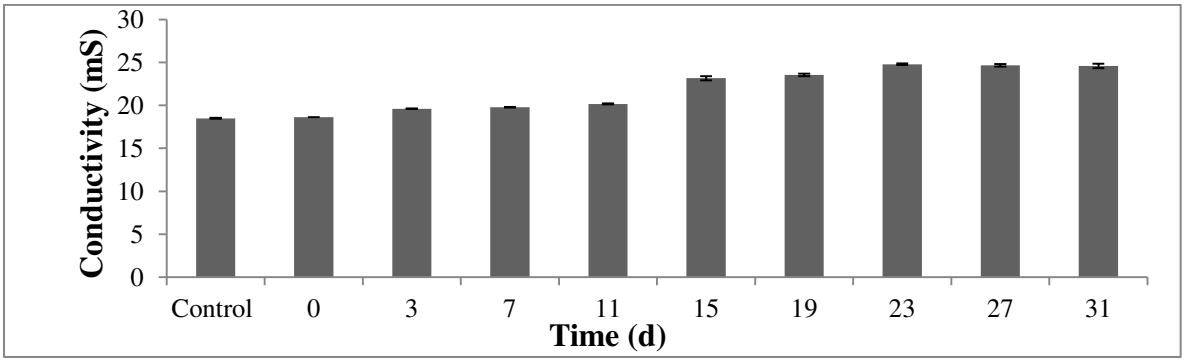
The higher value of volatile solids observed in the control medium (without microalgae) approximately 10.96±0.028 g/l and with treatment of microalgae it decreased up to of 8.71±0.275 g/l in dX.D. The reduction in the fixed solids also observed in the phycoremediated sample and it equivalents to 14.58±0.104 g/l and; 17.11±0.125 g/l value found in the control sample (without microalgae treatment).

5.2.1.7 Density and Conductivity profiling with phycoremediation

The density of dX.D depicted in Figure 5.7 (a), as the time of *D. armatus* cultivation increased in dX.D density decreased up to the extent of 988.43±0.057 g/l. The preliminary value of density 1052.8±0 g/l observed in dX.D without *D. armatus* treatment (control).



(a)



(b)

Figure 5.7: Depiction of (a) Density (b) Conductivity in the remediated samples of in dX.D. All values are represented as ± s.d of triplicate experiments.

The conductivity is ability to flow the current in particular solutions and it relies on the presence and absence of the ions. The higher conductivity value result in elevated concentration of ions

which approach latest oxygenated species in soil and water and afterward consequences is insufficiency of oxygen level in water. This O_2 insufficiency leads to disruption of flora and fauna in water [32].

The Figure 5.7 (b) shown the conductivity trends of phycoremediated dX.D with respect to control sample (no microalgae). The conductivity of phycoremediated dX.D increased up to 24.8±0.1 mS extent and where as in control sample conductivity found to be 18.5±0.05 mS. Heterotrophic cultivation of *Desmodesmus armatus* in cassava wastewater reduce the electric conductivity 998.10 ± 0.00 μs cm^{-1} from the initial value of 3680.00 ± 0.01 μs cm^{-1}. While mixotrophic cultivation in cassava wastewater of same microalgal species reduce the conductivity 621.92 ± 0.02 μs cm^{-1} from initial value 3680.00 ± 0.03 μs cm^{-1} [26].

5.2.1.8 Biological oxygen demand (BOD) profiling with phycoremediation

The BOD of various solutions signifies the pollution extent, and it's mandatory to decrease the BOD to its admissible limits before discharge in to the water sources. The BOD studies (Figure 5.8) of the dX.D showed the value of control medium (no *D. armatus* treatment) approximately 1.140±0.005 g/l and with treatment of *D. armatus* in dX.D it decreased up to 0.238±0 g/l. The organic pollutant present in dX.D utilized with time via the elevated microalgal biomass [33].

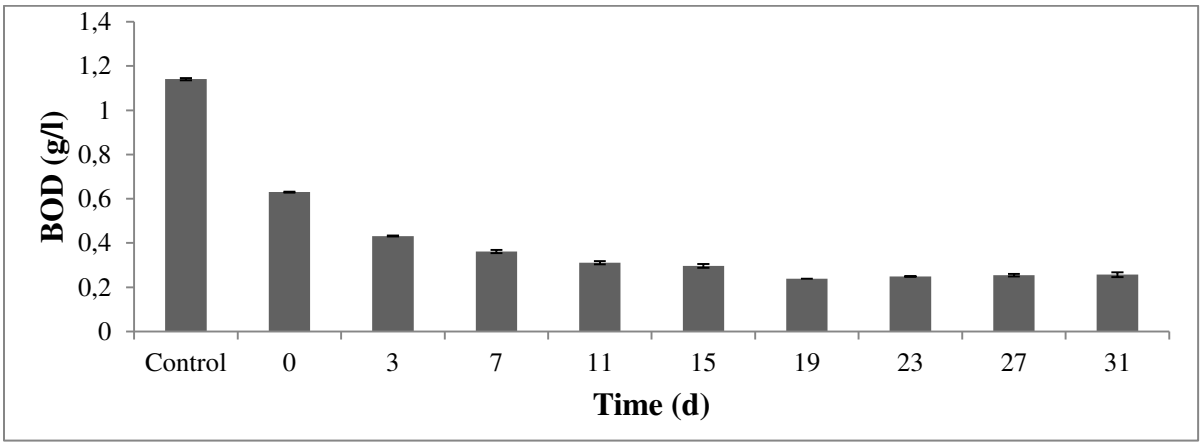


Figure 5.8: Depiction of biological oxygen demand (BOD) in the remediated samples of dX.D. All values are represented as ± s.d of triplicate experiments.

In accordance with Okpozu et al. 2019 [26] analysis, the reduction in BOD limits also occurred in *D. armatus* based remediation approximately up to 76% and 87% in heterotrophic and

mixotrophic cultivation respectively. The distillery waste water removal with microalgae exhibited promising outcome with 53% biological oxygen demand (BOD) reduction [34]. The microalgae *Chlorella salina* and *Chlorella vulgaris* demonstrated adequate results for remediation of waste with reduction in BOD values 87.01 to 90.75 % and 83.17 - 90.63 % [30].

5.2.1.9 Chemical oxygen demand (COD) profiling with phycoremediation

The increased amount of COD is consequence of contaminants present in solutions, released from the various refinement industries. The inorganic and organic chemicals existence in the dX.D, formulates the solution more hazardous with higher extent of COD. The Figure 5.9 depicted the significant decrease of COD value in phycoremediated dX.D around 1.331±0230 g/l and in control medium (no microalgae treatment) COD found to be 9.588±0.199 g/l. The process of phycoremediation reduces the COD, because organic matter biologically converted (microalgae degraded) which present in waste solution. The CO_2 release and chemically-oxidizing of organic matter reduces the value of COD [35].

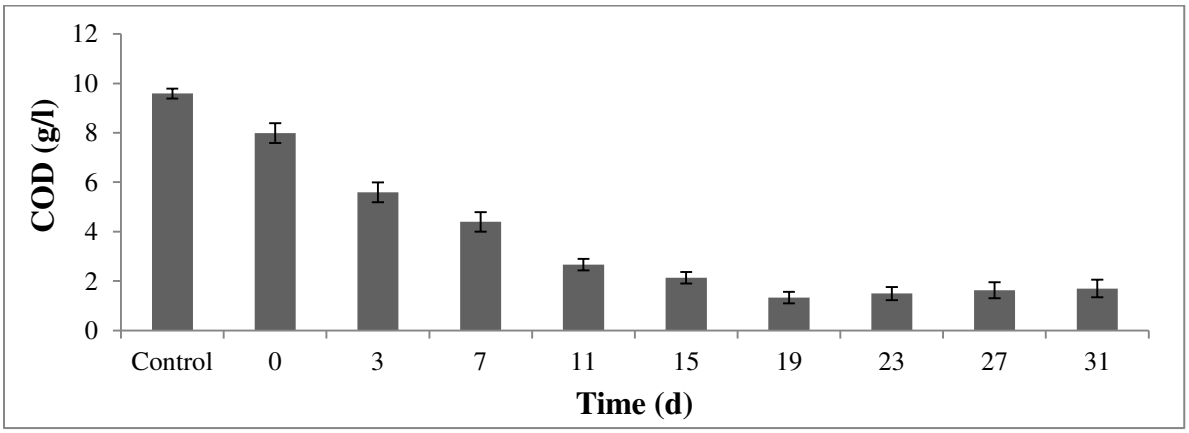


Figure 5.9: Depiction of chemical oxygen demand (COD) in the remediated samples of dX.D. All values are represented as ± s.d of triplicate experiments.

The decrease in the COD values 89-99% found in the microalgae *Spirulina's* and *Chlorella* treated cassava processing waste solutions in bioremediation parameters investigation [36]. The mixotrophic approach of *D. armatus* culturing showed 92% reduction in COD value: where as heterotrophic cultivation has 72% reduction in cassava water solution [26]. The waste solution from edible oil factory remediated through microalgae Desmodesmus sp.*1* which has enormous potential to reduce the chemical oxygen demand approximately 82% [37]. The

promising results showed by the *chlorella vulgaris* to remediate and decrease the COD level in textile industry waste. The process of phycoremediation involves the highest potential to remove the toxic chemicals and concurrently production of microalgal biomass and others co-value added products [38].

5.2.1.10 Total Kjeldahl Nitrogen (TKN) removal with *D. armatus* treatment

The Figure 5.10 represented the TKN content in the dX.D (control) around 0.276±0.001 % which decreased up to 0.254±0.001 % when treated with *D. armatus*. The nitrogen considered as one of the essential element for growth of microalgae. The microalgae *Chlorella* and *Desmodesmus* utilized for the nutrient and nitrogen removal from the domestic waste solutions [37, 39]. Microalgae have its own adeptness to produce the different biological substance through nitrogen utilization from waste water sources and this waste provide the enough amounts of nutrients for additional microalgal growth. The protein synthesis directly involve with the nitrogen uptake through microalgae system, the nitrogen availability in medium affects the abundance/ reduction of protein synthesis [40]. The microalga effectively makes use of existing N_2 in the waste solution and showed the 91%-N decline with nutrient removal in waste water [41]. The total amount of 35.9 mg/L of total nitrogen uptake by the microalgae *M. reisseri* in mine solution [42]. The Scendesmus sp. cultured in synthetic BG-11 medium and exhibited N_2 removal approximately 83-99% [43]. The reduction of all forms of nitrogen i.e. nitrite and nitrate observed through *Chlorella vulgaris* treatment [44].

