Transforming recalcitrant wastes into biodiesel by oleaginous yeast: An insight into the metabolic pathways and multi-omics landscape

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ABSTRACT

The escalating challenge of waste disposal and the potential threat to global energy supply have sparked renewed interest in repurposing waste materials for the production of sustainable and renewable fuels. In line with this objective, there has been a growing focus on biodiesel production from oleaginous yeast through the valorization of waste. While numerous reports have been published on this subject, only a limited number of studies provide a comprehensive overview of recent advancements. To address this gap and the economic viability challenges associated with yeast-derived biodiesel production, the present review aims to highlight the opportunities offered by various recalcitrant wastes as a renewable feedstock for oleaginous yeast cultivation. The review also delves into extensive knowledge about the metabolic pathways that facilitate the conversion of different recalcitrant wastes into single-cell oil (SCO), which has not been extensively covered in a single platform before. Moreover, the most promising species of oleaginous yeast are described, taking into consideration economic aspects and the sustainability of the overall process. Furthermore, the review emphasizes the application of omics techniques to advance waste bioconversion into lipids for the purpose of commercialization. In summary, this study contributes to expanding our current understanding of the topic and facilitates the future upscaling and commercialization of biodiesel derived from oleaginous yeasts.

1. Introduction

Escalating world’s population, urbanization, rapid industrialization and economic growth led incessant consumption of fossil fuels to meet the rising demand of energy. Due to which, unremitting emissions of greenhouse gases and rapid depletion in fossil fuel reserves have been seen presently. It is assumed that environmental safety and threat to global energy supply are two major threats which humanity going to face in near future [1]. Consequently, great attention has been given to lessen the world’s dependency on fossil fuels by finding substitutes in form of clean, renewable, and green energy which advocated as one of United Nation’s Sustainable Development Goals (Affordable and Clean Energy, SDG 7) [1,2].

Biofuels, especially biodiesel is one such alternative that not only has the capability to substitute petroleum-based diesel but among other desired properties this fuel is sustainable, biodegradable, non-toxic, non-flammable and has high flash point, and low exhaust emission [3]. Chemically it encompasses fatty acid alkyl/methyl esters formed by chemical reactions of lipid with suitable catalyst such as alcohols, etc. Biodiesel is primarily produced from lipids (or oils) acquired from various sources such as plants, vegetables, animals, microbial cells and waste oils. The edible oil obtained from conventional food crops (soybean, canola, sunflower, rapeseed, corn) are considered as first-generation feedstocks. Whereas, non-edible animal fats and residues, waste cooking oil, agricultural residues, various industrial wastes (yellow greases etc.) are categorised into second-generation feedstocks [4,5]. First-generation feedstocks have raised the “food versus fuel” debate and contributed to the upsurge in food price, and reduced land accessibility for edible crop. To mitigate these issues, second-generation feedstocks have been used which, owning to the lack of a passable supply in the long run together with high production cost, hindered their development towards affordable biodiesel production [6]. Photosynthetic oleaginous microalga with similar lipid composition to animal or plant lipids categorised under third-generation feedstocks.

Microbes accumulating 20–50% dry cell biomass in lipids are named oleaginous, and the lipid produced from them known as single cell oil
(SCOs). Oleaginous microbes store their lipid in oil vacuole as triacylglycerols (TAGs), that serve as a promising source for biodiesel production as well as human and animal nutrition. SCOs have several advantages over vegetable or plant oils, like easier cultivation, simple upstream and downstream processing, higher productivity, independency on climate conditions for their cultivations and easier genetic alternation regard to a target product [1,7,8]. From different categories of lipid-producing microbes, oleaginous microalgae and yeasts have gained more attention from researchers these days. Despite, numerous advantages that microalgae present, the long cultivation period and the necessity of more land area for their phototrophic cultivation raises technical and economic issues and hinders their further use towards biodiesel production [9,10]. Considering these facts, oleaginous yeasts are often pronounced as superior for commercial lipid production over microalgae owing to high intracellular TAG, fast growth, shorter processing time, high volumetric productivity and high production of endotoxin-free cell biomass [11,12]. Being enriched in C16/C18 fatty acids, yeast lipids are considered more apposite for biodiesel production.

However, for yeast lipid derived biodiesel production, approximately 70% of the production cost is connected to the raw materials used as substrates and thus, waste streams as well as biomass residues that are available at no or low cost has being recognized as valuable alternative [13]. For instance, agricultural waste streams (wheat straw, corn stover, sugarcane molasses), paper mill sludge and effluents from wastewater treatment plant have being widely explored for cultivation of divers oleaginous yeast genus including *Yarrowia*, *Rhodotorula*, *Lipomyces*, *Rhodosporidium*, and Cryptococcus [14,15]. Several industrial effluents encompassing recalcitrant compounds like aromatic hydrocarbons, toxic dyes, and other xenobiotic components have also been explored towards their potential as feedstocks [16]. Rapid advancement in researches associated with yeast biodiesel production from these renewable sources especially recalcitrant wastes have also proposed various interlinked biosynthetic pathways involved in waste metabolization and their conversion into SCO. Articles addressing detailed mechanistic insight on single waste valorization via oleaginous yeast are numerous however, no such review dissecting metabolic pathways allied with multiple recalcitrant waste valorization has reported till date.

