Chapter-1

Introduction

1.1 Background:

In the existing epoch, an intensifying population & huge expansion of industries, overutilized the non-renewable resources, such as natural gases, minerals, coal, oil, and water etc. Among these resources, water is one of the major limited resource available on earth. These industries had produced excessive load of wastewater or effluent. This effluent released into the natural water streams which adversely affect flora, and fauna available there. Hence, the accessibility of potable water has become a crucial threat globally. Various types of industries generate a variety of effluents or waste water, in between, the dairy industry is the particular one which produce an enormous yield of effluent or waste water. These effluents contain plentiful of organic & inorganic pollutants.[1, 2] Though the industries are an essential component of economy, but rapid industrialization has become a matter of major concern, due to their deteriorating activity on the environment. As these industries produced life threatening or toxic compounds which released into the environment, without proper treatment. This could be a cause of different types of hazardous diseases in human population.

The dairy industry is the major user of freshwater and generator of enormous amount of wastewater although it has a remarkable act to generate Indian economy. Additionally, dairy industry is flourishing constantly. Hence, it has become the chief developer of wastewater, while on the other hand, has massive impression on nation’s economy. Our dairy industry's growth contributes significantly to water contamination and adversely impacts water bodies by releasing significant amounts of pollutants into the water.[3] According to FAO 2020, India, has grade one in milk production by contributing 22% of the world's milk manufacturing, followed by USA, Pakistan. Dairy industry produced huge amount of effluent and hence, become the root cause of environmental destabilization. These industries swallow huge amount of fresh water when compare with other agronomical industry. This has documented, one litre of milk processing uses 03 litres of potable water.[4] It was estimated that the production and utilization of dairy products, surprisingly escalating constantly. Globally, it would be increasing up to 13.7% by 2023.[5] If this forecast comes accurate, and then effluent production is expected to enhance remarkably.

Although, Milk has been regarded as the most significant source of nutrients needed by the body and one of the main food elements in the world. While water is the primary ingredient utilized in the dairy industry to produce milk products, which generates large volumes of wastewater, making disposal a difficulty that cannot be disregarded.[1] According to reports, the dairy sector produces 2 to 10 L of DWW for every liter of milk manufacturing and packaging.[3, 6, 7] These industries primarily produce liquid effluent during the processing of different useful products from whole milk; like buttermilk, condensed cream, sweet whey, curd, cheese, butter, and ice cream, etc. The distinguishing features of dairy wastewater might be varying according to the formation of final products. Despite the compositional variations, dairy effluents are characterized by a significantly higher temperature, excessive organic components, and varying pH range that necessitate particular treatment to prevent or minimize ecological damage. The loss of dissolved oxygen is the prominent issues brought by discharging untreated or semi treated dairy effluent into the environment. Because dairy effluents are high in oil and fat molecules, they create a thin coating on the surface of water that prevents transportation of gases (oxygen and carbon di oxide) into water bodies. Hence, making it difficult for the local flora and wildlife to survive.[4, 8]

Additionally, majority of the whey, particularly acid whey, has been discarded without being properly treated, due to having elevated cost for its treatment and disposal operations which leads to an excessive financial burden.[3, 4] It also has detrimental effects on the survival of living beings. The environment has been influenced by each and every step of the dairy sector, involving the manufacturing, packaging and storage of milk products, and afterwards their successful marketing, and distribution process. The dairy waste water from various manufacturing processes are not discharged at the same time, hence it has significant fluctuations in its physico-chemical parameters.[4, 9] Hence, this industry has been introducing huge amount of toxins into the food chain and untreated wastewater into the freshwater streams, which further damages the whole ecology of freshwater resources. If the dairy industry's wastewater production levels are not properly dumped of, it can create a number of ecological issues.[10] Consequently, for a sustainable environment, wastewater management and treatment are very essential. Generally, dairy effluents containing high concentration of lipids such as triacylgycerides, phospholipids, unsaturated and saturated fatty acids, carbohydrates like; lactose, glucose and galactose etc. The dairy waste water also contains two major soluble proteins casein and whey protein etc., resulting in high BOD and COD levels. Its excessive BOD (biological oxygen demand) depletes the dissolved oxygen content and create anaerobic conditions very rapidly under the aquatic system. Dairy effluents contained various inorganic and organic compounds of N and P, available in higher concentration and responsible for the alkalinity of the dairy waste and other than it also contains trace amount of some mono-valent and bi-valent cations like Na, Mg, Ca, Fe, K, Ni, Mn etc. Use of detergents and chlorine in the dairy industry further deteriorates the quality of effluent.[11] Dairy effluents are biologically active because of the presence of heavy organic load and when it is discharged either untreated or semi-treated into water streams leads to deficiency in oxygen level. Hence, the proper treatment before the disposal of dairy waste effluent has become the major problem for the vitality of the dairy industry and environment.

