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Oily waste to biosurfactant: A path towards carbon neutrality and environmental sustainability

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ABSTRACT

Tons of oily waste accumulating worldwide has led to severe environmental problems and an increase in carbon footprint. The oily waste is rich in carbon and therefore its utilization as a substrate for the production of value-added products can aid in the concept of carbon neutrality. Oils can be directly utilized as substrate and microorganisms can catabolize them to produce biosurfactants. Biosurfactants being biodegradable and less toxic than synthetic surfactants are the molecules of the 21st century and are preferred candidates. Also, several fungal species can bio-transform oils to produce biosurfactants. Therefore, this study comprehensively summarizes different categories of oily waste generated worldwide, their sources, and environmental toxicity. The microbial efficiency towards oily waste utilization for the production of biosurfactants is reviewed. Following this, advance techniques including metabolic engineering, and omics approaches for biosurfactant production from this waste have been presented. Their global market and future perspective have been discussed to further emphasize the requirement for biosurfactants. The state-of-the-art information provided in various sections of this manuscript may aid the researchers to understand the relationship of oily waste utilization with carbon footprint generation. This directs attention and warrants future research towards the development of improved pathways/processes in oil waste based biorefineries.

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1. Introduction

In the modern world, oily waste management has become a significant challenge worldwide (Gaur et al., 2022a,b; Medeiros et al., 2022). Employing oily waste as substrates for the production of industrially important biosurfactants can serve as a tool to aid in the waste disposal and may reduce the enduringly detrimental environmental consequences. The chemically derived surfactants have historically been produced using petroleum or its derivatives. These surfactants have been extensively utilized for their various applications in textile, petroleum, food, and pharmaceutical applications. They pose severe toxicity as a result of their production and improperly controlled environmental discharge (Rebello et al., 2014; Rodríguez et al., 2021). Chemical surfactants adversely affect microalgae and other microbes by interacting with cell membranes and causing the breakdown of cell structure. Once the levels are high enough, negative effects are seen in fish, which take in chemicals through their skin, as well as in animals and individuals that consume meat (Ciurko et al., 2022), suggesting the negative impact on target and non-targeted organisms as well as human *via.,* the food chain. Consequently, low-cost feedstock, capable microorganisms, and appropriate bioengineering techniques are required to produce biosurfactants to overcome the economic challenge to compete with synthetic surfactants.

Oily effluents are produced on a daily basis by industries and households. These waste products are generated during several industrial processes such as maintenance, production, and transport (Medeiros et al., 2022). The used oil contains hazardous substances such as phenols and hydrocarbons like polycyclic aromatic hydrocarbons (PAHs), which prevent the development of both plants and animals. Many countries have enacted strict laws requiring businesses to manage their effluents, with maximum oil concentration restrictions in wastewater discharge falling between 5 and 100 mg/L (Abuhasel et al., 2021). A sustainable contribution to the circular bioeconomy is made by using approximately 29 million tonnes of lipid waste that is produced worldwide as a cheap substrate for the synthesis of biosurfactants in biorefineries (Liepins et al., 2021a). In developed countries, the primary source of oily waste is waste cooking oil which typically accounts for about 50 kg of per capita production annually (Sharma et al., 2022; Gaur et al., 2022a), because of the high demand for food concerning the increasing global population. With 3.3 billion tonnes of carbon dioxide (CO2) emitted into the environment, food wastes have a carbon footprint that accounts for around 8% of worldwide greenhouse gas (GHG) emissions (Mgbechidinma et al., 2022). Thus, it is urgent to apply the concepts/terms of net-zero (carbon neutrality) and environmental sustainability to the goal of lowering greenhouse gas emissions.

Carbon neutrality refers to the equilibrium between carbon emissions and carbon absorption from the environment in carbon sinks. Refineries and the food processing industry both produce significant volumes of oily waste and stimulate the emission of CO_2. The excessive emissions of greenhouse gases from the decomposed wastes also contribute to climate change and a high carbon economy (Mgbechidinma et al., 2022). Furthermore, it is also essential to develop methods or strategies for using waste as a cheap substrate that encourages the waste to turn into value products with a low carbon footprint. To achieve this biosurfactant has been previously reported as a key role player in minimizing atmospheric carbon dioxide emissions (Rahman and Gakpe, 2008) and the oily waste can potentially be exploited as a rich substrate, due to the structural composition of the fatty acids (Das and Kumar, 2018).

Several microbial genera such as *Bacillus, Pseudomonas, Acinetobacter,* and *Candida* have been extensively reported for biosurfactant production. Numerous studies have identified the microbes that can utilize vegetable-derived oils like soybean and sunflower oil for producing biosurfactants (Gaur et al., 2019). In addition to this several approaches such as metabolic engineering and system biology, and omics (metagenomics, metatranscriptomics, and metaproteomics) have been currently used for the effective production of biosurfactant and remediation of oily waste (Gaur et al., 2022c). Enhanced production and design of rhamnolipids were achievable by metabolic engineering of both regulatory and biosynthetic pathways (Dobler et al., 2016). Similar to this, omics techniques have been utilized for the identification of desired microorganisms (metagenomics), their respective gene expression (metatranscriptomics), and control at the protein level (metaproteomics), for the synthesis of biosurfactant from waste (Gaur et al., 2022c). The omics method is useful, but there are still a lot of problems to be solved. However, to increase the applications of biosurfactants, the multi-metaomics strategy, which combines two or more approaches, can be considered to achieve better data and in-depth insights into genes. Biosurfactants are excellent products for use in farming, food processing, water, and soil restoration, microorganisms-based oil recovery, biomedicine, nanomaterials, and other diverse fields, including cleaning agents. The characteristics that make biosurfactants suitable include low toxicity, specificity, ability to perform under a variety of harsh and extreme environments, as well as biocompatibility and biodegradability (Singh et al., 2019). Although, ineffective bioprocessing and bioengineering have made it difficult to produce these compounds at a reasonable cost. Despite extensive study, there is a scarcity of literature on developing bioprocess optimal control methodologies, their accomplishments, and the prospective for cost-effective biosurfactant synthesis based on the use of low-cost feedstock in their process of production. Fig. 1 highlights the network visualization of keywords co-occurrence of 'oily waste', 'biosurfactant', 'environment', 'sustainability', and 'carbon' (Figure is constructed based on 216 papers (of search of the keywords) published between 2019 and 2023 and accessed from SCOPUS on 26th November 2022).