Considering these research gaps, present review furnishes a comprehensive analysis on most promising recalcitrant waste effluents and discussed their benefits and drawbacks as feedstocks. Further, provides a single platform to discuss in-depth all possible natural pathways interlinked with recalcitrant waste conversion into biotechnology relevantly microbial oil. Insights on robust oleaginous yeast species capable to metabolize toxicants and the latest information on multi-omics strategies are some additional highlights of this review.

### 2. Recalcitrant waste effluent as renewable feedstocks for oleaginous yeasts

Synthetic compounds that are non-biodegradable in nature like polymers, toxicants, and organic contaminants primarily termed as recalcitrant wastes, mainly result of human activities. High chemical oxygen demand (COD) and low biochemical oxygen demand (BOD) are main characteristic of recalcitrant waste consisting wastewater. Depending on their sources, production these can be categorised into 1) industrial waste effluents and 2) municipal wastewater. Municipal/domestic wastewater generally collected through sewage systems, encompasses 99.9% water and 0.1% suspended as well as dissolved inorganic, organic solids like detergents, proteins, soaps, fats, lignin, and carbohydrates [17]. Compared to domestic one, industrial wastewater is considered much more complicated in terms of pollutants nature (heavy metals, recalcitrant substances, pesticides, etc.) and their abundance (Table 1). Thus, their treatment is a major concern from an environmental point of view. Innumerable electro and thermochemical conversion techniques have been assessed to tackle this but oleaginous yeast cultivation as a waste valorization approach is found better [16]. More specifically, yeast have the ability to metabolize structurally diverse waste compounds and route them towards a single product such as lipids, something that is very difficult by using purely chemical technologies [16]. This approach has also proven to be helpful for attaining a

<table>
<thead>
<tr>
<th>Source</th>
<th>COD (g/L)</th>
<th>BOD (g/L)</th>
<th>TSS (g/L)</th>
<th>pH</th>
<th>N</th>
<th>P</th>
<th>Other parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum waste water</td>
<td>15-51</td>
<td>7.8</td>
<td>–</td>
<td>6.16</td>
<td>0.06</td>
<td>–</td>
<td>Alkalinity, 0.7 g/L; VFA, 2.3 g/L; C/N, 109</td>
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<td></td>
<td>55-60</td>
<td>30-32</td>
<td>0.02-0.3</td>
<td>2.5-2.7</td>
<td>0.05-0.212</td>
<td>0.102-0.227</td>
<td>Phosphol, 0.36 g/L; VFA, 93.95 g/L; grease and oil, 0.013 g/L; acidity in total, 45-46 g/L</td>
</tr>
<tr>
<td>Palm oil mill effluents</td>
<td>77-82</td>
<td>20-23.4</td>
<td>26.25-28.92</td>
<td>4.25-4.48</td>
<td>0.4-0.49</td>
<td>–</td>
<td>VFA (acetic acid), 8.49-10 g/L; grease, oil, 3.167-5.193 g/L; VS total, 32.5-38.7 g/L; COD (soluble), 27.5-40 g/L</td>
</tr>
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<td></td>
<td>15-100</td>
<td>10.5-43.75</td>
<td>5-54</td>
<td>3.4-5.2</td>
<td>0.18-1.41</td>
<td>1.281-1.928</td>
<td>Color (ADMI) &gt; 500; TS, 11.5-79; oil and grease, 0.13-18; total VS, 9-72</td>
</tr>
<tr>
<td>Slaughter house wastewater</td>
<td>2-6.2</td>
<td>1.3-2.3</td>
<td>0.85-6.3</td>
<td>6.3-6.60</td>
<td>0.07-0.24</td>
<td>0.015-0.04</td>
<td>Turbidity, 90-190 NTU; FSS, 0.34-1.4 g/L; grease with oil, 0.04-0.6 g/L; VSS, 0.66-2.5 g/L; acidity, 0.9-1.78 g/L</td>
</tr>
<tr>
<td>Dairy industry wastewater</td>
<td>10.25</td>
<td>4.84</td>
<td>5.802</td>
<td>8.34</td>
<td>0.663</td>
<td>0.153</td>
<td>Chlorides, 0.616 g/L; Grease and fats, 0.3-9.44 g/L; VS, 0.53-2.6 g/L</td>
</tr>
<tr>
<td></td>
<td>2-68.6</td>
<td>1.2-40</td>
<td>0.3-59</td>
<td>4-11</td>
<td>0.065-1.12</td>
<td>0.009-0.5</td>
<td>Turbidity, 90-190 NTU; FSS, 0.34-1.4 g/L; grease with oil, 0.04-0.6 g/L; VSS, 0.66-2.5 g/L; acidity, 0.9-1.78 g/L</td>
</tr>
<tr>
<td>Paper and pulp industry wastewater</td>
<td>0.95–38.588</td>
<td>0.14–13.08</td>
<td>0.037–23.319</td>
<td>4.2–11.6</td>
<td>0.002–0.35</td>
<td>–</td>
<td>Color (Pt-Co), 0.0166-4.667; TS, 1.16–51.583</td>
</tr>
<tr>
<td>Paint industry wastewater</td>
<td>10-17</td>
<td>–</td>
<td>9.5</td>
<td>7.6</td>
<td>–</td>
<td>–</td>
<td>Color, gray; COD (dissolved), 7.43 g/L; VSS, 5.42 g/L; Suspended solids, 0.015-8 g/L; grease, oil, 0.01-0.03 g/L; chlorine, 1-6 g/L; SO4, 0.6-1 g/L</td>
</tr>
<tr>
<td>Textile wastewater</td>
<td>0.15-12</td>
<td>0.08-6</td>
<td>0.015-8</td>
<td>6-10</td>
<td>0.07-0.08</td>
<td>–</td>
<td>Phenolic compounds total, 1.450 g/L; TS, 0.748-18.332 g/L; Alkalinity, 0.05–0.1 g/L; Phenols, 0.065–0.072 g/L</td>
</tr>
<tr>
<td>Distillery/ winery wastewater</td>
<td>0.32–49.105</td>
<td>0.203–22.418</td>
<td>0.066–8.6</td>
<td>2.5–12.9</td>
<td>0.01–0.415</td>
<td>0.28</td>
<td>–</td>
</tr>
<tr>
<td>Pharmaceutical wastewater</td>
<td>2–3</td>
<td>1.2–1.7</td>
<td>0.3–0.4</td>
<td>6.5–7.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
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### Table 1