1.2 Proposal:

There are different technologies which can be used for the treatment of dairy waste before being released into the natural habitat or surroundings.[12] Dairy effluents are treated using mechanical, physicochemical, and biological techniques. To balance variations in both volumetric and mass flow, mechanical treatment is required. Additionally, some of the suspended particles are reduced. While emulsified compound removal can be accomplished with physicochemical procedures from dairy waste.[13] Several Physico-chemical treatments are used for remediation of dairy effluent, such as chemical precipitation, coagulation, coagulation/flocculation,[14] adsorption, electrocoagulation, electro flocculation,[15] membrane bioreactor, combined electrode system, etc.[16] But there are various demerits regarding the use of physicochemical treatments such as its higher cost, due to adding chemical reagents and generating secondary pollutants. These chemical reagents only perform partial treatment.[3, 4, 17] Extremely low to moderate, elimination of soluble chemical oxygen demand, (COD) is another drawback.[1, 4]

In order to compensate for the negative aspects of physico-chemical approach, biological methods are a suitable alternative for the remediation of dairy wastes. These are eco-friendly, cost-effective, potential to mitigate the contaminants from dairy effluents.[1, 3] The highly biodegradable pollutants make biological wastewater treatment systems the preferred method. Microorganisms such as bacteria, fungi, microalgae, etc. have been employed in biological treatment methods. Various attempts have been already made to solve the effluent problem by reusing the nutrients rich dairy waste with microorganisms to metabolize organic matter.[12] Among these microorganisms, the microalgae are well-known bioremediator because of their ability to grow photoautotropically and mixotrophically.[3,5,18] Dairy effluent having huge content of phosphorus, nitrogen, and organic matter thus, the nutrient-rich dairy waste effluent proved to be a suitable medium for proper growth and development of microalgae.[3,19]

Employment of microalgal strains for remediating the dairy waste water results in decontamination of dairy effluent along with mass culture of microalgae.[20] Mass cultivated microalgal biomass, harvested for lipids produced which are utilized in diverse industrial applications. Earlier researches suggested that development of microalgae for generating lipid components from dairy waste, had provide better results than any other wastewater used as microalgae cultivation medium.[17,21]

Dairy effluent has huge quantity of phosphorus, nitrogen, and organic contents thus, the nutrient rich dairy waste effluent proved to be a marvellous medium for the progression of microalgae. Microalgae can be considered as a self-sustainable alternative, providing a solution to curb both these problems i.e., dairy effluent treatment & energy crisis in most significant, eco-friendly and inexpensive way. Microalgae are one of nature's most important natural resource, which grow rapidly and produce several useful primary & secondary metabolites such as lipids, proteins, carbohydrates, pigments as well as other valuable by-products. Breeding in effluents, microalgae can survive and bloom in contaminated sites, utilizing inorganic and organic contaminants of effluents as a nutrient source for their own growth and flourishment. While in return, phycoremediating these sites. Effluent remediation by microalgae does not involve use of expensive and hazardous chemicals. Moreover, the generated microalgal biomass after phycoremediation, further utilized for bioenergy production. Generating bioenergy molecules from “negative-value” dairy effluent is now in vogue, and concurrently helps to meet the world’s energy requirements with decrease the pollution load.[21]

Many microalgae cultures displayed high growth on the diluted effluents from dairy.[20] Microalgae represents promising biological system for remediating dairy effluent because of having, their metabolic flexibility, perform as photoautotrophic, mixotrophic, or heterotrophic metabolic activity.[17,21]

Moreover, microalgae have the capability to utilize solar energy for its photosynthetic activity and combine water with carbon dioxide in order to make their food autotrophically, further; it converts the environmental carbon to oxygen. Due to this ability of microalgae, they have been used to remediate the dairy waste as the nutrient sources for their growth, and to reduce the concentrations of phosphorus, nitrogen, and other inorganic substances. It has been reported that microalgae are the better source for manufacturing of bio-fuel due of its elevated photorespiration activity, and rapidly growing biomass activity.[22] Micro algal biomass has been utilized for generation of biological compounds like single cell protein, bio-ethanol, biogas, hydrogen gas and lutein or astaxanthin and can also be used as bio fertilizers.[23] The microalgal strain which contain large number of fatty acids, like polar and non-polar or neutral lipids, and also flourishing in the native domain of dairy waste effluent have yet to be used for bio-fuel production. Micro algal feedstock is one of the best medium for bio-fuel production and as a substitutional source of fossil fuel and also proved beneficial to eradicate greenhouse gas emissions. Mass cultivated microalgae are harvested for lipids production which utilized in diverse industrial applications. Microalgal derived biodiesel, which is high in C16-C18 hydrocarbons, may be considered a dawn of clean and sustainable energy. These environmentally-friendly green technologies highly used to recover lipids from microalgae using dairy effluent as cultivation medium.[7,9]