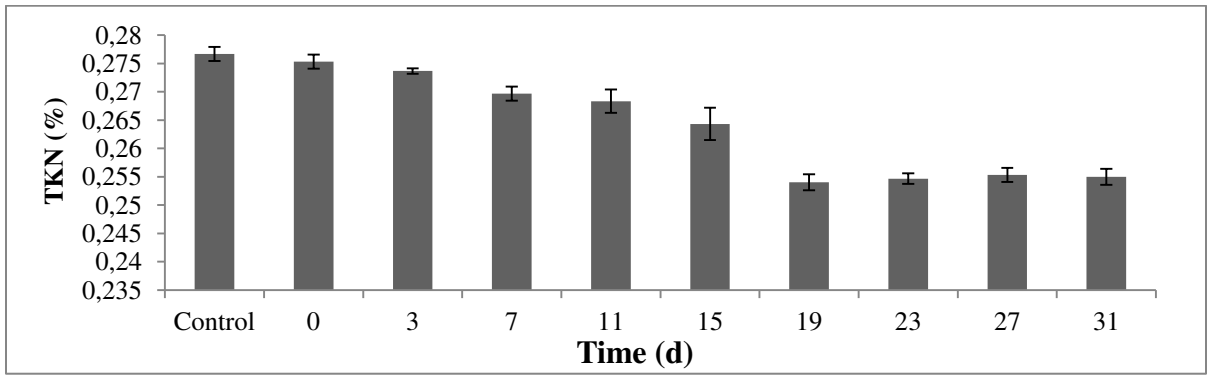


Figure 5.10: Depiction of total kjeldahl nitrogen (TKN) in the remediated samples of dX.D. All values are represented as ± s.d of triplicate experiments.

5.2.1.11 Total Phosphorus (TP) removal with *D. armatus* treatment

The *D. armatus* treatment to dX.D reduced the total phosphorus content up to 257±28.50 µg/ml extent with increase in biomass with time and when compared with control medium (without *D. armatus* treatment to dX.D) TP content is approximately 450±27.46 µg/ml (Figure 5.11). The algae eliminated the hazardous content of phosphorus in developer solution, which produce intimidation to the ecosystem. The microalgae have applicability of nutrients removal from the waste water through use of phosphorus as one of nutrient in the microalgal growth. The phosphorus has utility to produce phospholipids, nucleic acids and ATP [45]. The microalgae species such as *Chlorella vulgaris* and *Chlorella salina* have potential to remove the phosphorus from 76.97 to 82.48% and 87.14 to 90.08% respectively [30].

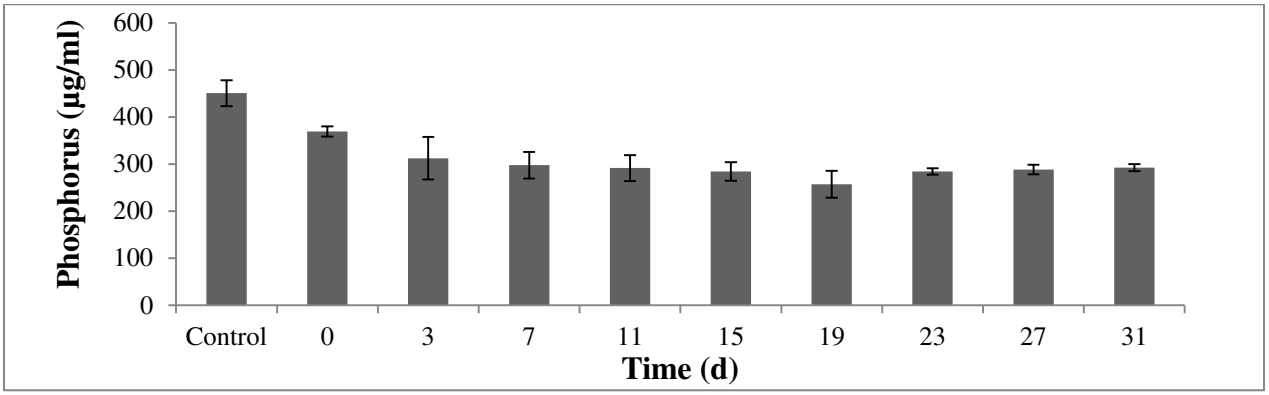


Figure 5.11: Depiction of phosphorus in the remediated samples of dX.D. All values are represented as ± s.d of triplicate experiments.

The microalgae *D. communis* has highest (100%) removal propensity of phosphorus and ammonia in solution [27]. The microalgal strains *C. quadricauda* and *C. vulgaris* also showed sufficient exclusion of phosphate in the waste water as 81.34% and 62.73% respectively [46]. The efficient eradication of phosphate through two dissimilar microalgal species i.e. *Chlorella* and *Spirulina* estimated around 89-99% in cassava waste solution [36]. The microalgae treatment to carpet refine solution showed the efficiency to highest removal of phosphorus [47]. The maximum value of phosphorus uptake attained around 4.9-5.4 mg/l in diluted mine solution treated with microalge *Micratinium reisseri* [42]. The Scendesmus sp. cultivated in the supplemented medium with extra phosphorus resource, and microalgae shown the highest elimination around 0.27 mg/l of phosphorus content [43].

5.2.1.12 Silver removal efficiency with concomitant lipid production with the phycoremediation of X-ray developer solution.

The dX.D has been used as cultivation medium for microalgae *D. armatus* and its characteristic properties evaluated before and after microalgae treatment with silver removal estimation and as well simultaneously lipids formation. The Ag is the only metal which abundantly present in the radiographic developer solution. The sampling of the treated sample (dX.D treated with *D. armatus*) were done at various time periods and further used for analysis. The silver uptake through microalgae increased with increase in microalgal biomass and lipids production. The log period of *D. armatus* growth showed maximum lipid content 1.392±0.002 %, attained from the initial content 0.006±0.003 %. The Figure 5.12 represented the relativity percentage of silver removal in comparison with the control. The utmost relativity percentage of silver removal 44.06% obtained in the log phase of the microalgae cultivation. The research conducted by Leonardo et al. 2016 [48], specified that microalgae *Coccomyxa actinabiotis* accumulated the silver in micro molar concentrations within the cytosol. The absorptive properties of *Chlorella vulgaris* efficiently remediate the 2.5 mg/l of silver ions/ thiosulfate complex with 94.5% removal rate [49].

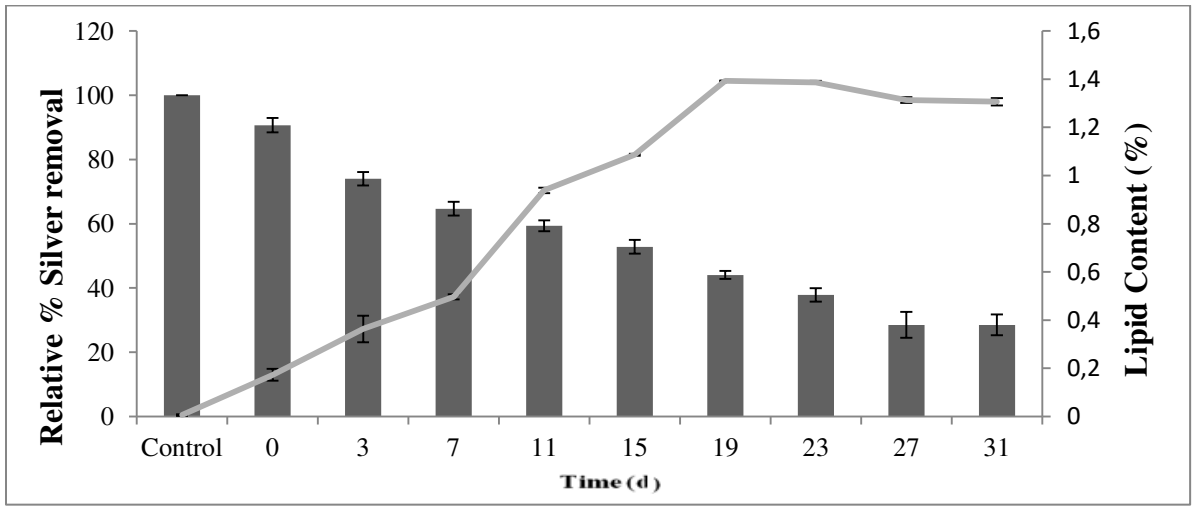


Figure 5.12: Depiction of concurrent silver removal (relativity %) and lipid production in *D. armatus* based remediation of dX.D. All values are represented as ± s.d of triplicate experiments.

The microalgae efficiently executed the heavy metal removal [50], *Desmodesmus pleiomorphus* remove the cadmium metal from its initial value 85.3 mg Cd/g to maximum removal value 61.2

mg Cd/g [51]. The presence of metal-chelating groups on microalgal cell surface adsorbs the metal ions on it.

Table 5.1: Relative bioremediation potential (%) of *D. armatus* of dX.D.

Parameters	Control value	19th day value of parameter	Relativity (%)
pH	8.63±0.02	8.09±0.01	-----
Total solids (g/l)	28.08±0.104	23.78±0.076	84.68
Total dissolved solids (g/l)	21.41±0.028	18.41±0.0577	85.99
Total suspended solids (g/l)	6.66±0.115	5.36±0.104	80.49
Total volatile solids (g/l)	10.96±0.028	8.71±0.275	79.48
Total fixed solids (g/l)	17.11±0.125	15.06±0.305	88.02
Conductivity (mS)	18.5±0.05	23.56±0.15	127.35
Density (g/l)	1052.8±0	988.43±0.057	93.88
Biological oxygen demand (g/l)	1.140±0.005	0.238±0	20.86
Chemical oxygen demand (g/l)	9.588±0.199	1.331±0.230	13.88
Total Kjeldahl Nitrogen (%)	0.276±0.001	0.254±0.001	92.02
Total phosphorus (µg/ml)	450.66±27.46	257.33±28.50	57.10
Silver (g/l)	0.0118±0.000	0.0052±0.000	44.06
Lipids (%)	0.006±0.003	1.392±0.002	23200

The microalgae biomass straightly compares with the metal removal efficiency, as increased in amount of biomass generally elevated the metal removal [52]. The fresh water microalgae Desmodesmus sp. stated the efficient Cu and Ni removal which seems around 90% and 43% respectively, and simultaneously lipids formation. Several researchers reported that cultivation medium with higher quantity of metals concentrations result in the low lipids formation because of metal toxicity. This necessitates the significance of toxicity studies of microalgae in remediation experiments [20, 53]. The relativity % of characterization parameters of phycoremediated samples with removal of silver and lipids production have been put in to a tabular form in Table 5.1.

5.3 Conclusion

The radiographic developer solution's analysis tells about the various types of organic, inorganic, physical and chemical contents and toxic silver amount, and its urgency to remove these content before discharging in to the water resources through an environment friendly way. The maximum growth of *D. armatus* found on the 19^{th} day of one month periods where ever 3:1 ratio (basal medium: waste radiographic developer solution) used for microalgae cultivation. The *D. armatus* treated waste developer solution illustrated the desirable outcomes in decreasing the values of silver, organic, inorganic, physical and chemical content with respect to the control (no *D. armatus* treatment). The remediation through microalgae *D. armatus* benefits in evaluation of relative % (i.e. 44.06%) of silver removal in comparison with control sample. Highest lipids content 1.39% has achieved, which calculated 23200% in terms of relative % and value enhanced from initial value (control). With increase in the cultivation period, microalgae enter in the stable phase of growth cycle and consequently microalgae remediation potential is not commendable. Comprehensively, *D. armatus* exhibited remarkable potential in the treatment of developer solution and maximal *D. armatus* growth and microalgal remediated values scrutinized on the 19^{th} day of microalgae culturing coupled with Ag elimination and lipids formation.