Characteristics of recalcitrant wastewaters from several industries. TS: total solids; BOD: biochemical oxygen demand; TRN: total Kjeldahl nitrogen; COD: chemical oxygen demand; FOG: fat, oil, and grease; VS: volatile solid; VFA: volatile fatty acids; TSS: total suspended solids; N: nitrogen; P: phosphorus.


The enormous utilization of crude petroleum for the production of fuel and chemicals is gradually leading to the global expansion of petrochemical industries, which continually generates ~33.6 million barrels wastewater per day [31]. This wastewater comprises complex matrix of organic pollutants like grease, resins that choke/drain pipes, cause unpleasant odors, and phenolic compounds which shows long term persistent in the environment [17]. A survey done by U.S Environmental Protection Agency (US EPA) stated that over 150 processes applied in petroleum refineries originate considerable amounts of these hazardous wastes [28]. Furthermore, accidental spillage or leakage during the exploration, refining, production, storage, and transportation of crude petroleum/petroleum products results in severe environmental damage through the discharge of aromatic, polycyclic aromatics, asphaltines, cycloalkanes, alkanes and naphthenic acid. Naphthenic acid is categorized as neurotoxic and oncogenic and may cause damage on asphaltines, cycloalkanes, alkanes and naphthenic acid. Naphthenic acid metabolites (methyl group containing dicarboxylic acid), soluble cellular matic hydrocarbons, branched alkanes and n-alkanes [33,36]. These carbon and energy source, however, increasing complexity in n-alkanes also been found to be capable of using mono-branched alkanes as sole Y. lipolytica the impact of environmental factors like temperature, pH, nutrient and activities were also conducted on PG32 respectively after 7 days cultivation [37]. Extensive research ac as well as dodecane (40%) post 48 h of cultivation [36]. Crude oil degradation [32,33,34,35,36,37,38,39]. For instance, Y. lipolytica NCIM 3589, isolated from oil contaminated seawater was found capable to degrade various hydrocarbons like tetradecane (50%), decane (40%), hexadecane (60%), octadecane (45%), as well as dodecane (40%) post 48 h of cultivation [36]. Crude oil degradation up to 68% and 58% was observed by Y. lipolytica PG20 and PG32 respectively after 7 days cultivation [37]. Extensive research activities were also conducted on Y. lipolytica PG20 and PG32 to monitor the impact of environmental factors like temperature, pH, nutrient and oxygen availability on their biodegrading capability. As a result, 28°C temperature leading 90% crude oil degradation was found optimum in Y. lipolytica cultivated on 0.1% (v/v) petroleum [38]. This species has also been found to be capable of using mono-branched alkanes as sole carbon and energy source, however, increasing complexity in n-alkanes structure hinders the degradation process. Further research on yeast utilizing petroleum components as sole carbon source demonstrated a pattern of increasing biodegradability from high molecular weight polar/aromatic compounds to cycloalkanes, low molecular weight aromatic hydrocarbons, branched alkanes and n-alkanes [33,36,38]. These hydrophobic substrates are incorporated into lipids, intermediate metab melalogenous yeast belong to the genera Rhodosporidium, Debaryomyces, Rhodotorula, Tri-chosporon, Yarrowia, Pichia were explored lately [33]. Amongst defined species, Yarrowia lipolytica, is mainly isolated from hydrophobic structure (alkanes, oil and fatty acids) rich environments. It is known to produce different metabolites of industrial interest (microbial lipids, organic acids, aroma compounds, and enzyme), and has being exten- sively studied for hydrophobic substrates bioremediation [34,35]. For example, Y. lipolytica strains were developed to efficiently degrade crude oil [32,36,37]. Y. lipolytica has been found to be a suitable organism for bioremediation of crude oil contaminated water [38]. This species has also been found to be capable of using mono-branched alkanes as sole carbon and energy source, however, increasing complexity in n-alkanes structure hinders the degradation process. Further research on yeast utilizing petroleum components as sole carbon source demonstrated a pattern of increasing biodegradability from high molecular weight polar/aromatic compounds to cycloalkanes, low molecular weight aromatic hydrocarbons, branched alkanes and n-alkanes [33,36,38]. These hydrophobic substrates are incorporated into lipids, intermediate metabolites (methyl group containing dicarboxylic acid), soluble cellular compounds (amino acids, proteins), and partly oxidized to CO₂ [33].