This review addresses a critical issue of the effect of waste oil valorization for biosurfactant production on environmental sustainability and carbon footprint reduction. The sources, fate, and negative impacts of oily waste in the environment, as well as different aspects of producing biosurfactant for managing oily waste, are summarized. Some of the most cutting-edge and promising technologies, like metabolic engineering and omics approaches, have been discussed to improve and optimize biosurfactant production. Recent market research and advancements in the field of ideally manufactured

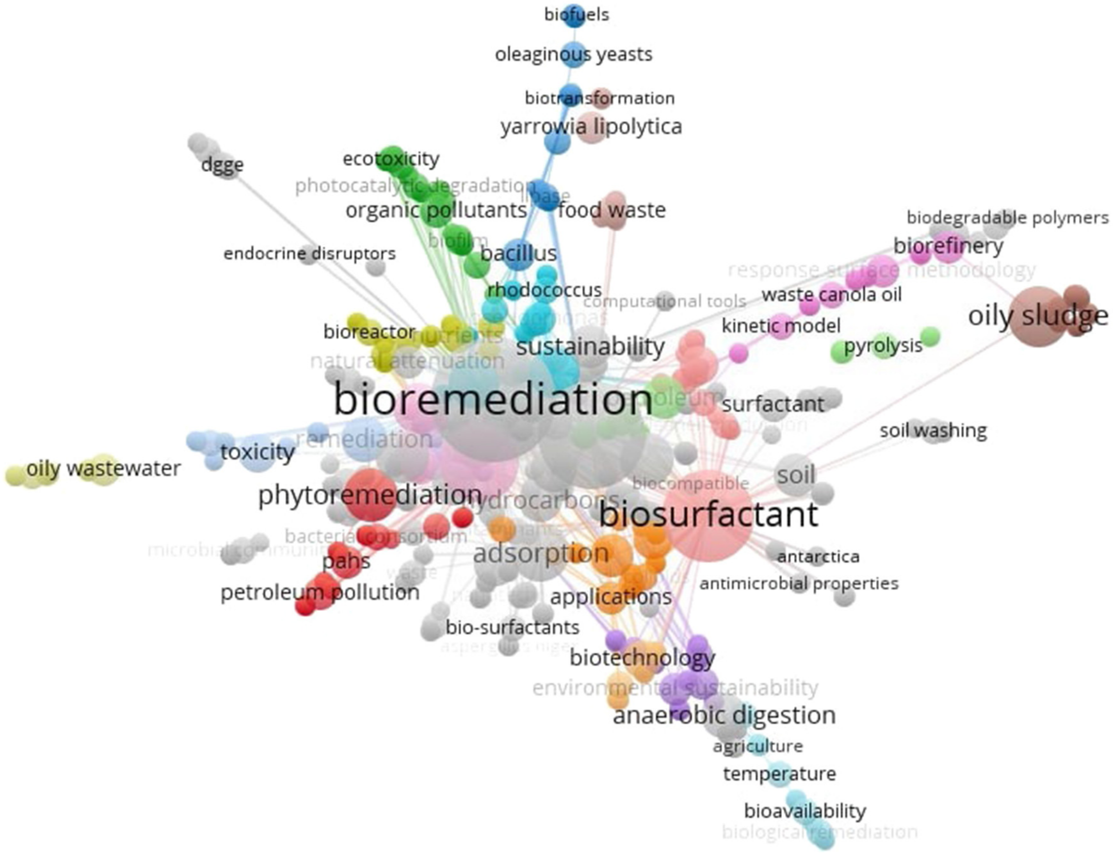

Fig. 1. Network visualization of keywords co-occurrence of 'oily waste', 'biosurfactant', 'environment', 'sustainability' and 'carbon' (Figure is constructed based on 216 papers (of search of the keywords) published between 2019 and 2023 and accessed from SCOPUS on 26th November 2022).

cost credit substrates for advanced product development and economization in subsequent processes are among the key factors highlighted along with future considerations. The recent advancement included in this review would not only provide an overview of important factors for economically producing biosurfactants, but they would also highlight several research-opening initiatives towards carbon neutrality and environmental sustainability.

2. Occurrence, sources, and fate of oily waste

Wastes are mainly generated by various human activities throughout the world mostly in developing countries. Increased resource utilization in turn increases the generation of waste products which may contain hazardous components. This waste enters the environment and causes severe detrimental effects (Nilsson, 2000). Oily waste such as waste or used cooking oil has (WCO or UCO) been generated from vegetable oils employed by various sources including household kitchen activities, hotels, restaurants, cafes, catering, and other commercial activities as shown in Fig. 2. WCOs are classified as "municipal wastes" in Europe, according to the European Waste Catalogue, and are collected by urban waste networks or directly transported by the ultimate user to a recycling station (Mannu et al., 2019). Several harmful pollutants, such as polyaromatic hydrocarbons (PAHs) and petroleum hydrocarbons, including cooking oil, are classified as hydrocarbon compounds since they are produced from various anthropogenic sources and have contaminated the environment (Zahri et al., 2021). Waste cooking oil or vegetable oil that is obtained after frying at relatively high temperature generate toxic compounds such as hydroperoxides, fatty acid oligomers, and volatile compounds (i.e., low molecular weight ketones, acids, aldehydes, and alkanes) (Kim et al., 2021). Worldwide, the production and consumption of total vegetable oil were estimated to be 20% to 32% that resulted in the various gastronomic practices and consumption patterns in various regions that significantly affect the nature, composition, and quantity of impurities present in the UCO (Orjuela and Clark, 2020; Awogbemi et al., 2021). In addition to this the used cooking oil market was 5.16 billion USD in 2020 and is projected to rise to 10.08 billion in 2028 at 7.76% CAGR. Due to insensible behavior, lack of regulations, and law enforcement, waste oil is disposed into drains, sewers, open areas, rivers, and forestland which leads to the blockage

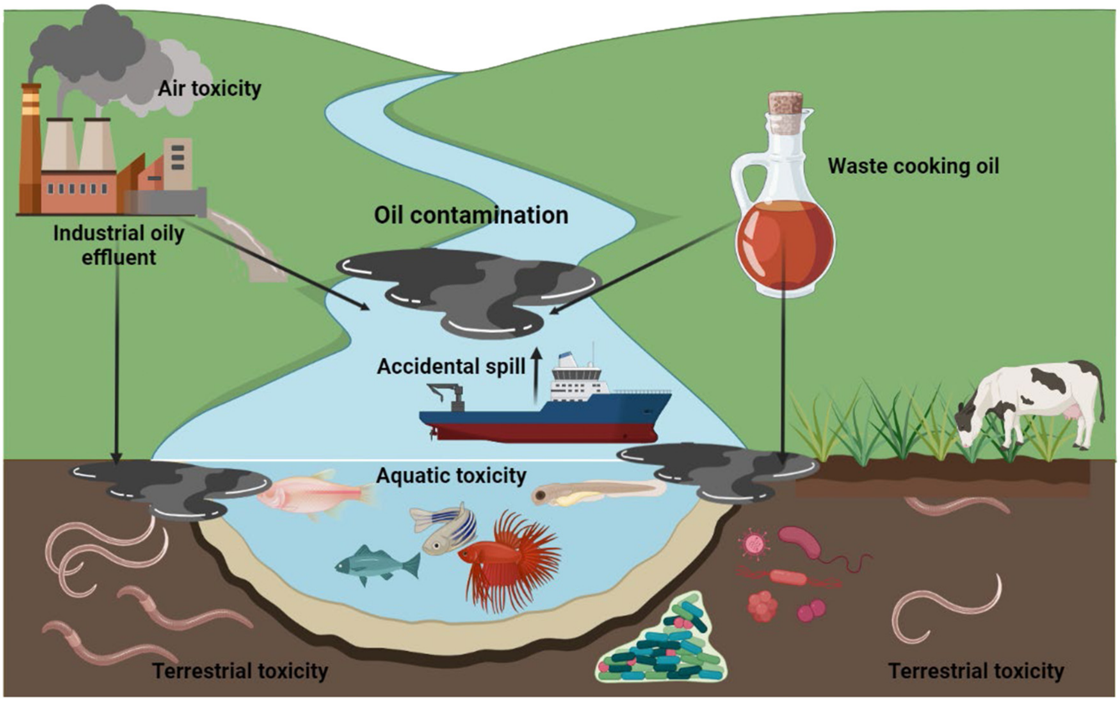

Fig. 2. Schematic representation of sources, fate, routes and toxicity of oily waste to environment.