CHAPTER 6

Bioremediation Studies of X-ray Developer Solution by *D. armatus* using Food Waste & Agri-Compost Media

CHAPTER 6

Bioremediation Studies of X-ray Developer Solution by *D. armatus* using Food Waste & Agri-Compost Media

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6.1 Introduction

Along with the rapid increase in the population and change in the living trend, there is high wastage of food in the different sources such as household, industrial, and agriculture [1]. Food waste is one of the emerging waste-producing worldwide has a high amount of lipids, proteins and carbohydrates, and other organic compounds. The conventional methods used to dispose of the food waste include anaerobic digestion, incineration, animal feed, and landfill provoke high environmental pollution and economic imbalance [2]. According to the food and agriculture organization, 1.3 billion of food is wasted worldwide. Wasted food is 1/3rd of the food produced worldwide, and agricultural land utilized is 28% [3, 4]. The UN Development Programme report stated that approximately 40% of the food is wasted in India, valued at around 92,000 crores per annum. The wasted food has an immense proportion of carbohydrates (20-45%), lipids (10-40%), proteins (5-10%), along with its moisture content [5]. Food waste considers as one of the precarious origins of municipal solid waste, and it's inappropriate way of dumping generate environment-related troubles like water contagion by its organic deterioration, odour, lethal gases formation, the shelter of bugs [6–8]. The inadequacy of governing the food waste guidelines in most countries causes problems in properly recognizing management rights. The ultimate resolution to discontinue the lethal effects of the wasted food is to craft a suitable procedure to utilize the wasted food with minimum damage to the environmental sustainability and can be further recycled and efficiently used in assorted relevance [9].

The organic dissipated expulsion into the environment enhanced since inevitable urbanization and the burden for agriculture and industrial manufacturing has been constantly improved. The augmented the quantity of organic waste from the agricultural movement to fulfil the needs and energy requirements. The process of composting accomplished the function of management of organic waste and putrefied into the humus-like materials that utilized as bio fertilizers and

conditioners for soil [10–12]. According to research of food and agriculture organization [13, 14] the production of agriculture sources enhanced about three times from the past 50 years to complete the needs of consumption. The expansions in agriculture activities generate loads of environmental pressure and originate the pessimistic impact on the soil, water, and air resources [15]. The utilization of the appropriately composted organic waste as feedstock for the cultivation of plants and microalgae followed the leads from past years [16, 17]. The food waste and agriculture compost medium utilized for the culturing of microalgae because of the existence of phosphorus and nitrogen content, reducing the cost of cultivation medium. The great quantity of waste, economical and renewable character of the food waste and agriculture waste makes them a suitable medium for sustainable growth of microalgae [18, 19].

Radiographic processing is an effective method in the domain of diagnosis inside hospitals and other medical centre. Various kinds of solutions (fixer, developer, lead foils, wastewater, and other chemicals) are generated during the processing, and all these chemicals are utilized during the radiographic processing. The developer is one of the solutions used during the manual processing of the radiographs in the practices of X-ray and CT scans. This wasted developer solution has an elevated amount of inorganic and organic contaminants, which is hazardous and entails severe intimidation to the environment. It is necessary to treat the waste before discharge into the domestic/ industrial sewage treatment plant [20, 21]. The spent developer solution is not recognized in the category of hazardous waste. The higher pH (~12.5), corrosive nature, alkalinity, higher amount of BOD and COD, presence of other chemical compounds such as hydroquinone, sulphites, acetate, and silver (more than five ppm) are a few properties that make them hazardous and necessitate proper handling or treatment before disposed of into the environment [20, 22, 23].

Along with various chemical compounds (organic/ inorganic), the silver metal is one of the toxic compounds that should be retrieved or treated from the processing solution before discharge into the drainage system—the threat associated with the silver when it is exposed to the environment and later to the human body. There is a varied number of chronic diseases occurred when somehow soluble/colloidal silver enters into the body and produces undesirable effects such as argyria (discoloration of skin- blue/grey patches), argyrosis in the eye, kidney and liver problems, and also mount up inside the muscles and brain [23, 24]. There are diverse types of

treatment for the radiographic solution viz oxidation, sedimentation, reverse osmosis, adsorption, biological oxidation, precipitation, electrochemical and dual chemical-biological, etc. [25]. Furthermore, the silver recovery process includes oxidation, electrolysis, adsorption, chitin, and ion exchange, and several of the recovery processes are on-site and off-site [26–31]. These methods have their drawbacks from its high equipped cost to the production of the immense amount of derived waste, perilous byproducts, detrimental to the organic species, and slight effectiveness to eliminate the traces contaminates from the solution [32–34]. The biological methods of remediation of the solution come forward as one of the eco-friendly and efficient approaches for the futuristic period. The microalgae seem to be an excellent competitor with varied effortless cultivation and remediation characteristics with by products production. The indeed properties of microalgae include rapid growth, easily cultured in a barren area, accumulate the heavy metals within the cells, uptake the nutrient from various waste and utilizing it's for self-growth, biomass used for valuable products formation and auto and as well heterotrophic form of cultivation [35–39].

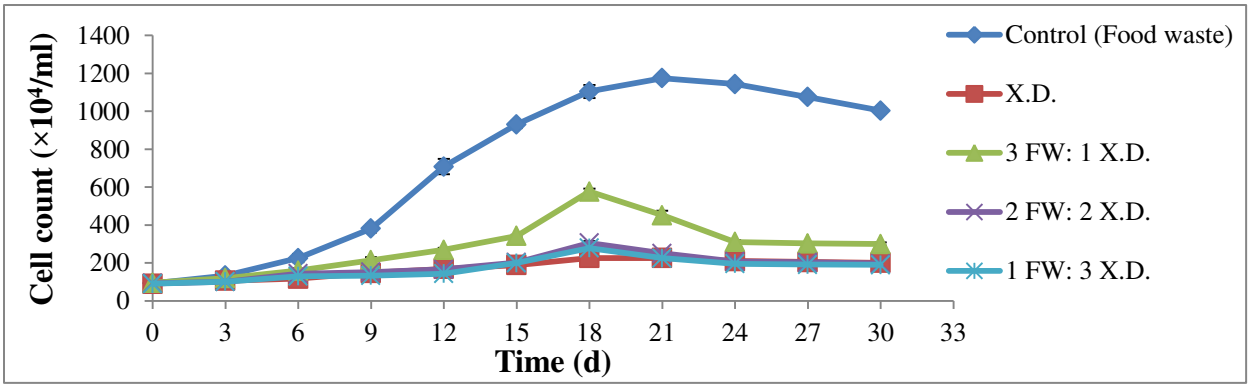
The microalgae Desmodesmus sp. has varied relevance in removing nutrients from wastewater and is further exploited for biodiesel production, carbon-neutral fuel [40, 41]. In another research, the *D. armatus* has a vital role in treating the radiographic solution, mainly the developer solution with its toxicity evaluation, silver removal, and lipids production, while microalgae *D. armatus* cultivated in the BBM [42]. The current studies intend to use an organic waste (food waste medium, FWM/ agri-compost medium, ACM) diluted with varied ratios of radiographic developer solution for the culturing of *D. armatus* and simultaneously treatment or elimination of nutrients and silver from developer solution and additional biomass consumption for lipids production. The present study intended to showcase the low-cost waste media's (FWM and ACM) in phycoremediation potential in handling the X-ray developer solution.

6.2 Results and Discussion

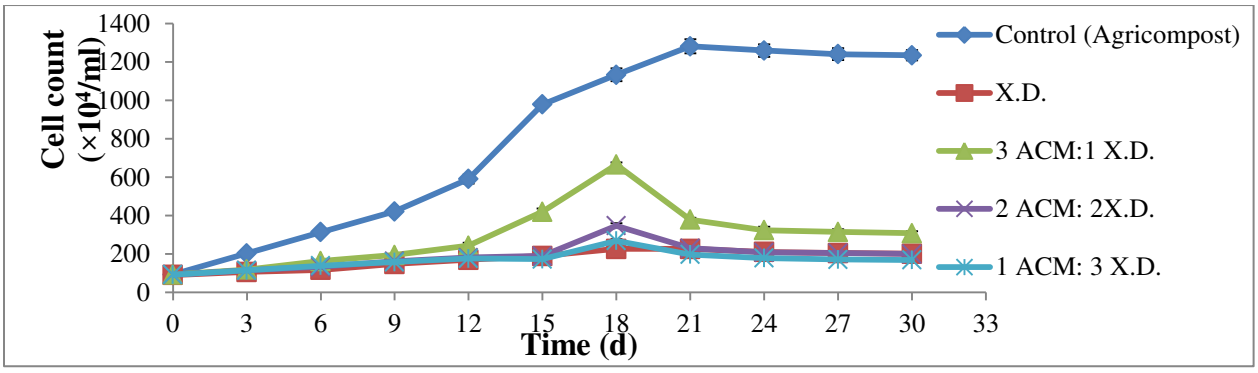
6.2.1 Evaluation of *D. armatus* toxicity tolerance in different dilutions of X.D with FWM and ACM

The microalgae *D. armatus* has diverse applicability in removing waste nutrients from various solutions [40, 43]. The microalgal-based remediation primarily relies upon the tolerance limits exhibited by the microalgae in various industrial and other solutions [44]. The *D. armatus* was

grown in the different ratios of X.D in dilution with FWM and ACM for one month. The cell count was observed in each proportion of X.D diluted with FWM and ACM along with control medium (without X.D) (Figure 6.1). The maximum cell count was observed in the ratio of 3:1 ratio of dFWM (3FWM:1X.D) and dACM (3ACM:1X.D) compared to the other dilutions of X.D with FWM and ACM (Fig. 6.1). Among the different dilution medium (FWM and ACM), the utmost cell count of *D. armatus* observed in the dACM (Figure 6.1 (b)). The presence of less nutrients in the other dilutions of X.D and FWM/ACM hampers the microalgal growth. The *D. armatus* growth displayed adequate cell count throughout the cultivation period and can be proven effective in treating X.D (Figure 6.1). The maximum growth of *D. armatus* showed in the 15-19th d of cultivation period in the dFWM and dACM. Enough nutrients present in the dFWM and dACM supports the maximum growth of *D. armatus* compared to the other dilutions of FWM/ACM with X.D.