2.1. Metabolic pathway of hydrocarbon biodegradation

Oleaginous yeasts have the ability to utilize both hydrophilic and hydrophobic substrates (HS). Hydrophilic carbon sources like sugars, alcohols, organic acids, etc. produce fatty acid precursor acetyl-CoA and integrates it into Kennedy pathway for TAGs synthesis (commonly known as de novo pathway) [6]. Whereas, HS like alkanes, free fatty acids (FFAs), TAGs once transported inside the cell are metabolized by several multigene families that reassemble them into neutral lipid (ex novo pathway) [6]. Till date, limited yeast species are reported to sur vive under hydrophobic environments and synthesize lipid via ex novo pathway. Y. lipolytica is such an example and considered as a model oleaginous microbe to study the mechanistic insights allied with HS degradation [39]. It owns several multigene families that are directly contribute in degradation pathway. Multiplicity and complexity of these genes also aid this yeast to metabolize a broad range of lipophilic substrates [40]. Some articles have summarized the uptake and biochemistry of HS metabolism by Y. lipolytica [6,39-44]. The initial step involves HS transportation from media into the cells by inducing various modifications in substrates to improve their accessibility [45]. For example, Y. lipolytica produce surfactants like glycolipid which reduce the HS droplets in size and enhance the contact area between substrate and cells [46]. It also secretes extracellular lipase and “lipo- osan,” an extracellular emulsifier that facilitates TAGs hydrolysis into fatty acids, hence enable surface-mediated substrate transport [47]. Alternatively, alkane can be bonded to hydrophobic outgrowths formed on yeast surface and transported into the cell (however, transport mechanism is not yet well described). Then it migrate to the endo plasmic reticulum (ER), where fatty acid synthesis take place via the enzyme catalytic system including cytochrome P450 reductase, fatty alcohol oxidase, and fatty aldehyde dehydrogenase [41]. Further, by binding to coenzyme A, the released fatty acids activated into acyl-CoAs in presence of enzyme acyl-CoA synthetase (FAAS1) which are subse quently reassembled into TAGs and stored in lipid bodies [44]. These lipid bodies serve as an energy source for the cell as inadequate supply of carbon source lead to TAG degradation (β oxidation in peroxisomes) and acetyl-CoA formation for maintaining cellular metabolism. However, in excess intracellular substrates, these are stored in TAG and sterol ester (SE) [40]. An outline of neutral lipid synthesis via HS utilization in Y. lipolytica is presented in Fig. 1.

2.2. Fatty acid distillates (FADs)

FADs are another significant water pollutant released by food processing industries (such as edible oil manufacturing, slaughterhouse, dairy, restaurants). For example, palm fatty acid distillates (PFAD) annual production is estimated to 600,000 tons amounting 3.6% of crude palm oil [48,49]. FADs mainly constitute > 85% of FFAs (like palmitic acid), 5–15% triglyceride and unsaponifiable compounds ster rol, squalene and volatile compounds [50]. When discharged into the water, they form a layer on water surface, hence reduce sunlight pene tration and ultimately interrupt photosynthesis in marine plants, which in turn result to a significant drop of dissolved oxygen. Moreover, oil trapped in feathers of marine animals blocks respiratory system and thus can lead to their death. This event negatively affects food chain and cause health risk in humans too [51]. In past decade, researchers have tested the potential of oleaginous yeast for utilizing oil containing wastewater as feedstock.