of drainage systems, stinking, impurities in the terrestrial and marine environment, infrastructure damage, overflow of wastewater, etc. Moreover, the improper disposal of WCO also resulted in the formation of foam, enhancing the organic burden on water sources, reduction in dissolved oxygen concentration, and alteration in ecological balance. Therefore, the huge amount of the generated waste cooking oil when not disposed of properly causes detrimental health problems for humans, animals, and the environment (Orjuela and Clark, 2020; Awogbemi et al., 2021). WCO generally obtained by frying contains several organic, toxic, and volatile compounds such as aldehyde, acrylamide, and 4- hydroxymethyl furfural possess carcinogenic and mutagenic properties. Moreover, these compounds present in the oil, when dissolved in water and absorbed in living cells cause various harmful effects such as inflammation, hypertension, neurodegenerative diseases, and endothelial dysfunction (Zahri et al., 2021). At large scale, waste cooking oil is largely generated from the deep fryers used in restaurants. As reported by the Energy Information Administration, 378 million L of WCO is produced per person in the United States each year. Other countries namely Canada, United Kingdom, Japan, China, Ireland, and other European countries generated 0.135, 0.2, 4.5, 0.45–0.57, 0.153 and 0.7–1.0 million ton/year of WCO respectively. As a result, there are issues with waste collection, waste treatment, and waste disposal due to the significant amount of WCO that various countries generate (Panadare, 2015). In 1994, EPA released a regulation under Oil Pollution Act in which vegetable oil and animal fat cannot be excluded from the rules establishing regulations for oil spill clean-up (Kumar and Negi, 2015). Thus, the improper disposal practices of UCO causes global warming, freshwater eutrophication, scarcity of fossil resource, and freshwater, marine, and terrestrial eco-toxicity.

3. Composition and environmental toxicity of oily waste

A large amount of WCO is generated throughout the world. Generally, glycerol esters containing several essential fatty acids are referred to as cooking oils, which are mostly found to be dissolved in organic solvents (Suzihaque et al., 2022). During the cooking procedure, the affluent chemical composition of the oil undergoes various physical and chemical transformations generating toxic substances that may lead to cancer. During the frying process, significant chemical processes like oxidation, polymerization, hydrolysis, and heat degradation take place, resulting in changes in the contents of saturated and unsaturated fatty acids (Panadare, 2015). The number of frying cycles, which lowers the smoking point value and the unsaturation level of fatty acids, increases the degree of polymerization and adversely affects the properties of the oil, also has an impact on the formation of volatile organic compounds (VOCs), and particularly of off-flavor compounds (Mannu et al., 2019). More often, WCO possesses a high amount of fatty acids, ∼60% monosaturated free fatty acids and 26% polyunsaturated fatty acids that produce odor and causes corrosion of metals and solid materials. The two primary saturated fatty acids found in WCO are stearic and palmitic acid (Chhetri et al., 2008). WCO consumption results in major human health problems, including mutagenesis and possible gastrointestinal disturbances (Suzihaque et al., 2022).

Table 1
Summary of maximum reported fatty acid composition in waste cooking oil.

S. No.	Type of fatty acid	Acronym	Maximum composition (%)		
			Awogbemi et al. (2021)	Paul et al. (2021)	Awogbemi et al. (2019)
1.	Oleic acid	C18:1	58.57	23.96	18.02
2.	Palmitic acid	C16:0	54.75	–	43.2
3.	Linoleic acid	C18:2	56.98	39.10	33.89
4.	Stearic acid	C18:0	5.59	–	1.14
5.	Myristic acid	C14:0	1.03	–	17.04
6.	Lauric acid	C12:0	0.34	–	–
7.	Linolenic acid	C18:2	0.76	5.25	–
8.	Eicosanoic acid	C20:1	0.8	–	–
9.	Arachidic acid	C20:0	0.79	–	–
10	Palmitoleic acid	C16:1	0.25	–	–
11.	Erucic acid	C22:1	–	–	0.26
12.	Caprylic acid	C8:0	–	–	0.20
13.	Undecylic acid	C11:0	–	–	1.85
14.	Nonadecylic acid	C19:0	–	–	9.76

Vegetable oil is typically present in the cis-configuration, which is nutritionally advantageous, but when fats are partially hydrogenated, the cis form is converted into the harmful trans form of fatty acids (He and Liu, 2019). These trans fatty acids were reported to have detrimental effects on human health and significantly increase the risk of coronary heart disease. Oil undergoes partial hydrogenation, which enhances its flavor and oxidative durability by lowering the amount of unsaturated fatty acids. However, this processing is undesirable because it adds additional costs and simultaneously creates trans-double bonds (He and Liu, 2019). The used frying oil is mostly composed of mono-, di-, and tri-glycerides, with small amounts of free fatty acids, and a larger concentration of polar hydrocarbons than fresh oil. Vegetable oils are typically triacylglycerols, which comprise glycerol and a variety of fatty acids esterified at the hydroxyl residue; sn1 and sn3 are located at the ends, while sn2 is located in the central position of the glycerol molecule (Zambelli et al., 2015). It has been reported that WCO contains a large amount of free fatty acids that is helpful in the formation of soap and water (Gnanaprakasam et al., 2013). Based on the free fatty acids (FFA) content; WCO is categorized into yellow fat and brown fat where the former is known to have FFA of less than 15% while the latter has more than 15% FFA and water. The composition of fatty acids in WCO is shown in Table 1. The most widely used source of biodiesel is yellow fats, which can be used after washing and filtration. Biodiesel that has been derived from waste oil differs in the fatty acid composition, its properties, and the level of toxicity (Pikula et al., 2019). The frying of oil in open air modifies the structure of cooking oil through an oxidation reaction by producing the hyperoxide that was further oxidized to produce a hazardous substance 4-hydroxy-2-alkenals. The hydrolysis process takes in vegetable oil with unsaturated and short-chain fatty acids more easily because of their high water solubility rather than saturated and long-chain fatty acids. The formation of several chemical compounds may decrease or increase depending on the duration of frying the oil where oil may also get contaminated with the traces of metals from used equipment (Liepins et al., 2021a).

In WCOs, the concentration of PAHs was reported to be very high These PAHs are mutagenic and carcinogenic substances posing threat to human health and environment. When WCOs are used repeatedly, they form volatile and non-volatile components which causes serious harm to humans such as tumor development (Afwanisa'Ab Razak et al., 2021). Studies by Mulyati et al. (2020) and Ambreen et al. (2020) demonstrated that consuming WCO high in saturated fat causes elevated blood triglyceride levels that impaired the liver function of Wistar rats and rabbits. Shuguang et al. (1994) studied the toxicity of cooking oil fumes on Chinese women where a high concentration of PAHs was recorded. Dibenzo(a,h)anthracene and benzo(a)pyrene, which are highly carcinogenic chemicals found in cooking oil, have been linked to the development of lung adenocarcinoma in Chinese women who were exposed to cooking oil vapors in the kitchen. Not only for humans, but the PAHs were also found to be toxic for the non-targeted organisms of aquatic and terrestrial biota. Water-repellent conditions are created by oil waste pollution in the soil, which reduces soil fertility. Since soil is a habitat for a variety of microorganisms and other living things, oil contamination caused serious harm to soil. The functioning of an ecosystem is disrupted when soil is contaminated with petroleum-based lubricants because these lubricants have negative and destructive impacts on biological life (Nowak et al., 2019). A study showed that following crude oil exposure, zooplankton larval and adult stages changed via. necrosis, feeding, growth, and reproduction as well as other detrimental effects (Almeda et al., 2013). They proposed that zooplankton larvae were more vulnerable to crude oil than adult zooplankton. Because gasoline combustion products have been developed during the thermal breakdown of motor oil, the amount of PAHs in the oil increases, hence increasing its ability to cause cancer and cause mutations (Tripathi et al., 2020; Gaur et al., 2022b).