Many microalgal strains have been analyzed for their lipid compositions, approx 20-50 % of dry biomass including components of lipids. Some microalgae such as species of *Nannochloropsis*, *Neochloris*, *Tetraselmis*, *Isochrysis*, *Dunaliella*, *Crypthecodinium*, *Cylindrotheca*, *Nannochloris*, *Schizochytrium*, *Phaeodactylum*, *Nitzschia*, *Porphyridium*, and *Chlorella*have been recognized for containing certain classes of polar and non-polar lipids. Nonpolar lipids like sterols, free fatty acids acylglycerols, etc. and polar lipids like glycosylglycerides, phosphoglycerides, and sphingolipids have been reported in microalgae.[24]

There are very few researches which represented the complete characterization of lipids of a particular micro alga. Due to the great differentiation among the species of microalgae and their cultural conditions, frequently, more challenging when compared the outcomes for biochemical profiling of bio molecules such as lipid, protein, and carbohydrates etc. present in all microalgae species. Because of this reason, there is still a need for the whole characterization of different bio molecules of each and every one microalga. This helps in demonstrating the suitable analytical method which can be employed for further use. The complete characterization of fatty acids in microalgae, is also indespensable for the selection of good species. Thereafter, the production of fatty acids can be utilized as bio-fuels and other high-value compounds in the field of food, pharmacy.[25]

There are various strains of microalgae (*Chlamydomonas, Chlorella* sp. etc.) which have been employed as an experimental design to examine its anabolic and catabolic activities and functions. Genomes of *Chlamydomonas reinhardtii* have been sequenced and its molecular and genomic tools is also available. It can also accumulate considerable value of triacylglycerols under salt stress or nitrogen deprived conditions. Micro-algal species collected from the dairy waste indicated great prospective for low-cost cultivation and producing lipid from dairy waste, without modifying the procedure. It was documented that the *Chlorella* strain isolated from the dairy waste water showed good biomass production when grown on dairy effluent, reduced its chemical oxygen demand, and nutrients.[20]

Microalgal culture has the Potential to contributing in bioremediation of environment and also in the formation of lipid byproducts such as saturated and unsaturated fatty acids, B-carotenoids, oxylipins etc. microalgae culture could also be modified to synthesize various types fatty acids, hydrocarbons, sterols etc. These bio products have huge applicability in various fields like; biofuel, pharmaceuticals, nutraceuticals, food and nutrition, (aqua feed and animal feed) and cosmetics. Microalgae biomass could be modified energy containing fuels through thermal processes. In future with continued progress being made in the field of systems biology metabolomics will play an important role as a holistic approach in generation of bio-fuels which produce lower emissions, for green energy production and thereby maintaining environmental sustainability.

1.3 Research Hypothesis

In the literature, we have observed that the utilization of autochthonous microalgal species isolated from dairy wastewater for cultivation purposes demonstrates significant potential in terms of bioremediation efficiency, biomass production, and lipid yield. Hence, keeping that thing in mind, we hypothesize that these microalgae, when cultured on dairy wastewater, will exhibit effective remediation of pollutants (BOD, COD, nitrogen, and phosphorus), resulting in higher biomass and lipid production. Additionally, we posit that specific strains with superior lipid production capabilities can be identified and characterized through morphological analysis, phylogenetic investigation, and examination of their lipid composition. The outcomes of this research aim to provide valuable insights into the sustainable use of microalgae for dairy effluent treatment and the potential production of energy-rich biomolecules for future applications.

1.4 Aims and Objectives:

The current study has been carried out to explore the capability of autochthonous microalgal species. Isolation of different autochthonous microalgae species from dairy waste water which enormously employed as a cultivation medium for the growth and development of microalgal species, had been done by using standard protocol. After that Microalgal species were screened for dairy effluent remediation, higher biomass and lipid production. When Microalgae cultured on dairy wastewater, the following parameters were studied; Bioremediation of DWW, lowering of BOD and COD, nitrogen and phosphorous content. Isolated microalgae were effectively utilized for the eradication of nutrients from dairy wastewater and used as an efficient biodegradative agents as well as incredible resources for the production of energy rich biomolecules. Different concentrations of DWW were applied for optimization of microalgal biomass and lipid production. Higher lipid producing microalgal species were characterized and identified by studying their morphological features using compound microscope. After morphological characterization of microalgae, the phylogenetic analysis was carried out by employing 18S rRNA sequence analysis. Extracted microalgal lipids were characterized for constitution of fatty acids in total lipids, hydrocarbons, and sterols through GC-MS. This information revealed about type of lipids to be exploited as future biomolecule.

The present study offers the sustainable solution for microalgal-mediated bioremediation of dairy effluents along with production of lipids, which could be exploited for the bio-suitability (as the precursor of biofuel, food or nutraceuticals, pharmaceuticals etc.) with multifaceted applications. The study was started with the following objectives;

1.4.1. OBJECTIVES:

1. Isolation & identification of different autochthonous microalgae species from dairy waste water.
2. Screening of isolated microalgal sp. for dairy effluent remediation, higher biomass and lipid production.
3. Identification of higher lipid producing microalgal species.
4. Microalgal lipids characterized by GCMS for its bio-suitability.