(a)



(b)

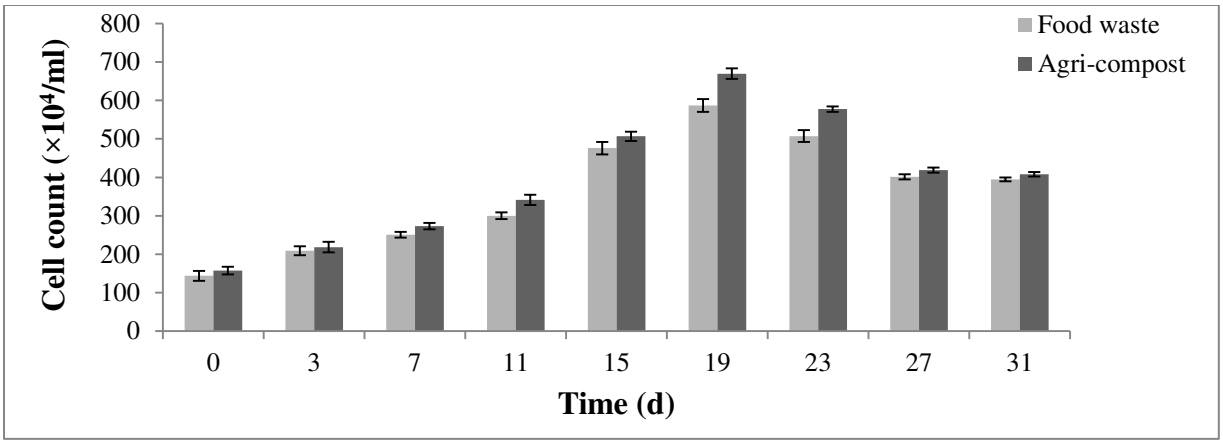
Figure 6.1: Cell count (×10^4/ml) of *D. armatus* in the different dilutions of X.D with (a) FWM (b) ACM. All values are represented as ± s. d of triplicate experiments.

From the microscopic examination, the microalgal cells show healthier in dFWM and dACM in contrast to other dilutions. The X.D (no *D. armatus*) without any dilution with FWM and ACM exhibited slighter growth of *D. armatus* (Figure 6.1). The X.D consist of a higher amount of chemical substances with toxic properties towards environmental sustainability [20, 42]. The elevated concentrations of waste nutrients in developer solution probably hinder the proper growth of microalgae. The microalgae rapidly grow in the suitable culturing medium with appropriate nitrogen, phosphorus, and other micronutrients presence [45–47]. The diluting cassava wastewater fills the nutrient absence with BBM for the growth of *D. armatus* [40].

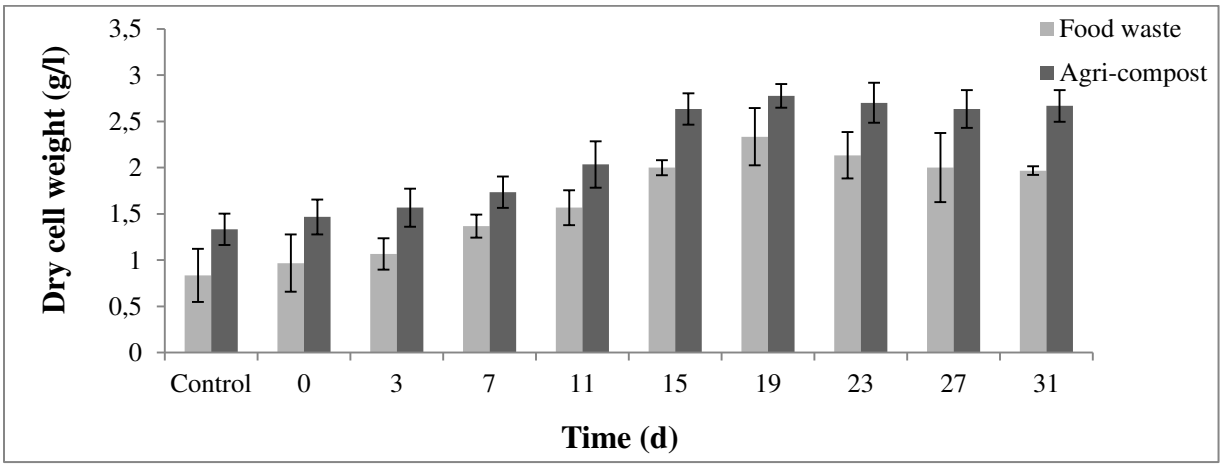
Microalgae have the adaptability to thrive in any severe environment because of their capability to tolerate any inconsiderate condition [48]. The microalgae *D. quadricauda* growth in industrial wastewater (12%-24%) reduces with increasing wastewater concentrations [49]. The microalgae *C. vulgaris*, *M. aeruginosa*, and *E. gracilis* are cultivated in the animal waste treatment plant [50]. The raw tannery solution diluted with distilled water (25%-60%) was used to grow microalgae Scendesmus sp., *C. sorokiniana*, and *C. variabilis* and simultaneously produce lipids from microalgal biomass [51]. The solution from dairy waste diluted with BG-11 medium (1:3, 3:1, 1:1) utilized as cultivation medium for the *A. ambigua*, *S. abundans*, *C. pyrenoidosa* growth [52]

6.2.2 *D. armatus* growth in dFWM (3FWM:1X.D) and dACM (3ACM:1X.D)

The preliminary assessment of *D. armatus* growth in various concentrations of X.D with FWM and ACM demonstrated the highest growth in 3:1 (FW/ACM: X.D.). Among the two media, the dACM showed maximum biomass growth (dry cell weight- 2.7 g/l) and cell count- 669 ($\times 10^4$/ml) on the 19th d of cultivation. However, the dFWM exhibited lesser biomass growth and cell count than dACM. The *D. armatus* come in the stable point (no microalgae growth) after the 19th d in treated X.D. The selected dFWM and dACM were further utilized for the imminent phycoremediation studies of X.D. For forthcoming remediation experiments, the dFWM and dACM without microalgae, was utilized as a controls (Figure 6.2).



(a)



(b)

Figure 6.2: Depiction of *D. armatus* in optimized ratio of radiographic developer solution with food waste and agri-compost medium (a) Cell count ($\times 10^4$/ml) (b) Dry cell weight (g/l). All values are represented as $\pm$ s.d of triplicate experiments.

The *D. armatus* growth is strictly depends on the pH, temperature, N and P concentration, time, and light conditions [53]. The microalgae growth varies with deprivation in nutrients, opaqueness, and less light penetration inside the medium, so microalgae cease their growth after a certain period [54, 55]. The *D. armatus* exhibited maximum biomass of 1.65 g/l after 12 d of cultivation and light around 108 µmol m^{-2} s^{-1} in the photo bioreactor [53]. The medium type is the primary aspect for microalgae growth; the *D. armatus* illustrated a maximum growth rate of 0.423/ day in the 5% leachate medium in12 h light exposure [56].

6.2.3 Phycoremediation studies of X.D with dFWM and dACM

The physical and chemical parameters explored in *D. armatus* treated and untreated dFWM and dACM for one-month results are summarized in the below lines.

6.2.3.1 Fate of pH with the phycoremediation

The radiographic developer solution has a considerable quantity of inorganic and organic compounds (hydroquinone, sodium sulfite, phenidone, and potassium bromide, etc.), which impart the alkaline nature to the solution [20]. The interpretation of pH reduction in the dFWM and dACM with *D. armatus* treatment is depicted in Figure 6.3. The control media of dFWM has a pH around 9.7±0.01 whereas control media of dACM has a pH of 9.65±0.01 (Table 6.1 and 6.2). The reduction in the pH was observed after the *D. armatus* treatment in both media. As the *D. armatus* initiated to cultivate in the diluted X.D, the decline in pH values was observed concerning the control medium (no *D. armatus* treatment). The highest reduction in the pH value was observed on the 19th d of cultivation in dFWM and dACM, which has a net reduction to pH 9.39±0.04 (dFWM) and 9.37±0.02 (dACM)(Figure 6.3).

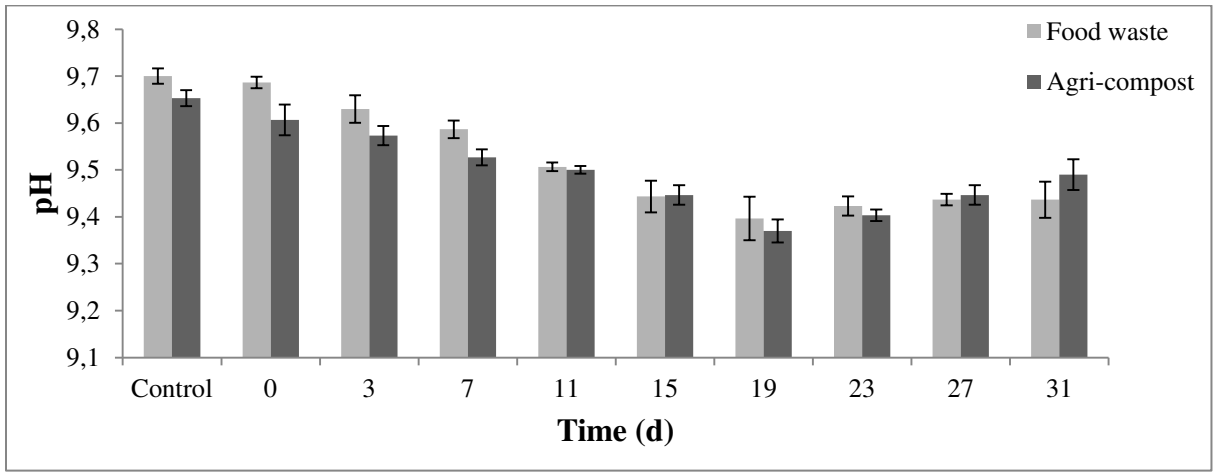


Figure 6.3: Depiction of pH profiling with the phycoremediation studies in dFWM and dACM. All values are represented as ± s.d of triplicate experiments.

The reduction in pH after microalgae treatment was observed in dFWM and dACM. The microalgae have the potential to utilize nitrogen sources, either ammonium or nitrate form from the waste. The utilization of ammonium as a primary source of nitrogen and inhibiting the nitrate

reduction step ultimately decreases pH in the waste solution medium [57]. The pH decrease was also observed in the *C. vulgaris* treated glucose-rich primary settled wastewater, as microalgae use NH_3-N as a nitrogen source and liberated more H^+ ions in the medium [58]. The microalgae *D. armatus* in the treatment of developer solution diluted with BBM medium exhibited the same pattern of pH reduction concerning the control medium [42].

6.2.3.2 Total solids (TS) profiling with phycoremediation

The high amount of TS in the X.D contributed to the presence of a dissolvable and suspended form of solids. The characterization of X.D discloses the higher solids amount in solution and its effectual removal with *D. armatus* treatment [42]. The dFWM and dACM has approximately solids content (control) of 25.03±0.46 g/l and 27.16±0.21 g/l, respectively. The *D. armatus* remediated samples of dFWM and dACM exhibited a noteworthy decrease in solids content on the 19th d, which is around 20.75±0.28 g/l (dFWM) and 22.84±0.51 g/l (dACM) (Fig. 6.4) (Table 6.1 and 6.2). The most important basis of total solids removal in X.D was the utilization of inorganic and organic contaminants in the solution for the growth of *D. armatus*. The microalgae are adaptable to grow in various industrial and other solutions with a reduction in physico-chemical parameters.