4. Microbial production of biosurfactant using oily waste

4.1. Catabolism by bacteria

Several bacterial species such as *Pseudomonas, Bacillus, and Rhodococcus* are some of the genera that have been potentially used for the production of different classes of biosurfactants. Amongst various bacterial species, *Pseudomonas*

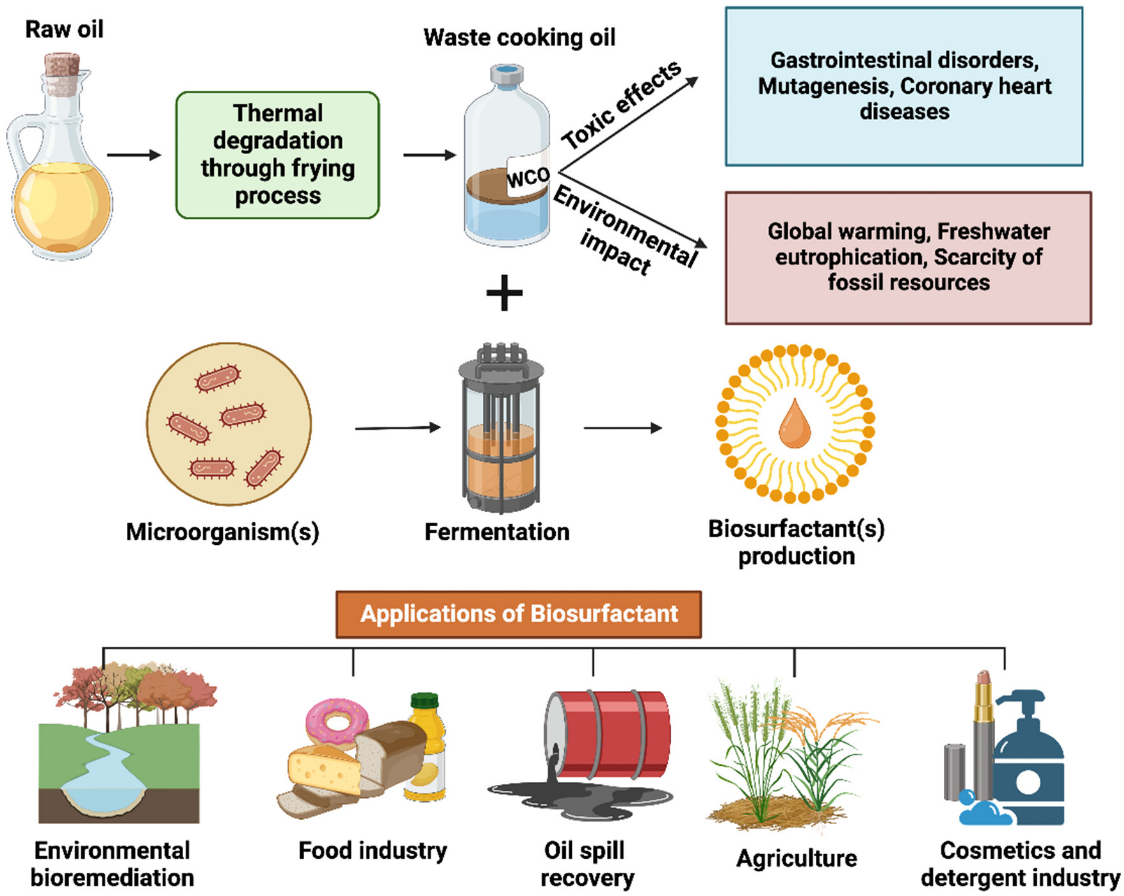

Fig. 3. Utilization of oily waste for biosurfactant production via microbial fermentation approach.

is majorly used for biosurfactant synthesis. Biosurfactant is gaining attention due to several advantages such as low toxicity, easy biodegradability, eco-friendly, and cost-effectiveness (Mohanty et al., 2021a; Gaur et al., 2020). A wide variety of waste such as waste from different industries (oil, fruit, dairy) has been potentially used for the synthesis of biosurfactants as shown in Fig. 3, *via.,* fermentation technology (Table 2). Mainly sunflower oil and olive oil were reported to be employed as a carbon source by *P. aeruginosa* for biosurfactant production (Mohanty et al., 2021a). Similarly, various studies have investigated the utilization of waste frying oil as a medium by different bacterial species namely *Bacillus stratosphericus*, *Pseudomonas cepacia*, *Bacillus subtilis* and *Mucor circinelloides* are found effective for biosurfactant production. *Bacillus subtilis* and *Pseudomonas spp.* have been investigated extensively for their ability to manufacture biosurfactants by processing vegetable oil, which generates enormous amounts of waste that comprise oils, fats, and associated materials (Mohanty et al., 2021a). Compared to refined sunflower oil, the rate of waste oil consumption and the biosurfactants yield was higher (Partovi et al., 2013). Therefore, it is probable that waste oil includes more nutrients including fat, nitrogen, carbohydrates, protein, calcium, starch, magnesium, and phosphorus that are good for microbial growth than refined oil. When sunflower WCO was pre-treated with activated earth, the content of rhamnolipids increased from 2.8 g/L without pre-treatment to 7.5 g/L with pre-treatment for rhamnolipid production by *P. aeruginosa* (Wadekar et al., 2012). In contrast, when crude oil is used as the only source of carbon by the bacterial strains *B. subtilis* and *P. aeruginosa*, they produce biosurfactants that can break down the aromatic and aliphatic hydrocarbons that are present in the crude oil (Parthipan et al., 2017), resulting into the less toxic by-products. The growth of biosurfactant-producing microorganisms, the improvement of the efficiency of recovery and fermentation methods, and the improvement of production medium with alternative sources can all unlock the means to their low-cost production (Parthipan et al., 2017). For example, biosurfactant synthesized by the bacterium *Bacillus cereus* UCP1615 through fermentation in a medium enriched with 2% used cooking oil from soybeans as a cheap substrate (Durval et al., 2021). A similar study by de Oliveira and Garcia-Cruz (2013) also reported that *B. pumilus* utilized waste frying oil as a sole carbon source showing good efficiency for biosurfactant production i.e. up to 5.7 g/L at 5% concentration of waste frying oil. Another study reported that *Bacillus* sp. HIP3 produced 9.5 g/L surfactin (lipopeptide biosurfactant) in 7 d by utilizing 2% WCO. The synthesized biosurfactant has the potential to remove heavy metals like copper (13.57%), zinc (2.91%), lead (12.71%), cadmium (0.7%),

Table 2
Microbial species producing biosurfactant by utilizing waste oil as substrate.