Consequently, several yeast species including Candida lipolytica, Saccharomyces lipolytica, Candida tropicalis, and Candida rugosa have been identified to metabolize FADs [52,53,54]. Lipomyces starkeyi was shown to synthesize 21% lipid content (with C16/C18 predominant fatty acids) when grown on diluted (50%) palm oil mill effluent [53]. Likewise, refined soybean oil wastewater treated by Trichosporon fermentans was removed 89% oil in 40 h with 94.7% COD reduction. The obtained biomass, lipid concentration and content were 7.9 g/L, 3.4 g/L and 43% respectively [53,54]. Wickerhamiomyces anomalus EC28 showed adequate growth on two different substrates, cheese whey and olive mill-based wastewater medium and produced substantial amount of intracellular lipid content of 40% and 75% respectively [55]. Data suggested that oily effluents are a potential raw material for biodiesel production, however, their scale up requires high energy due to oil immiscibility in water, which demands excess shear force to attain proper mixing in the reactor [55]. Various studies have shown that oleaginous yeast may use internal enzymatic mechanisms known as “ex novo” lipid synthesis (explained in section 2.1.1) to transform waste cooking oils into value added fatty acids. According to Papanikolaou and Aggelis (2003), Y. lipolytica can uptake fatty acids in a selective manner, and synthesize lipids which have a fatty acid composition very different compared to the provided substrate [56]. This species was also studied for synthesis of tailor-made lipids by using chemically hydrolysed rapeseed oil, and found synthesized lipids with similar fatty acid
composition to cocoa butter, valuable for the food industry and specifically for chocolate manufacturing [57]. Recently, Patel et al. (2019) demonstrated the successful cultivation of Cryptococcus curvatus on waste cooking oil, and fatty acid composition of resulting lipids was more favourable for biodiesel production as compared to the waste cooking oil itself [58].

2.3. Saline wastewater

Industries associated with seafood, textile, tanning, pharmaceutical, fermentation, pickle and vegetable mainly generate a large quantity of high organic strength wastewater with salinity ranges from 0.2 to 15%. If left untreated, these high salinity (>10 g/L) and organic matter (proteins, cleaning agents, biomaterials, carotenoids, and biopolymers) containing effluents can cause serious environmental problems. Elevated BOD (500–1500 mg/L), COD (>30 g/L), and odor produced by the formation of various compounds like mercaptans, hydrogen sulphide gas, ammonia significantly pollute both water and air [60–62]. Thus, research attention has been paid to investigate eco-friendly ways for saline wastewater treatment. In previous literatures, osmotolerant yeast having capability to survive under higher salinity has found more competent over bacteria for aerobic treatment of saline wastewater [48,63–66].

It is noteworthy to mention the studies conducted by Hamimed and co-workers on Y. lipolytica’s tolerance to high salt, osmotic stress, and a wide pH range (from 3.5 to 11.5) [60,67]. Recently in Tunisia, it was explored to mitigate crude as well as diluted tuna wash processing wastewater (TWPW) which exhibited a significant reduction in the pollution levels after 7 days treatment under optimized experimental conditions (29°C, pH 6.50). This biological treatment led to 66% reduction of the COD and salinity, 69.8% in total organic carbon, and 100% of total phosphorus from the crude TWPW. Diluted TWPW treatment resulted in 75% COD and 74% total organic carbon reduction, along with complete removal of nitrogen, phosphorus, and 68% decrease in salinity. In this study, Y. lipolytica produced 5 g/L biomass with high protein and lipids contents of 302 ± 5.44 mg/g and 336 ± 12.2 mg/g respectively [60]. Biotreatment of saline fish canning wastewater with concurrent biodiesel and animal feed supplement production was also evaluated by Azin et al., (2022). Y. lipolytica EBL13 noticeably reduced COD (85%), BOD (59%), sulphite (50%), nitrate (51%), total hardness (15.6%), total suspended solids (85%), and total phosphorus (91%), whereas pH and ammonia increased post-treatment. The yeast biomass was rich in protein with a protein content of 50.2%, in which 16.5% to be essential amino acids. Fatty acids such as 0.15% linolenic acid, 0.21% omega-3, and 22.15% omega-6 were obtained and the predicted physicochemical parameters for biodiesel produced from those fatty acids, such as iodine value (IV: 76.4 g I2/100g), and cetane number (CN: 55.7), meet the EN 14214 specifications [68]. L. starkeyi HL cultivated on fistmeal wastewater attained 5.34 g/L biomass with 20.8% lipid content, and the supplementation of this wastewater with glucose boosted biomass and lipid by 3.36- and 2.43-folds, respectively [1]. Laboratory adaption process made Trichosporon cutaneum resistant to high salinity up to 130 g/L NaCl, while achieving 31.7 g/L lipid concentration, approximately 36% greater than freshwater [69]. The oleaginous yeasts Rhodotorula glutinis and Y. lipolytica were also tested in seawater (typical salinity of 3.5%) but their cell growth found to be significantly suppressed. However, Rhodotorula mucilaginosa cultivation on seawater (using crude glycerol as carbon source) synthesized 17.2 g/L biomass and 65 ± 5% lipid content in which oleic, linoleic acids, and palmitic were the predominant fatty acids [69–71].