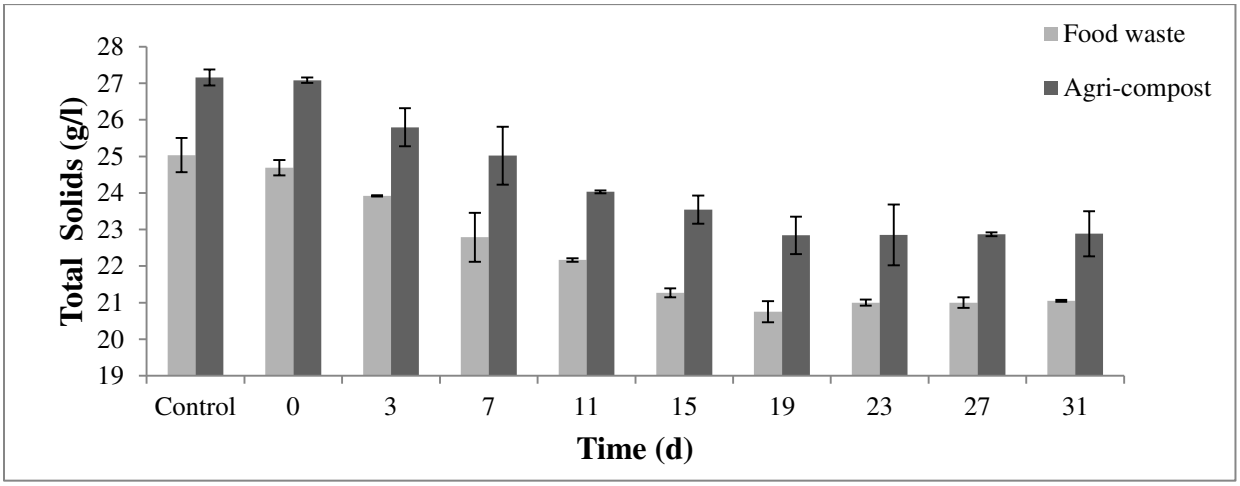


Figure 6.4: Total solids (TS) profiling with the phycoremediation of dFWM and dACM. All values are represented as ± s.d of triplicate experiments.

The household wastewater treated with *C. vulgaris* also demonstrated the drop in total solids amount and appeared as an eco-friendly approach to treat wastewater [59]. The efficient

elimination of total solids was examined with Scendesmus sp. and Chlorella sp. treatment in various carton box wastewater [60]. The *C. vulgaris* also reduced the total solids amount treated solution in raw textile wastewater [61].

6.2.3.3 Trend of total dissolved solids (TDS) with phycoremediation

The dissolved solids, including organic and inorganic compounds, are a toxic waste of concern to aquatic life and human health. The microalgae with tremendous application in eliminating dissolved solids from different solutions utilized these compounds for their growth [62]. The *D. armatus* treated dFWM and dACM has reduction in TDS to 11.71±0.79 g/l (from14.36±1.12 g/l, control) and 15.19±0.8 g/l (from 18.35±0.52 g/l . control) on the 19th d of microalgae growth, respectively (Figure 6.5) (Table 6.1 and 6.2). The developer solution with a higher amount of dissolvable solids makes the usable water more precarious and reduces the dissolved oxygen level [42]. The algal biofilm reactor for the wastewater treatment decreased the dissolved solids content and removal efficiency by about 27% [62]. The microalgae *D. subspicatus* efficiently reduce the concentration of dissolved solids by approximately 80%-84.04% from the wastewater and cassava waste [43, 63]. The *D. armatus* also reduce the dissolved solids concentration in cassava wastewater in heterotrophic (72%) and mixotrophic (83%) cultivation [40].

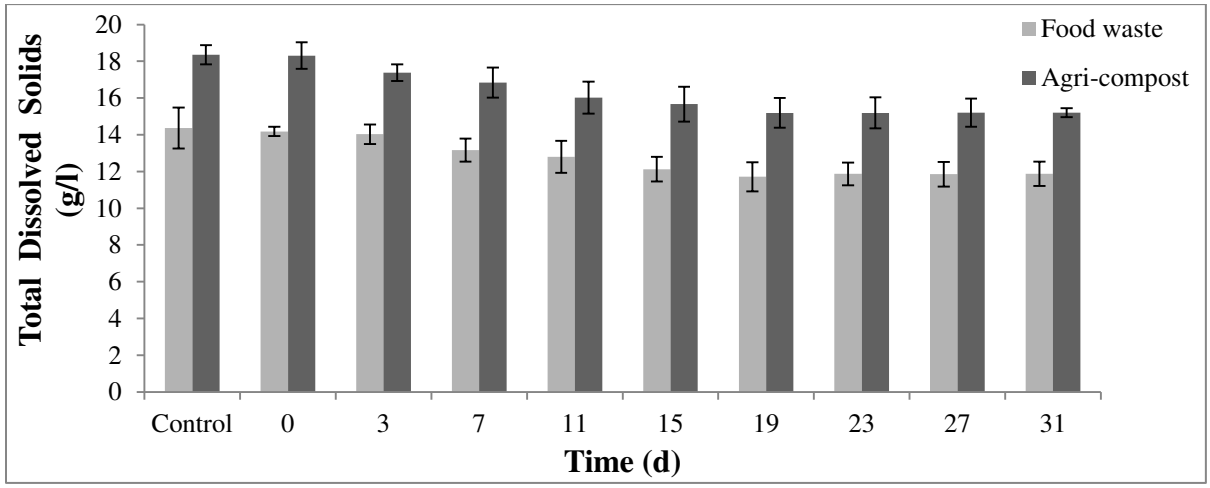


Figure 6.5: Depiction of total dissolved solids (TDS) profiling with the phycoremediation of dFWM and dACM. All values are represented as ± s.d of triplicate experiments.

6.2.3.4 Total suspended solids (TSS) profiling with phycoremediation

The suspended solids generally stay in floating form and enhance the temperature in the solution through obstructing light dispersion inside them. The suspended solids diminish the water clarity and affect the photosynthesis process in the aquatic system. The microalgae can grow in diverse solutions and remove suspended solids. The dFWM and dACM consists of a considerable amount of suspended solids 10.66±0.08 g/l and 8.88±0.15 g/l, respectively (Figure 6.6). The *D. armatus* treated developer solutions (dFWM and dACM) illustrated a decline in suspended solids content with increased cultivation days. The maximum reduction in suspended solids observed on the 19th day of cultivation was around 9.03±0.04 g/l and 7.65±0.33 g/l in dFWM and dACM, respectively (Table 6.1 and 6.2) (Figure 6.6). The decrease in suspended solids is observed in household wastewater through *C. vulgaris* [59].

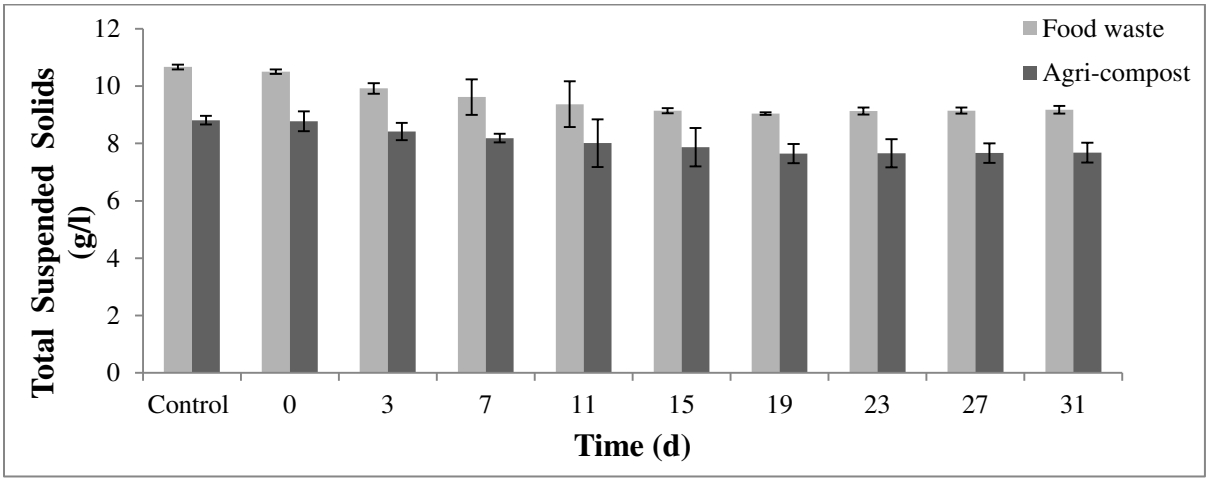
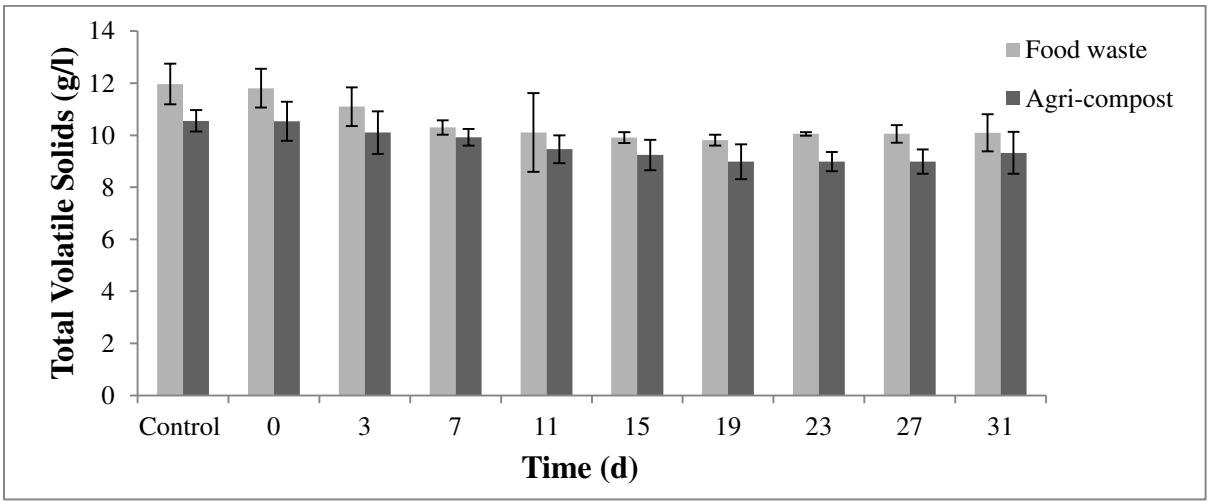


Figure 6.6: Depiction of total suspended solids (TSS) profiling with the phycoremediation of dFWM and dACM. All values are represented as ± s.d of triplicate experiments.