Type of oily waste	Concentration of oily waste used	Microorganisms involved	Biosurfactant type	Surface tension (mN/m)	Biosurfactant yield (g/L)	References
Waste frying oil	5%	*B. pumilus*	Surfactin	45	5.7	de Oliveira and Garcia-Cruz (2013)
Waste frying oil	4%	*Pseudomonas sp.*	Rhamnolipid	32–34	1.4	Haba et al. (2000)
Waste frying sunflower oil	4%	*B. subtilis*	Surfactin	31.9	3.67	Vedaraman and Venkatesh (2011)
Waste frying rice bran oil	4%	*B. subtilis*	Surfactin	34.5	4.67	Vedaraman and Venkatesh (2011)
Used cooking oil	2%	*Bacillus sp.*	Surfactin	38	9.5	Md Badrul Hisham et al. (2019)
Waste frying oil	5%	*P. aeruginosa*	Rhamnolipid	26.4	2.77	Wadekar et al. (2012)
Used olive oil	4%	*P. aeruginosa*	Rhamnolipid	–	2.7	Wadekar et al. (2012)
Kitchen waste oil	–	*P. aeruginosa*	Rhamnolipid	–	2.47	Chen et al. (2018)
Soybean oil refinery wastes	–	*P. aeruginosa*	Rhamnolipid	–	14.55	Partovi et al. (2013)
Waste cooking oil	–	*Pseudozyma aphidis*	Mannosylerythritol lipid	32.83	–	Niu et al. (2019)
Waste frying oils	–	*C. bombicola*	Sophorolipid	–	50.0	Fleurackers (2006)
Restaurant oil waste	–	*C. bombicola*	Sophorolipid	–	34.0	Shah et al. (2007)
Restaurant oil waste	–	*P. aeruginosa*	Rhamnolipid	–	20.0	Zhu et al. (2007)
Residual sunflower oil frying oil	3%	*R. erythropolis*	Trehalolipid	31.9	–	Sadouk et al. (2008)

and chromium (1.68%) (Md Badrul Hisham et al., 2019). Thus, this oily waste comes into sight is an excellent alternative source for the biosynthesis of biosurfactants by different bacterial strains. The waste used as a substrate help in reducing the cost of biosurfactant and makes it more economically competitive.

4.2. Fungal-mediated biotransformation

The process by which a biological system converts chemical molecules into new structural analogs is known as biotransformation. Fungi are known as one of the promising producers of different types of biosurfactants including xylolipids, sophorolipids, hydrophobins, cellobiose lipids, polyol lipids, and mannosyl erythritol lipids using various renewable resources (Da Silva et al., 2021). The fungi *Candida batistae, Candida lipolytica, Candida bombicola, Candida ishiwadae, Trichosporon ashii, Ustilago maydis,* and *Aspergillus ustus* have all been thoroughly investigated for the production of biosurfactants on affordable raw materials (Bhardwaj et al., 2013). Lipids constitute a large proportion of biosurfactant therefore lipid-rich substrates can be utilized for the high yielding product. For example, because of their high lipid content, lipid-rich agro-industrial wastes such as peanut oil cake, leftover frying oil, and coconut oil cake can be employed as low-cost substrates for biosurfactant synthesis (Suryawanshi et al., 2021). Lipid wastes such as oils, grease, and fats from different sources have grown to be a significant source of biogenic waste in urban areas. Plant-based lipids such as rapeseed oil, olive oil, sunflower oil, soybean oil, corn oil, coconut oil, palm oil, and canola oil, etc. and animal-based lipids such as fish oil, animal fat, cheese, butter, and ghee, etc. are the two main sources of lipid waste. WCO after frying process is one of the more well-known types of waste lipids (Liepins et al., 2021a). The maximum yield of 120 g/L of sophorolipid biosurfactant was reported to be produced by *Torulopsis bombicola* and *Candida bombicola* utilizing waste vegetable oil and glucose sugar as carbon sources (Bhardwaj et al., 2013). It has been reported that most of the fungal biosurfactants are lipid derivatives and have a protein–lipid–carbohydrate complex (Bhardwaj et al., 2013). Shah et al. (2007) studied the synthesis of biosurfactant by *Candida bombicola* using restaurant waste oil and reported 34 g/L sophorolipids production in batch fermentation conditions. It has been suggested that animal fat, a rich supply of lipids, acts as a simulator for the synthesis of sophorolipids. Santos et al. (2013) and Deshpande and Daniels (1995) suggested that animal fat waste has been effectively utilized by *C. lipolytica* and *C. bombicola* respectively for the synthesis of sophorolipid biosurfactant. The wastewater generated by several dairy industries contains huge amounts of oils and fats that are not easy to degrade. Daverey et al. (2009) reported that *C. bombicola* produced 38.76 g/L sophorolipid by utilizing valuable by-products obtained from dairy wastewater containing sugarcane molasses and soybean oil.

5. Advancement in biosurfactant production for waste utilization

5.1. Metabolic engineering in biosurfactant production

Metabolic engineering helps to satisfy the urged demand for biosurfactants along with offering sustainable solutions to the challenges occurring while large-scale production and its commercialization (Abdel-Mawgoud et al., 2008).

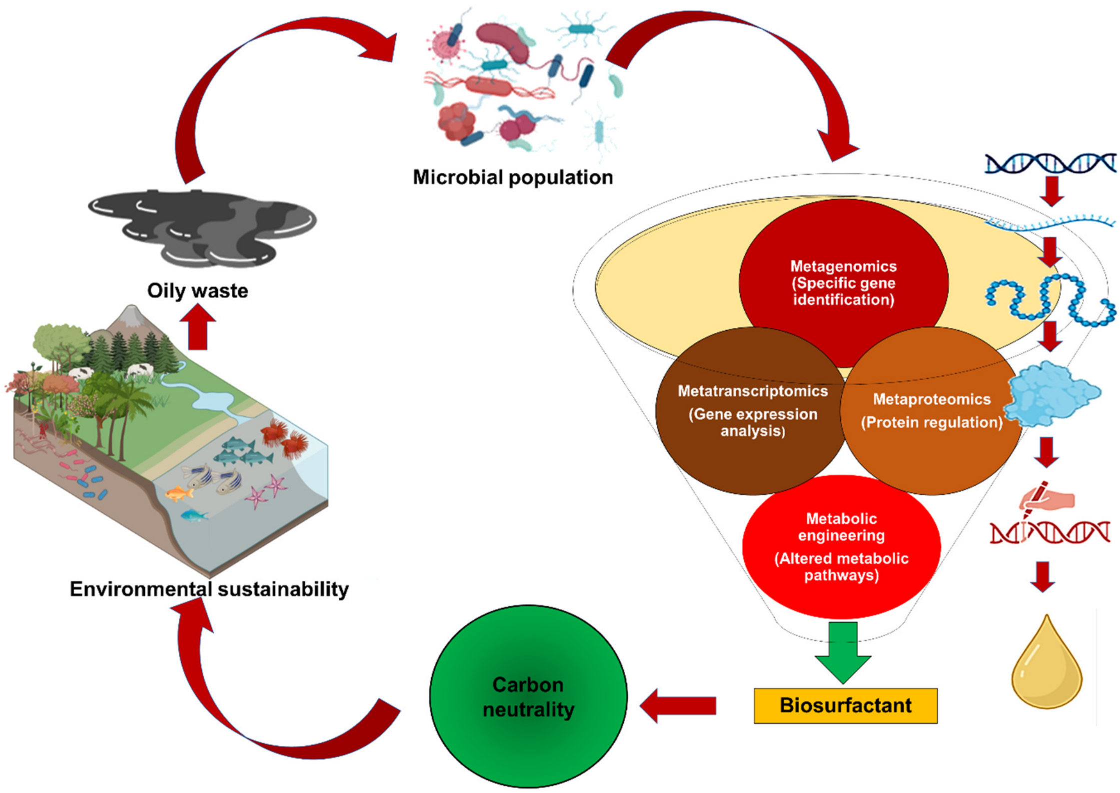

Fig. 4. Metabolic engineering and omics approaches in the production of waste derived biosurfactant aiding in carbon neutrality.