A plethora of reports discussed the mechanistic insight underlying yeast adaptation into saline stress. In brief, salinity induced pathway is triggered by the accumulation of osmotically active compounds, particularly glycerol, trehalose, erythritol and compatible ions to equilibrate an increased external osmotic pressure. These events create osmotic stress by activating plasma membrane H + -ATPase and Na + /H +
antiporter through which excessive intracellular Na\textsuperscript{+} out from the cell by using plasma membrane’s proton electrochemical gradient as a driving force. On the other side, accumulated osmolytes transformed into glycerol-3-phosphate which further contribute into the pathway of TAG synthesis. Experimental data suggests that altering membrane viscosity is also a respond to high salinity, achieved through deviations in the content of saturated/unsaturated fatty acids in the membranes or increased length of fatty acyl residues. These alterations ultimately depend on the level of TAGs in the cells (Fig. 2) [72].

2.4. Heavy metal and radioactive containing waste effluents

Metals with density > 4 g/cm\textsuperscript{3} are termed heavy metals, can cause widespread environmental pollution and recently attracted the public interest due to associated health risks [73]. Mining, ore processing, foundries, smelters, dumpsites and other metal-based industrial operations are some notable examples of sectors emancipating heavy metals, radionuclides, metalloids, organic/inorganic compounds into the environment [74,75]. Among them, dissipation of radionuclides and heavy metals from anthropic practices is considered a forefront issue, and pose a severe threat to land as well as aquatic ecosystem. Radioactive contamination can cause even more severe effects into the environment, as contaminated areas can become inhabitable for very long time. Ingestion of heavy metals (including lead, Pb; arsenic, As; chromium, Cr; cadmium, Cd; copper, Cu; etc) above a critical limit can cause life threatening effects on humans by triggering mutagenic, carcinogenic and teratogenic effects, etc. Moreover, oxidative stress induced by metal ions impairs cellular organelles, and components like Cr, Cd, As, Cu may facilitate DNA breakage, aberrant gene expressions, hypomethylated DNA and Wilsons disease respectively [76–78]. Deteriorating effects like metabolic disruption, structural abnormalities, low productivity, unbalanced nutrition, death under extreme conditions have been seen in aquatic life form on contaminated sites. Along with heavy metals, these sites often possess diverse inorganic pollutant such as N, P, S, Cl, radionuclides (e.g. radon, uranium, plutonium), and metalloids (As, selenium) etc. [79]. According to US EPA, 40% of assessed water of the nation does not meet the permissible water quality parameters and even Antarctica is not left untouched from heavy metal pollution [80,81]. Water samples from river Benue, Niger, Yamuna, Shenzhen, etc., were found heavily contaminated with multiple metal elements and same also reported in mangrove ecosystem in China [80–86].

Several conventional methods like oxidation-reduction, electro-chemical treatment, membrane technology, chemical precipitation, active carbon adsorption and ion exchange have been explored to treat contaminated waterbodies, however the low metal removal efficiency and associated operational cost became a major challenge [87]. At this juncture, microbes mediated bioremediation has attracted research interest as an alternative method for heavy metal entailing wastewater treatment owning to its eco-friendly and cost-saving features [88]. Yeasts encompassing adaptability to endure high saline conditions have been proven very competent for metal decontamination [89]. However, oleaginous yeast has been poorly exploited due to the negative impact of heavy metals on their lipid productivity, although in some cases induced lipogenesis was also observed. For example, *R. glutinis* ISO A1 achieved complete removal of heavy metals (Cr, Cd, zinc (Zn)) from municipal wastewater and enhanced lipogenesis (lipid content ~40.66%) with remarkable abatement in nitrate (73.2 ± 3.95%), COD (88.33 ± 1.88%) and phosphate (85.1 ± 2.97%) [90]. Aibeche et al., (2022) isolated *R. mucilaginosa* and *W. anomalus* yeast strains from Cd and Pb polluted areas of Dayet Oum Ghellaz lake (Algeria) and studied their bioremediating potential towards Cr, Pb, Cu, Zn, mercury (Hg), and iron (Fe) [91]. Unlike Cd\textsuperscript{2+} (41% w/w lipid in presence of Cd\textsuperscript{2+} as compared to 51% w/w obtained in control conditions) Zn\textsuperscript{2+} was reported to slightly enhance lipid accumulation in *L. starkeyi* cells (~52 % lipid