6.2.3.5 Fate of total volatile solids (TVS) and fixed solids (TFS) with phycoremediation

The volatile solids assessed the organic material's existence in the wastewater. Figure 6.7 (a) (b) in developer solution diluted with food waste and agri-compost medium, the volatile and fixed solids inclination. The volatile solids have a massive amount, i.e., 11.96±0.78 g/l, and 10.55±0.41 g/l with with dFWM and dACM, respectively. The *D. armatus* treated samples to reduce the volatility by approximately 9.8±0.21 g/l and 8.98±0.66 g/l in dFWM and dACM, respectively (Figure 6.7 (a) and Table 6.1 and 6.2).

On the other hand, the amount of fixed solids (inorganic contaminants mainly) in developer solution is more than volatile solids around 13.06±0.78 g/l and 16.61±0.48 g/l in dFWM and dACM, respectively. The *D. armatus* treatment to dFWM and dACM reduces the fixed solids values to 10.94±0.89 g/l and 13.86±0.44 g/l, respectively (Figure 6.7 (b) and Table 6.1 and 6.2). The same trend (the decrease in volatile solids and fixed solids) was reported examined in the developer solution diluted BBM medium with *D. armatus* treatment [42].



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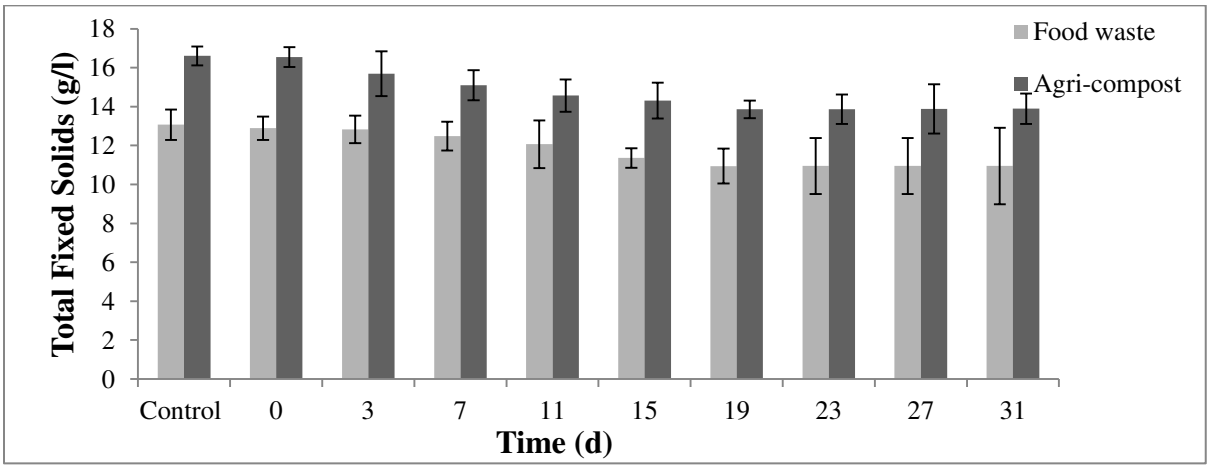
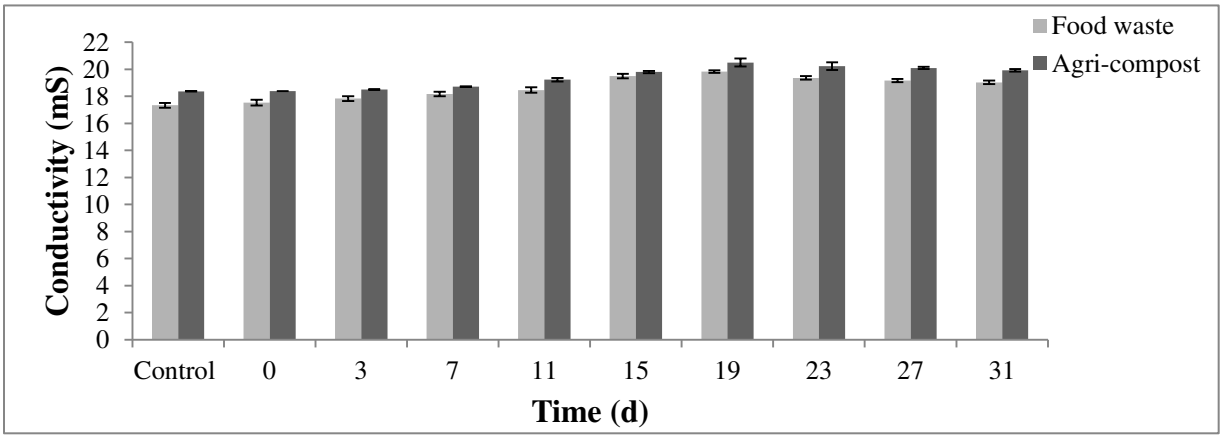


(b)

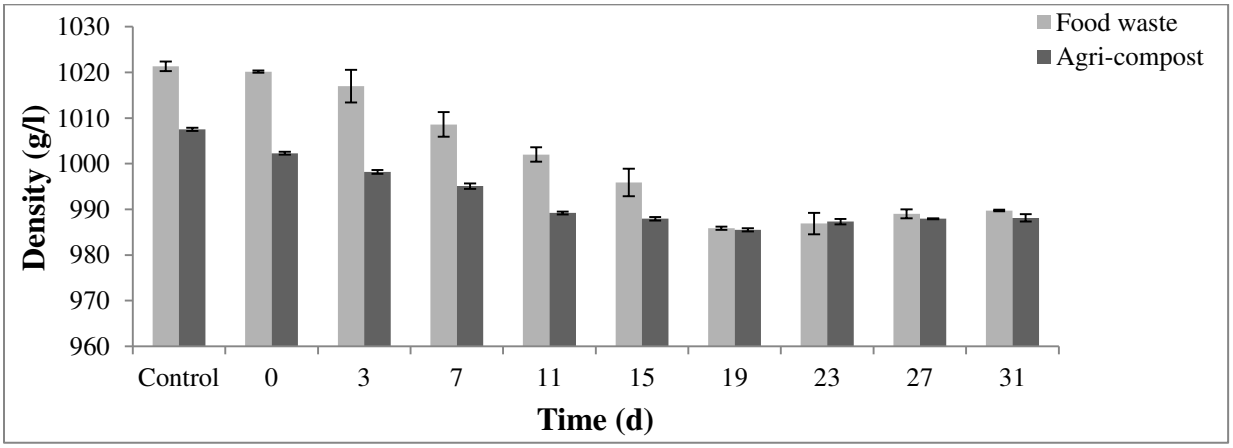
Figure 6.7: Depiction of (a) total volatile solids (TVS) (b) total fixed solids (TFS) profiling with the phycoremediation of dFWM and dACM. All values are represented as ± s.d of triplicate experiments.

6.2.3.6 Fate of conductivity and density with phycoremediation

The conductivity of phycoremediated dFWM and dACM was depicted in Figure 6.8 (a). The conductivity of solution increased with enhancement in dissolved solids content. Increasing the cultivation period and the microalgae growth enhanced the solution conductivity [42, 64].



(a)



(b)

Figure 6.8: Depiction of (a) conductivity (b) density profiling with the phycoremediation of dFWM and dACM. All values are represented as ± s.d of triplicate experiments.

The dFWM and dACM (controls, without algal treatment) has conductivity around 17.33±0.16 mS, and 18.36±0.01 mS, respectively. After the *D. armatus* treatment to developer solution, the conductivity increase with the increase in the algal cell density in the solution, and it reaches up

to 19.83±0.09 mS and 20.5±0.29 mS on the 19th day of cultivation in dFWM and dACM, respectively (Figure 6.8 (a) (Table 6.1 and 6.2).

The density of developer solution gets reduced with an increase in *D. armatus* growth in dFWM and dACM. The maximum reduction in density was attained in the 19th d around 985.86±0.32 g/l (from 1021.37±1.04 g/l, control) and 985±0.32 g/l (from 1007.53±0.33 g/l, control) in dFWM and dACM, respectively (Figure 6.8 (b) (Table 6.1 and 6.2). The reduction in density was also observed in the developer solution treated with *D. armatus* in dilution with BBM medium [42].

6.2.3.7 Biological Oxygen Demand (BOD) depiction trend with phycoremediation

The BOD illustrated the amount of dissolved oxygen in the waste solution, indicating the level of pollution in particular solution. Before discharge in the environment, the BOD reduction is imperative in wastewater treatment [65, 66]. The X.D, which has an elevated level of BOD, imparts a negative effect to the environment. The *D. armatus* treated dFWM and dACM showed the highest reduction in BOD level up to 0.26±0.01 g/l and 0.20±.000 g/l, respectively (Figure 6.9). The Dacm exhibited the highest relative 17.61% % removal of BOD concerning control (Table 6.2). The various strains of green microalgae utilized the waste nutrients and reduced the BOD 88% in urban waste solution [67].

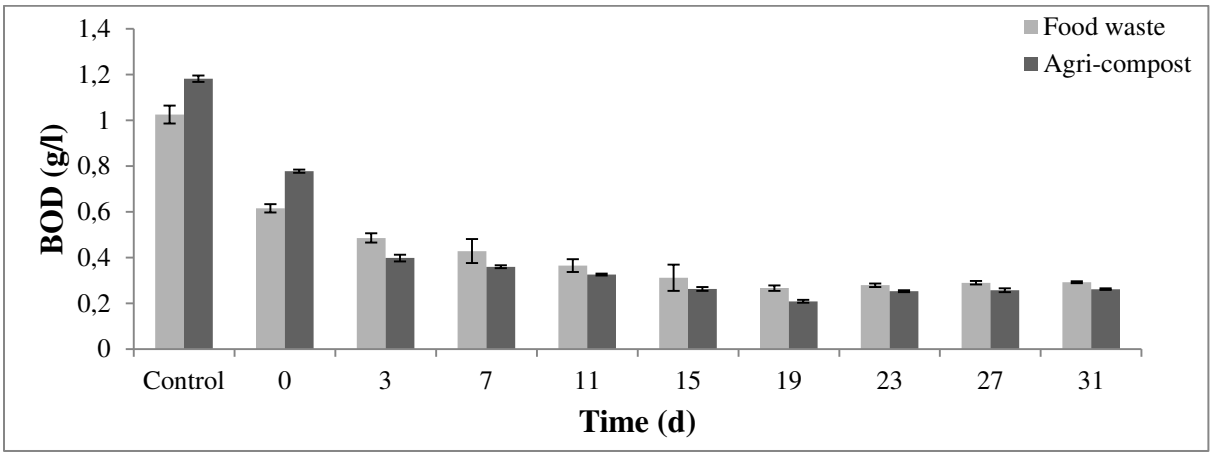


Figure 6.9: Depiction of biological oxygen demand (BOD) profiling with the phycoremediation of dFWM and dACM. All values are represented as ± s.d of triplicate experiments.