Traditional techniques such as fermentation, breeding, and statistical optimization are insufficient to accomplish the demand and commercial feasibility of biosurfactants. Therefore, genetic engineering approaches (mutation, extracellular peptide overexpression, and recombinant DNA technology) are promising techniques in satisfying the demand for novel, competitive, and eco-friendly biosurfactant production from improved microbial strains along with a significantly low production cost. These gene manipulation techniques are helpful in introducing heterologous genes in an organism or altering the existing genes in an organism. In addition to this, new biosynthetic pathways can be designed and introduced in the organism of interest to improve the production of biosurfactant (Table 3) (Jimoh et al., 2021; Jimoh and Lin, 2019) as depicted in Fig. 4.

The higher manufacturing cost of pure biosurfactants is also a limiting factor in their widespread consumption. Metabolic engineering provides a better understanding of regulatory pathways and can aid in the biosynthesis of high-producer strains to slacken the production cost of biosurfactants. Metabolic engineering has been extensively employed to produce a diverse range of modified strains, with a focus on the regulatory proteins that assist in gene expression and the biosynthetic enzymes that help produce biosurfactants (Dobler et al., 2016; Occhipinti et al., 2018). For example, the wild-type *rhlI* gene was cloned into a suitable *E. coli* strain or introduced into a *Pseudomonas aeruginosa* cell-free spent supernatant carrying the signal transduction molecules to significantly increase rhamnolipid output (Dusane et al., 2010). Gene editing and molecular dynamics are expected to be ground-breaking biotechnological technologies for biosurfactant generation, improved manufacturing, and determining their uses. The characteristic, quality, and quantity of biosurfactants, were found to be improved through the effective designing of mutants and the development of bumper-producing recombinants (Jimoh et al., 2021).

Rhamnolipid biosynthesis enzymes are encoded by the genes *rhlA, rhlB,* and *rhlC* in *P. aeruginosa*, and overexpression of these particular genes may result in mutant strains with increased rhamnolipid output. In microbes, random mutagenesis can enhance their biosurfactant synthesis capacity up to twelve times more as compared to their original wild-type. While planned and targeted alteration (overexpression of genes, ribosome engineering) via genetic engineering can further enhance productivity (Dobler et al., 2016). Genome shuffling in *Bacillus amyloliquefaciens* was found to increase surfactant production up to 10 times more in the recombinant strain as compared to the wild strain (Geys et al., 2014). A mutant strain of *Rhodococcus erythropolis* SB-1 A developed through random mutagenesis caused by ultraviolet radiation in offshore oily water was isolated. The method of spreading oil demonstrated the capacity of the strain to produce approximately four times higher levels of critical micelle dilutions and biosurfactants as compared to the parent

Table 3
Metabolic engineering in microbial strains for improved biosurfactant production.

Organisms	Strain	Gene modified or introduced	Biosurfactant produced	Substrates used	Remarks	References
Bacteria	*P. aeruginosa NRRL B-771*	*VHb gene (Vitreoscilla hemoglobin)*	Rhamnolipid	Cheese and olive oil waste	VHb increases dissolved oxygen tension within cells during oxygen limited growth conditions	Kahraman and Erenler (2012)
	P. putida	*rhlAB* gene	Rhamnolipid	Soybean oil	Heterologous biosurfactant production	Cha et al. (2008)
	B. subtilis 168	*sfp*	Surfactin	Sucrose	Integrating a complete sfp gene improved the synthesis of surfactin	Wu et al. (2019)
	P. putida iJP962	*rhlA* and *rhlB*	Rhamnolipid	Glucose	Heterologous biosurfactant production	Occhipinti et al. (2018)
	E. coli	*rhlAB* and *rhlC*	Rhamnolipid	LB + glucose	Di-rhamnolipid production	Du et al. (2017)
	E. coli	mutant *rhlB*	Rhamnolipid	–	Rhamnolipid production	Han et al. (2014)
	E. coli ATCC 8739	*rhlAB* and *rhaBDAC*	Rhamnolipid	–	Rhamnolipid production	Gong et al. (2015)
Fungus	*S. bombicola*	Δcyp52m1 Δ faa1 Δmfe2	Sophorolipids	Glucose + rapeseed oil	Sophorolipid biosynthesis and β-oxidation	Jezierska et al. (2019)
	S. bombicola O-13–1	*VHb* gene *(Vitreoscilla hemoglobin)*	Sophorolipids	–	Alleviate oxygen limitation	Li et al. (2021)
	S. bombicola	Δugta1/Δpox/Δ fao1	Sophorolipids	Glucose	Blockage of three catabolic pathways	De Graeve et al. (2019)
	U. maydis	*Mac1* and *Mac2*	Mannosylery-thritol lipids	–	Genes coding for acyl-transferases are involved in MEL production	Becker et al. (2021)
	C. bombicola	ΔugtB1	Lactonic diacetylated SL	Rapeseed oil	Deletion of bacterial glycosyltransferases led to sophorolipid production	Saerens et al. (2011)

strain and the thin layer chromatography results demonstrate that there were no changes among both the mutant and parent-derived biosurfactants (Cai et al., 2016; Occhipinti et al., 2018; Yadav et al., 2019)

Through the chromosome integration of the *rhlAB* operon from *P. aeruginosa* PAO1, transposons were used to develop an improved strain of *P. aeruginosa* for increased rhamnolipid output. The HPLC/MS analysis of rhamnolipids confirmed the composition and construction similarity with the wild strain's productivity, albeit the percentage of the various structural constituents may differ. This new variant showed a maximal rhamnolipid production of 1.819 g/L when cultivated in soybean oil. Using glucose as a carbon source is enticing even though productivity has decreased because it is less expensive than soybean oil (Dobler et al., 2016).

A novel strain for rhamnolipids production was developed by using transposome biotechnique where the desired gene was successfully introduced into *P. aeruginosa* and *E. coli* cell chromosomes. In several bacteria, stable insertion mutations could be produced using transposon-mediated chromosome integration. In contrast to plasmid-based engineered strains, transposon-based engineered strains could exist steadily in the absence of drug selection pressure. Although, DNA sequencing, inverse PCR, and matching with a massive store of genome data available in public sources may help to confirm the gene integration site of the targeted genes in the newly developed strain. This gene regulation mechanism allows the controlled production of rhamnolipids (Dusane et al., 2010). The co-overexpression of a gene responsible for glucose catabolism in *S. roseosporus* had resulted in enhancing the rhamnolipid production by up to 43.2% (Geys et al., 2014). Strain *P. aeruginosa* NRRL B-771 co-expresses the *Vitreoscilla* hemoglobin (VHb) gene and had shown a beneficial result as this protein increases the oxygen accessibility of bacterial cells, therefore, raises the production levels of the gene of interest and the biosynthesis of rhamnolipids (8.4 g/L). Additionally, using industrial effluent from cheese and olive oil as a carbon source, *P. aeruginosa* NRRL B yields 13.3 g/L of rhamnolipids (Kahraman and Erenler, 2012).