![Fig. 2. Metabolic pathways evoked under high osmotic stress condition and bio-mitigation process of heavy metals leading to fatty acid synthesis. Abbreviations: Dihydroxyacetone phosphate (DHAP), Isocitrate dehydrogenase (ICDH), ATP-citrate lyase (ACL), acetyl-CoA carboxylase (ACC), reactive oxygen species (ROS), phosphatidic acid phosphohydrolase (PAH), phosphatidic acid phosphatase (PAP), diacylglycerol (DAG), diacylglycerol acyltransferase (DGAT), triacylglycerol (TAG).](image-url)
content) [92]. The oleaginous yeast *Rhodosporidium toruloides* K5 is isolated from floating water of a previous uranium mining site showed effectual uranium removal from surrounding. The strain tolerated high uranium concentration of approximately ~6 mM (350 mg uranium/g dry biomass, 48 h). Uranium uptake was affected by the growth temperature, in which at 30°C accumulation of needle like structures of uranium as well as lipid granules (quantitative lipid data not shown in study) were observed in cytosol [93].

In contrast to microalgae, limited information is available about metal detoxification mechanisms associated lipogenesis in oleaginous yeast. *S. cerevisiae*, *Candida* sp., *Schizosaccharomyces pombe* are the most studied, in which it was found that yeast evolved different metal detoxifying mechanism: 1) biosorption, mainly occur by metal interaction with negatively charged groups (such as sulphohydral, carboxyl and phosphate) present on yeast cell membrane; 2) biotransformation, which involves reduction of highly toxic forms to less toxic one; 3) bioaccumulation, intracellular uptake and transport of toxicants by living cells [94]. The mechanism of intracellular uptake is more complex than the other two discussed methods and not completely understood yet. However, Rajakumar et al., (2020) reviewed detailed the mechanistic insights of Cd bioaccumulation in *S. cerevisiae*. According to this, Cd penetration into the cell occurs via the bivalent cation transporters; high and low affinity Zrt1p, Zrt2p (zinc transporter) respectively. The Zrt1p (activated from Zn transcription factor ZAP1) is mainly responsible for zinc uptake however, Cd shares similar physico-chemical properties with Zn and thus, get enter into the cells via displacing Zn ions. These events reduce intracellular Zn concentration that activates ZAP1 which ultimately triggers a transcription factor, Zap 1p. This transcription factor leads activation of phosphatidic acid phosphohydrolase (PAH1) that forms phosphatidic acid, primarily contributes to TAG formation. On the other side, Cd internalization generates excessive reactive oxygen species (ROS) that further activates the discussed pathway and induces lipogenesis in cells. By accumulating intracellular TAG, *S. cerevisiae* protect themselves from both Cd toxicity and oxidative damage (Fig. 2) [95].

### 2.5. Xenobiotic biodegradation

Non-naturally produced chemical substances in the environment are known as xenobiotics. Fertilizers used in agriculture, waste generated from paint, dye and textile industry and plastics are few examples of xenobiotic compounds. These substances are accountable for severe environmental problems and long-term exposure of human beings can pose a risk for severe diseases such as cancer, mutation, abnormalities in kidney, brain, liver and cerebrospinal nervous system, etc. [48]. Owing to their recalcitrant nature, their degradation is very challenging as many chemical processes are not able to break down halogen, nitro, sulfonyl containing substances into the inorganic materials [77]. Microbial bioremediation could offer an alternative as it consists a mild and green method that can breakdown xenobiotic compounds. However, further research is still required for development of microbial bioremediation processes.

#### 2.5.1. Dye containing effluents

The release of dyes into the water bodies not only cause toxicity to the water but also add discoloration in water [96]. Approximately >7x10^5 tons dyes are produced annually which used in detergents, food colouring, pharmaceutical, cosmetic manufacturing, plastic, leather and textiles industries. Owning to deficiencies in dyeing process, a significant loss of dyes can occur (for example 20–50% is estimated for the textile industry) that result into the wastewater [96].Like crude oil, dye effluents obliterate the aesthetic quality of water, reduce sunlight penetration, consume dissolved oxygen, impede photosynthesis, suppress plant growth and even harm aquatic flora and fauna. Based on the various chemical structure of chromophores, dyes are mainly categorised into twenty-five classes and amongst all, azo dyes occupy ~70% of the annual production with 60% usage worldwide owning to their easy application, wide range of color shades and low energy demand. Besides existence of azo group (N=N), azo dyes have also comprised complex aromatic structure that persist for long time in the environment, release hazardous materials upon degradation and cause visible contamination even at 50 mg/L concentration. Moreover, azo dyes are also known to decrease urease activity and increasing ammonification rate in ecosystems [11].