The *C. sorokiniana* also exhibited the highest tendency of BOD reduction (80%) during tertiary treatment of waste solution [68]. On the other report, *D. armatus* during treatment of cassava

waste+ BBM medium reduces the BOD level up to 76% and 87% in heterotrophic and mixotrophic cultivation [40]. The microalgae *D. subspicatus* in wastewater treatment demonstrate maximum BOD reduction (76%) in 20 d of cultivation [43]. The developer solution treated with *D. armatus* also exhibited the same trend in BOD reduction and the highest reduction observed on the 19th day [42].

6.2.3.8 Trend of chemical oxygen demand (COD) with phycoremediation

The developer solution exhibited an enormous amount of organic and inorganic contaminants, which enhanced the COD of particular solution. The developer solution treated with *D. armatus* reduces the COD level to a great extent. The microalgae utilized the contaminants as a nutritive medium and ultimately enhanced the solution oxygen level [42]. The COD reduction in dFWM and dACM was depicted in Figure 6.10. As the *D. armatus* grew up in the X.D, it decreased in COD level of X.D, and the highest reduction was illustrated on the 19th d of cultivation. The dFWM reduces COD 1.73±0.49 g/l from control 9.45±0.82 g/l (no *D. armatus* treatment) and in Dacm reduction attained at 1.05±0.18 g/l from control 9.45±1.14 g/l (Figure 6.10).

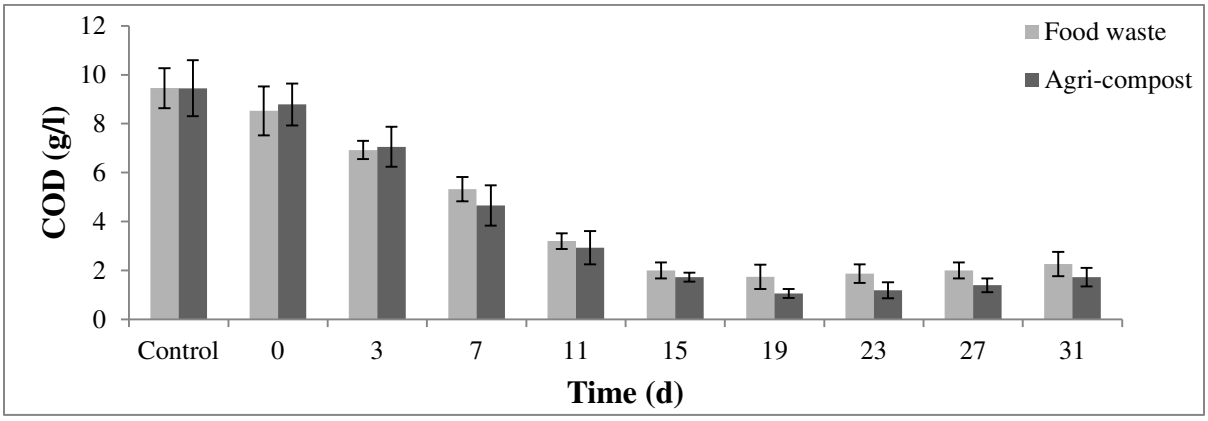
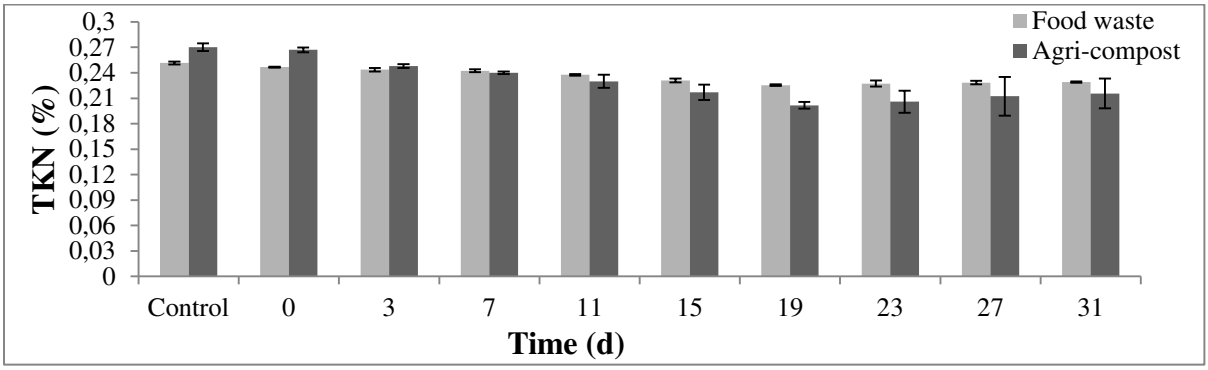


Figure 6.10: Depiction of chemical oxygen demand (COD) profiling with the phycoremediation of dFWM and dACM. All values are represented as ± s.d of triplicate experiments.

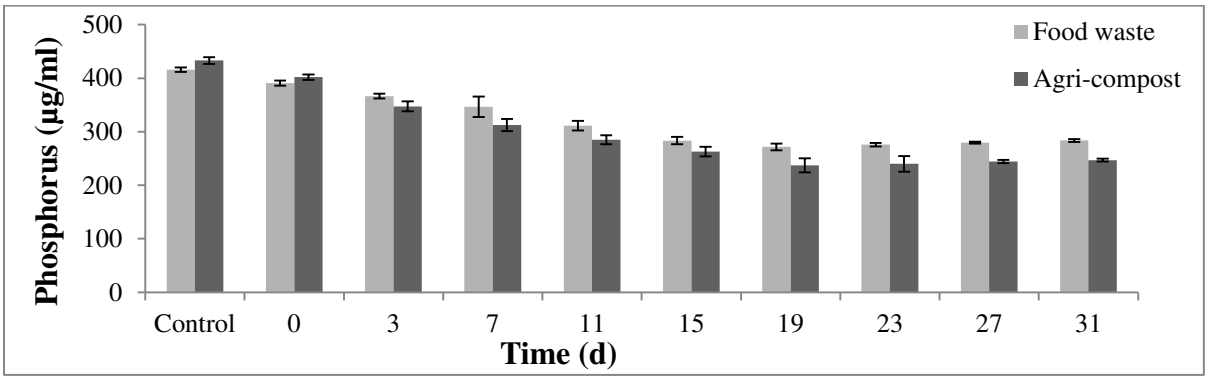
The relative % of COD removal was 11.11% achieved in the developer solution diluted with agri-compost medium (Table 6.2). The *C. vulgaris* and Scendesmus sp. show the highest COD removal, 80%-95%, in different types of waste generated from various industries [66, 69]. The microalgae *C. pyrenoidosa* also effectively reduced the COD values in synthetic waste solution

consist higher chemical oxygen demand initially [70]. The *D. subspicatus* and *D. armatus* immensely reduce the COD by 70%-94% in treating waste solution [40, 47]. The microalgae Scendesmus sp. efficiently removed the COD 93% in Agri and industrial waste solution [71].

6.2.3.9 Total Kjeldahl Nitrogen (TKN) and phosphorus removal efficacy of phycoremediation



(a)



(b)

Figure 6.11: Depiction of (a) Total Kjeldahl Nitrogen (TKN) (b) Phosphorus profiling with the phycoremediation of dFWM and dACM. All values are represented as ± s.d of triplicate experiments.

The microalgae consume the nitrogen and phosphorus nutrients from the diverse solution (municipal, household to industrial) and convert them into microalgal biomass and other byproducts. The microalgae maintain sustainability in the environment through the treatment of

wastewater [72, 73]. The TKN in dFWM and dACM is depicted in Figure 6.11 (a). The treated dFWM and dACM illustrated reduction in TKN around 0.22±0.00 % (from 0.251±0.00%, Control) and 0.20±0.00% (from 0.270±0.00 %, Control), respectively (Figure 6.11 (a). The maximum relative % of TKN is 74.44%, estimated in dACM (Table 6.2). The microalgae *C. zofingiensis* effectively utilized the nitrogen from wastewater with high COD and its removal up to 81% [74]. The *D. communis* exhibited an immense vitality in removing nutrients (nitrogen and phosphorus-100% removal) and enhancing biomass growth [47]. The microalgae consortium (Scendesmus sp. and Parchlorella sp.) proficiently removed the total inorganic nitrogen in rare acidic element tailing waste solution [75]. Generally, an overall reduction in nitrogen content (NH4+ -N-99% and TN-92%) was attained in wastewater treated with Scendesmus sp. [76]. The *D. armatus* inoculation in developer solution (diluted with BBM medium) also showed efficient removal in TKN content with the increased cultivation period [42].

The controls, dFWM and dACM (no *D. armatus* treatment) has total phosphorus contents of 416±4.32 µg/ml and 433±6.16 µg/ml, respectively. The *D. armatus* effectively removed/utilized the phosphorus content of 271.66±6.23 µg/ml and 237±13.13 µg/ml in dFWM and dACM (Figure 6.11 (b)). The maximum relative % of phosphorus removal, 54.73%, was observed in the phycoremediated dACM fraction (Table 6.2). The microalgae *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus quadricauda*, *Scenedesmus dimorphus* cultured in a photobioreactor with anaerobically digested solution efficiently removed the phosphorus for their growth [77]. The *Desmodesmus subspicatus* exhibited 71% phosphate removal in wastewater [43]. *Scendesmus obliqus* also showed a massive reduction in total phosphorus content in piggery wastewater [78]. The *Scendesmus armatus* showed a great extent of phosphorus removal in influent 36% and solution 51% in municipal wastewater treatment [79]. The *D. armatus* in remediation of developer solution diluted with BBM medium depicted a reduction in phosphorus removal [42]. The microalgae *Micratinium reisseri in* diluted mine solution exhibited a maximum value of phosphorus uptake around 4.9-5.4 mg/l [46].

6.2.3.10 Determination of relativity silver removal (%) and concurrent lipid production in phycoremediation of dFWM and dACM.

The silver with high reflectivity is abundantly present in the radiographic solution, and its amount varies with the solution category [80]. The developer solution generally contains less silver than other radiographic waste [20, 81]. The dFWM and dACM subjected to *D. armatus* treatment, and its relative % of silver removal and lipids production is depicted in Figure 6.12. The relative % of silver removal was 45.29%, and maximum lipids production was 1.37±0.04% attained in the dFWM (Figure 6.12) (Table 6.1). Further on, dACM achieved relative % of silver removal 43.69% and simultaneously lipids production around 1.42±0.07% (19^{th} d of *D. armatus* cultivation). *D. armatus* treated dACM shown maximum silver removal and lipids production than the phycoremediated dFWM (Figure 6.12) (Table 6.1 and 6.2). The *D. armatus* with increased biomass enhances the relative % of silver removal and concurrently lipids production when microalgae are in their log phase. The microalgae adapt diverse mechanisms for the elimination of heavy metals, and its metal uptake relies upon various factors (strain of microalga, concentration of heavy metals, nature, and cultivation conditions) [82–84]. The developer solution diluted with BBM and its treatment with *D. armatus* effectively removes the silver and simultaneously produces maximum lipids (1.39%) [42]. The silver accumulated within the cytosol in *Coccomyxa actinabiotis* and the highest concentration of silver ions disrupt the photo system path in the chloroplast [85]. The silver was liberated from the various resources (medical clinics, textile, and other industries), assimilated, and absorbed different microalgae strains. The silver was reduced to the silver nanoparticles through the microalgae remediation methodology and afterward switched to less toxic forms inside the cell [86]. The *C. vulgaris* show with high sorption capability to bind the Ag^{+} (97.5%) and silver thiosulphate complex (89%) and its efficient removal from the solution [87]. The *D. armatus* has also shown remarkable potential in producing fatty acid methyl esters (lipids) and remediation of waste cassava solution [40]. The relative % of physical and chemical parameters of treated developer solution (diluted with food waste and agri-compost) is tabulated in Tables 6.1 and 6.2.