It is widely acknowledged that, due to high manufacturing costs, biosurfactants cannot replace the chemically generated surfactants and other synthetic substances in the market. However, a wide range of less expensive renewable raw materials for biosurfactant production have already been proposed, but the ability of microbes to utilize these raw ingredients should be explored or the targeted genes should be induced in the preselected host cell via., genetic engineering. A genetically modified strain of *P. aeruginosa* carrying lac genes of *E. coli* via heterologous expression was found capable of utilizing whey waste for biosurfactant production. Similarly, for efficient utilization of low-cost lignocellulosic waste, glycolipid-producing bacterial and fungal cells could be modified at the gene level (Abdel-Mawgoud and Stephanopoulos, 2018).

5.2. Omics approaches in biosurfactant production

"Omics" techniques have become popular over the past few decades as an effective technology for comprehending the physiology and regulatory systems of microorganisms. With the realization of the potential of next-generation sequencing (NGS), various omics approaches, such as metagenomics, metaproteomics, and metatranscriptomics as shown in Fig. 4, had acquired popularity along with metabolomics, phenomics, and bionomics in predicting the efficacy of microbial secondary metabolites secretion and interactions (Yang et al., 2021). The combination of one or more approaches or multi-omics approaches could emerge as a promising tool for efficiently predicting the yield, growth, senescence, and effect of biotic and abiotic stress on diversified microbial populations present in the ecosystems (Gaur et al., 2022b,c).

Metagenomics is the study used for the identification of unknown microorganisms. Without the need for enrichment or culture methods, the genetic material is directly separated from the environmental samples. It entails the separation of DNA, 16S rRNA sequencing, construction of metagenome libraries, and data processing. The study of mRNA transcripts, or metatranscriptomics, helps us understand how genes function. This method shows how environmental stress and the over- and under-expression of genes in microorganisms are related. For example, *P. putida, E. coli and S. lividans* fosmid were used to create a metagenomic library. Using a paraffin spray experiment, biosurfactant activity was discovered in a *P. putida* clone, and the enzyme responsible for this activity was identified as ornithine acyl-ACP N-acyltransferase (OLSB). Although the fosmid remained dormant in *E. coli*, the T7 promoter was able to make the *olsB* gene overexpress, resulting in the formation of lyso-ornithine lipid with biosurfactant potential (Williams et al., 2019). Metaproteomics is the study of protein expression. It is useful to study the protein content found in cells. It brings information about functional genes and the effect of protein interactions in microbes (Gaur et al., 2022c). In one investigation, proteomics enabled the discovery of a comparable protein of the cerato-platanins (CP) family in two fungi species i.e., *Trichoderma harzianum* MUT290 and *Aspergillus terreus* MUT271 isolated from a marine area that was contaminated with oil (Pitocchi et al., 2020).

6. Carbon neutrality and environmental sustainability

Owing to the multifarious applications and growing demand for biosurfactants, the exploitation of waste as a substrate has been fuelled up to minimize production costs along with its aid in waste management as a bonus benefit, thereby closing the loop of leaving less waste unutilized (Makkar and Cameotra, 2002). Non-regulated discharge and disposal of wastes from different sectors not only pollute the environment but stimulate the emission of CO_2 (Eurostat, 2022). On the positive side, current advancements in waste recycling are gratifying to produce biosurfactants, yet above all, the sustained production of biosurfactants has improved "economic feasibility". Therefore, from an economic viewpoint, the utilization of industries-derived waste for biosurfactant production is more profitable as it advocates reasonability and environmental sustainability with low carbon footprints (Mgbechidinma et al., 2022). In this respect, using wastes as feedstock has two major benefits, (i) provides economic value to these wastes or by-products, and (ii) addresses the problem of their management. Moreover, the decomposition of waste significantly enhances greenhouse gas emissions and contributes to climate change with an elevated carbon economy. Therefore, new, affordable, secure, and renewable health-grade biosurfactants can be sustained by waste reduction, reuse, and recycling. In this context, the notion and practices of the circular and bio-economy have been developed as a system model to displace the prevalent "take, make, and dispose" economic development paradigm and implement environmental sustainability (Ahmed et al., 2022). In accordance with Gavrilescu and Chisti (2005), a product is considered sustainable if it outperforms its traditional counterparts in terms of durability, performance, recyclability, toxicity, and biodegradability. Biosurfactants fulfill all these attributes and thereby necessitate the production demand. However, biosurfactants made from sustainable resource materials are gradually making their way onto the market. To date, several wastes have been studied for the production of bio-based surfactants, but oils can be discreetly exploited for the efficient production of these products (Gaur et al., 2022a; Mohanty et al., 2021b). In many areas, it is not permissible to dispose of these wastes in landfills. Thus, oils as a most abundant substrate, derived from food waste, dairy waste, vegetable waste, and slaughter waste may act as an inducer to produce biosurfactants of different origins. Increasing the value of these wastes directly offer strategies for carbon neutrality by reducing greenhouse gas emission (GHG). Many international climate change treaties reflect global efforts to limit GHGs. The Conference of the Parties (COP21), where various nations decided to put the globe on a more sustainable course by preventing a rise in the average worldwide temperature not more than 2 °C above pre-industrial levels (UNFCCC, 2016). Rahman and Gakpe (2008) asserted that biosurfactants are crucial in lowering atmospheric carbon dioxide emissions, a significant greenhouse gas from the atmosphere. In this regard, the circular economy also can gain from the conversion of waste into biosurfactants in addition to reducing climate change and carbon sequestration. Sustainable perspectives acknowledge the need to balance environmental, economic, and social concerns while taking their long-term effects into account in an environmentally benign manner. However, concerns about environmental sustainability not only accentuate pollution but the depletion of natural resources from the environmental inventory. We must update our understanding of the current condition of carbon flux in the overall ecosystem as the world community moves towards carbon neutrality. To safeguard both human and environmental health, it is now crucial to convert non-renewables to renewables that maintain current production systems and solve climate change challenges. In the current scenario, residual waste from different industrial sectors is considered as a "green substrate" for biosurfactant production and their valorization reduces the global carbon footprints. As per Sustainable Development Goals (SDGs) 13, it is vital to improve the recycling of waste and its management to reduce the emission of greenhouse gases (United Nations, 2019)

The disposal of oily waste emits a huge number of carbons to atmosphere and their entrapment can be achieved by balancing emission and their absorption in carbon sinks to attain a state of net zero carbon (Solutions, 2018). To reach carbon neutrality in upcoming years, a resource sustainable economy should be called. To meet the green objectives, carbon-neutral biosurfactants delivering low CO_2 should be produced at large scale using fermentation-based approach with a Renewable Carbon Index (RCI; it is a measure of sustainability that can be calculated by dividing the number of carbons derived from renewable sources by the total number of carbons in an active ingredient) of 100%. Patel (2003) stated that increasing the production and usage of biological surfactants must be considered as part of a larger GHG (Greenhouse gas) emission reduction strategy that incorporates a variety of techniques to control both energy supply and demand. Biosurfactants generally have a lower carbon footprint, as a life-cycle assessment analysis concluded that 1 metric tonne (Mt) of a typical ethoxylated surfactant replaced with 1 Mt of sophorolipid reduces CO_2 emissions by 1.5 Mt (Bettenhausen, 2022). Therefore, waste derived substrates for biosurfactant production would be sought to resolve climate change challenges in coming future. The GHG emissions might be decreased by 2.1 to 2.8 billion tonnes CO_2/year by 2030, and waste management and recycling methods could reduce around 5% of global GHG emissions (Fletcher et al., 2021). Without any doubt, biosurfactants can be a significant player in this statistical goal which would efficiently improve both environmental sustainability and the circular bioeconomy. In near future, recycling oily wastes to value-added products will not only help in making waste free environment, but the production of biosurfactants from it is crucial in reducing atmospheric carbon dioxide emissions, a substantial greenhouse gas.

7. Global market, future perspectives, and practical significance

The biotechnological compounds of the 21st century that are most economically desired are biosurfactants. Synthetic surfactants produced from petrochemical or oleochemical sources account for the majority of sales in the worldwide surfactants market. Private surfactant manufacturers and government organizations are attempting to replace conventional chemicals in nearly all applications with renewable and sustainable alternatives. nevertheless, as a result of the growing worries for both the safety of human health and the environment. According to a recent estimate, the worldwide biosurfactant market would increase from USD 1.2 billion in 2022 to USD 1.9 billion in 2027, at a compound annual growth rate (CAGR) of 11.2% (Markets and Markets, 2022). In a different analysis, it was predicted that the worldwide biosurfactants market will increase from $4.18 billion in 2022 to $6.04 billion in 2029, with a CAGR of 5.4% over the projected period of 2022–2029 (Fortune business insights, 2022). Some of the critical factors driving the biosurfactants market worldwide are based on consumer acceptance of biobased surfactants, environmental concerns, regulatory compliance, and shifting oil prices. The biosurfactant market brought US$ 1.8 billion revenue in 2017, and it is anticipated that by 2023, this sum would climb to US$ 2.6 billion, with an increase of almost 8%, and that by 2024, 540 kilotonnes of biosurfactant will be produced (Gaur et al., 2022a). The industrial activity in the increasingly environmentally conscious industries of detergents and cosmetics is predicted to have a significant impact on the demand for biobased surfactants in 2029. The market can be divided into categories based on type, including polymerics, fatty acids, glycolipids, and lipopeptides. The market is divided into humectants, emulsifiers, and preservation agents, among other categories, based on application. Furthermore, this segmented, among others, into personal care, detergents, agrochemicals, and food processing depending on the end user. Country-by-country, the biosurfactant market is primarily divided into four regions: Europe, North and Latin America, East and Middle Africa, and Asia Pacific. Now the demand for biosurfactants has grown as a result of improved infrastructure and more awareness in Asian nations (Singh et al., 2019). Due to the rising demand for environmentally friendly alternatives to synthetic and traditional surfactants in the nation, the Indian biosurfactants market, for instance, is predicted to rise moderately over the years. Major corporations like Evonik India Pvt. Ltd., Mitsubishi India, Vetline India Pvt. Ltd. (Unit of Simfa Labs), etc. dominate the Indian biosurfactants market (Waghmode and Suryavanshi, 2020). But the expensive expense of biosurfactants is one of the main barriers to their broad usage in industry. The most efficient microbial biosurfactants, sophorolipids, have an average cost of $34/Kg, compared to the $1 to $4/Kg cost of synthetic surfactants such as plant-based amino acid surfactants and sodium dodecyl sulphate. Variables in the production process, such as lower yields, higher downstream processing costs, extended processing periods, energy requirements for disinfection and maintaining biological cultures, etc., contribute to the increased cost of a biosurfactant (Rodríguez-López et al., 2020).

Even though there has been a tonne of study over the past two decades on how to produce biosurfactants more cheaply, despite being more successful commercially than their synthetic counterparts, they nonetheless face financial obstacles. Globally, to encourage the growth of microbes and biosurfactant production, there has been an increase in the use of inexpensive feedstock/substrate with a precise ratio of lipids to carbohydrates. The manufacture of biosurfactants on a big scale still presents a challenge. Thus, in order for biosurfactant production to be commercially competitive, the following factors must be considered: the type of raw materials and microorganisms, industrial bioreactor design, the intended market, purification techniques, biosurfactant characteristics, the production environment, and the amount of time it takes for appropriate fermentation and accurate production yields (Gaur et al., 2022a). Industrial waste can reduce the production costs and improve both economic and environmental sustainability. Vegetable oils, oily biomass, and oil waste from refineries are the finest alternatives to pricey substrates. Unfavorable pH and temperature of the media also hinder the manufacturing process by preventing microbial development to sustain a greater yield. These issues related to the manufacture and processing of biosurfactants can be addressed with strategies. For large-scale biosurfactant production, it is advised to design practical fermentation conditions, optimize the fermentation composition, use strains that produce abundantly, choose waste that is high in sugar and carbohydrates, and develop strains that produce abundantly by genetic engineering (Varjani and Upasani, 2017). While waiting for legislation and regulations to be developed, guidelines should be formed to maximize the marketplace for biosurfactants while reducing adverse effects on the environment. A circular bioeconomy with a restorative and regenerative perspective will also be built through collaborations with researchers and decision-makers, making the social and economic sides work in harmony with one another for the biosurfactant industry.

Biosurfactants offer an increasingly valuable and diverse market capital with various applications. Whereas, an increase in the generation of environmental carbon footprint by oily waste is detrimental to climate, environment, and life on the planet. Therefore, this study practically demonstrates the limitations and relationship between biosurfactant production, oily waste remediation, carbon footprint reduction, and improved environmental sustainability. Furthermore, other concerns such as improved policy measures for waste generation and management, and the limitation in the development of efficient microbial strains can be taken into consideration for future research.

8. Conclusions

The global increase in the generation of waste oil increases the generation of carbon footprint. The microbial aided production of biosurfactants using this waste can significantly aid in the reduction of GHG emissions and aid towards carbon neutrality. In this lieu, the utilization of advanced techniques viz., metabolic engineering and multi-metaomics has led to the development of more robust microbial strains and identified the limitation in the biosurfactant production pathways respectively. In addition to this, drafting and implementation of policy regulations for source identification, and management of accidental and industrial oil spills is warranted. The exploitation of oily waste as a feedstock for the production of value-added products like biosurfactants further supports carbon neutrality and environmental sustainability. Therefore, the concept of oily waste biorefinery in oily waste transformation, mitigation, and conversion to value-added materials needs to be explored by researchers.

CRediT authorship contribution statement

Krishna Gautam: Literature review, Writing – original draft, Data curation. **Poonam Sharma:** Literature review, Writing – original draft, Data curation. **Vivek Kumar Gaur:** Writing – original draft, Data curation. **Pallavi Gupta:** Writing – original draft, Data curation. **Upasana Pandey:** Writing – original draft, Data curation. **Sunita Varjani:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing, Resources. **Ashok Pandey:** Writing – review & editing. **Jonathan W.C. Wong:** Writing – review & editing. **Jo-Shu Chang:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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