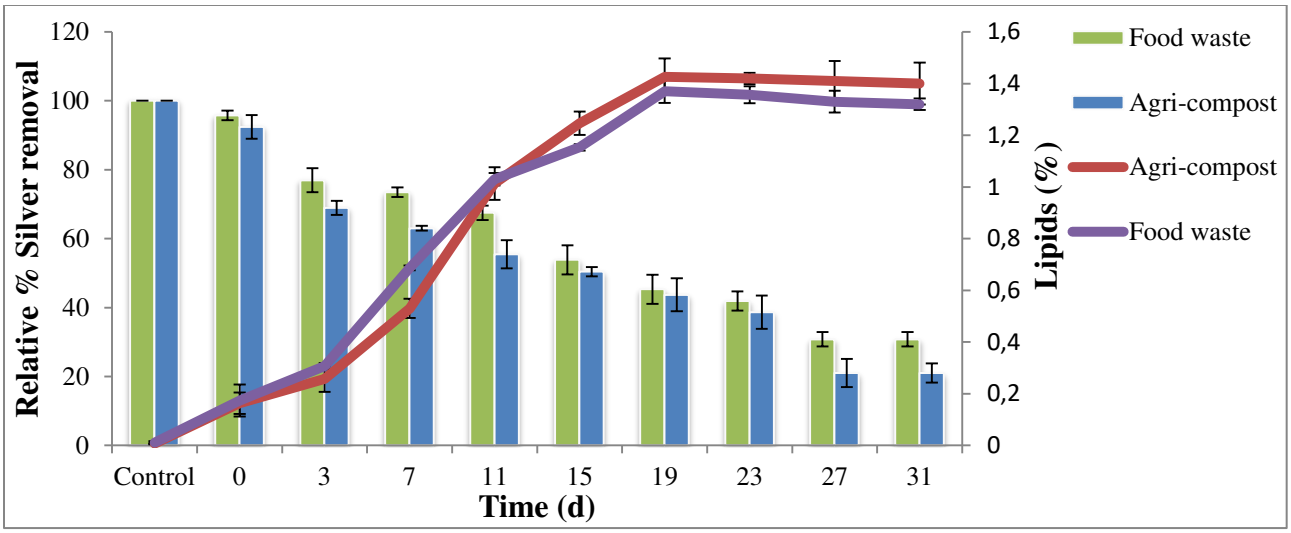


Figure 6.12: Depiction of concurrent relative silver removal (%) with lipid production with the phycoremediation of dFWM and dACM. All values are represented as ± s.d of triplicate experiments.

Table 6.1: Relative bioremediation potential (%) of *D. armatus* in dFWM.

Parameters	Control value	19th day value of parameter	Relativity %
pH	9.7±0.01	9.39±0.04	----
Total solids (g/l)	25.03±0.46	20.75±0.28	82.90%
Total dissolved solids (g/l)	14.36±1.12	11.71±0.79	81.54%
Total suspended solids (g/l)	10.66±0.08	9.03±0.04	84.70%
Total volatile solids (g/l)	11.96±0.78	9.8±0.21	81.93%
Total fixed solids (g/l)	13.06±0.78	10.94±0.89	83.76%
Conductivity (mS)	17.33±0.16	19.83±0.09	114.42%
Density (g/l)	1021.37±1.04	985.86±0.32	96.52%
Biological oxygen demand (g/l)	1.02±0.03	0.26±0.01	26.07%
Chemical oxygen demand (g/l)	9.45±0.82	1.73±0.49	18.30%
Total Kjeldahl Nitrogen (%)	0.251±0.00	0.22±0.00	89.64%
Total phosphorus (µg/ml)	416±4.32	271.66±6.23	65.30%
Silver (g/l)	0.0117±0.00	0.0053±0.00	45.29%
Lipids (%)	0.01±0.00	1.37±0.04	13700%

Table 6.2: Relative bioremediation potential (%) of *D. armatus* in dACM**.**

Parameters	Control value	19th day value of parameter	Relativity %
pH	9.65±0.01	9.37±0.02	-----
Total solids (g/l)	27.16±0.21	22.84±0.51	84.09%
Total dissolved solids (g/l)	18.35±0.52	15.19±0.8	82.77%
Total suspended solids (g/l)	8.81±0.15	7.65±0.33	86.83%
Total volatile solids (g/l)	10.55±0.41	8.98±0.66	85.11%
Total fixed solids (g/l)	16.61±0.48	13.86±0.44	83.44%
Conductivity (mS)	18.36±0.01	20.5±0.29	111.65%
Density (g/l)	1007.53±0.33	985±0.32	97.81%
Biological oxygen demand (g/l)	1.18±0.01	0.20±0.00	17.61%
Chemical oxygen demand (g/l)	9.45±1.14	1.05±0.18	11.11%
Total Kjeldahl Nitrogen (%)	0.27±0.00	0.20±0.00	74.44%
Total phosphorus (µg/ml)	433±6.16	237±13.14	54.73%
Silver (g/l)	0.0119±0.00	0.0052±0.00	43.69%
Lipids (%)	0.007±0.00	1.42±0.07	20285.71%

6.3 Conclusion

The X.D has a massive quantity of contaminants, and its hazardous nature requisite the proper discharge into the environment. The primary toxicity studies of *D. armatus* cultivation in different ratios of X.D with FWM and ACM show maximum growth of *D. armatus* in 3:1 (FWM/ACM: X.D). The 3 FWM/ACM: 1 X.D ratio has been utilized for with *D. armatus*-based phycoremediation studies. The dACM demonstrated the enviable outputs in reducing bioremediation parameters, silver removal, and lipids production concerning the control medium (without *D. armatus*). The promising bioremediation results with dACM has been observed on19th day with a 0.20 g/l BOD, 1.05 g/l COD with a 43.69 % relative silver removal with concomitant lipid production of 1.42%. The bioremediated dFWM showed a 0.26 g/l BOD, COD- 1.73 g/l COD values with a relative silver removal of 45.29% by simultaneous lipid production of 1.37% on 19th day of cultivation.

SUMMARY, MAJOR FINDINGS

&

FUTURE PROSPECTS

SUMMARY, MAJOR FINDINGS & FUTURE PROSPECTS

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SUMMARY:

- The waste X-ray developer and fixer solution have a higher amount of BOD, COD, silver, and other parameters, which is more than the permissible limits set by Govt. to discard the waste to the environment.
- The toxicity screening of both microalgae in various dilutions of X-ray developer and fixer solutions with BBM medium showed the highest cell count in the ratio of 3:1 (BBM: X-ray developer solution).
- Microalgae *D. armatus* showed more potential to treat the X-ray waste developer solution compared to *S. abundans.*
- Relative % of silver -44.06%, BOD-20.86%, and COD-13.88% and reduction in other parameters achieved at 19th day of *D. armatus* cultivation in treated X-ray developer solution (using BBM).
- The highest lipids content, 1.39% observed on the 19th day of *D. armatus* treated developer solution (using BBM)
- Agri-compost and food waste as an algal media source and its 3:1 ratio (FW /ACM: BBM) utilized for the bioprocess dynamic study and lipids, carbohydrates, and proteins formation.
- The agri-compost media (maximum biomass concentration-0.871) was used as alternative source compared with other media (food waste and BBM) from the bio-process dynamic study.
- Toxicity tolerance study of food and agri-compost medium showed highest cell count in the 3:1 (FWM/ACM : X-ray developer solution).

- Bioremediation results of X-ray developer solution with diluted agri-compost medium showed promising results on 19th day in physical and chemical parameters reduction (i.e., final BOD - 0.20 g/l; COD- 1.05 g/l; Total phosphorus- 237µg/ml).
- The relative removal (%) of bioremediation parameters in waste X-ray developer effluent diluted with agri-compost medium has BOD-17.61%, COD- 11.11%, silver removal-43.69% with a relative lipid production of 20285%.
- The promising bioremediation results have been attained with the X-ray developer solution using diluted food waste medium on the 19th day of *D. armatus* cultivation (with a final BOD- 0.26 g/l; COD- 1.73 g/l; Total phosphorus- 271 µg/ml).
- The relative (%) bioremediation potential of developer solution diluted with food waste medium has 26.07% of BOD, 18.30% of COD with a relative silver removal of 45.29% and relative lipid production of 13700%

MAJOR FINDINGS:

- Reported the first-time bioremediation of X-ray developer solution.
- Successfully implemented the phycoremediation of X-ray developer solution with concomitant lipid Production.
- The waste resources agri-compost and food wastes proposed to be a cost-effective media.
- The proposed agri-compost and food-waste media's also shown promising results in phycoremediation of X-ray developer solution.

FUTURE PROSPECTS:

- ✓ The mechanism of silver uptake in *D. armatus* treated waste developer solution can be studied.
- ✓ Cultivation of *D. armatus* and remediation of waste developer solution in scale-up level can be performed.
- ✓ Utilization of *D. armatus* in remediation of other types of wastewater and study its biochemical components.

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CHAPTER-1

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CHAPTER -2

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CHAPTER 5

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LIST OF PUBLICATIONS

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Journal papers

- **Sharma, S**., & Garlapati, V. K. (2020). Phycoremediation of X-ray developer solution towards silver removal with concomitant lipid production. Environmental Pollution, 268, 115837. http://dx.doi.org/10.1016/j.envpol.2020.115837 (**Impact factor: 8.071**)

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- **S. Sharma** and Garlapati VK (2021) Waste resources as an algal media constituents and its bioremediation potential of X-ray diagnostic effluents with integrated algal lipid production (Communicated).

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- ✓ **Sharma S** and Garlapati VK, "Characterization of Medical Waste towards Microalgal based Bioremediation" *Oral* presentation at 3rd Himachal Pradesh Science Congress, October 22-23, 2018, IIT Mandi, HP- 175005, India.

- ✓ **Sharma S** and Garlapati VK, "Screening of microalgae towards tolerance of various concentrations of radiographic waste solutions" *Poster* presentation at International Conference on recent trends in Biotechnology and Bioinformatics (ICBAB-2019), 01-03 August 2019 , Jaypee University of Information Technology, Waknaghat, Solan, H.P.

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