Various physico-chemical methods are available for their treatment including ion exchange, adsorption, filtration/coagulation, ozonation, photocatalytic reactions and fenton reagent etc [96]. Nevertheless, biological methods by using microbial cells and enzymes have gaining popularity. Earlier literature suggests that unlike monoculture, microbial co-culture with desired characteristics has proven best suited for this purpose. In line, Ali et al. (2021) explored multiple novel oleaginous yeast consortia for dye degradation, lignin valorization and biofuel production. Among them, a cold adapted oleaginous yeast consortium Y-MG-SH (*Yarrowia* sp., *Meyerozyma guilliermondii*, *Sterigmatomyces halophilus* halophilus) decolorized ~80.56% dye mixture. Moreover, high saturated fatty acid content (34 ± 0.95%) with low polysaturated fatty acid amount (21.48 ± 1.1%) in the synthesized lipids suggested their availability toward biofuel generation [97]. Group containing manganese-dependent peroxidase producing oleaginous yeast strains (*Debaromyces hansenii*, *M. guilliermondii*, *Vanrijia humicola* and *Meyerozyma caribbica*) found capable for azo dye degradation, whereas another consortium (*Mnp-YC4; M. caribbica* MPY-2, *V. humicola* MPY-1, *M. guilliermondii* MPY-4, *D. hansenii* MPY-3) showed tolerance to dye contaminated wastewater in the presence of 3, 5-Dimethoxy-4-hydroxybenzaldehyde and significantly reduce COD (7160 mg/L) with 91.4% azo dye decolorization [98,99]. Similarly, the Y.SH.BC consortium designated with *Yarrowia* sp., *S. halophilus*, *Barrettosxyx californica* promoted 82% reduction of Red HE3B dye in the presence of lignin, achieving a high lipic productivity (1.56 g/L/day), lipase activity (170 U/ml), while the produced lipids has 36.09% saturated fatty acids, 45.44%, 18.30% mono and polysaturated fatty acids respectively [100]. In another case, the oleaginous yeast consortium Y.SH.BC (*Yarrowia* sp., *S. halophilus* and *B. californica* isolated from termite guts) presented high xylanase, β-xylanohydrolase, CMCase, xylan esterase, lipase, cellulobiohydrolase, β-glucosidase activities that resulted to ~81% decolorization of a reactive dyes mixture (Reactive Black 5; Reactive Blue 19; Reactive Red 120; Reactive Violet 5; Reactive Orange 16; Reactive Green 19) [11].

Interestingly, azo dye catabolism combined with yeast lipid pathway, concurrently adds value to the process through biofuel production. Researchers working in this field have suggested multiple mechanisms for dye biodegradation. According to Tohamay et al., (2020), reducing power generated due to oxidoreductase and dehydrogenases (actively participates in gluconeogenesis, glycolysis and TCA cycle in oleaginous yeast) is used by NADH-DCIP reductase that asymmetryically cleaves the azo bond and regarded as the initial step towards azo dye biodegradation [101]. It was observed that induction of this enzyme was significantly enhanced in yeast consortia as compared to individual microbes [100]. In the next step, this cleavage results to the formation of two intermediates; 1,2,7-triamino-8-hydroxy-3,6-naphthalenedisulfonate (TAHNDs) and amines 2-(4-aminobenzene)sulfonyl) ethoxy sulfonic acid (compound IV), that are further subjected to desulfonation and oxidatively transformed into smaller compounds via routes A, route B respectively (Fig. 3). For route B and compound I (produced from desulfonation of TAHNDs) undergoes deamination and forms naphthalene-1,2,4-triol (compound II) that is then transformed into catechol (compound III). Further, catechol undergoes ring fission via cis-mucinic acid pathway and converts into succinic acid and acetyl-CoA, which is a TCA cycle precursor [102]. These final events of dye metabolism build up acetyl CoA, a precursor for *de novo* TAG biosynthesis (Kennedy pathway). Proposed by earlier studies on oleaginous yeasts, accumulated TAG can be effectively transesterified to
produce FAME (biodiesel) [103]. For route B, compound IV oxidatively transformed through three intermediate compounds V, VI, VII named as 2-[(4-aminophenyl)sulfonyl]ethanol, 4-ethanesulfonyl aniline, aniline by desulfonation. In the following step aniline deamination forms benzene (compound VIII) and transformed into cis-9-Octadecenoic acid (compound IX). Finally, cis-9-Octadecenoic acid transported into TCA cycle and is probably mineralized [101].

2.5.2. Waste effluents containing polyaromatic hydrocarbons (PAHs)

The fused ring aromatic compounds also knowns as PAHs are ubiquitous environmental pollutants which formed during natural and anthropogenic activities such as inadequate combustion of coal, wood, oil and petrol. Due to possessing mutagenic, genotoxic, carcinogenic and toxic properties, US EPA has classified them as priority pollutant agents [77]. Thus, abolition of these toxicants from domestic as well as industrial effluent is essential.

Rhodotorula, Rhodosporidium, Cryptococcus, Candida, and Trichosporon are some examples of oleaginous yeast genus that are being potently explored for biodegradation of multiple aromatic compounds like anthracene, phenol, and phenanthrene [104]. Patel et al., (2017) investigated phenol (1 g/L) biodegradation by the oleaginous yeast Rhodosporidium kratochvilovae, resulting to 64.92% lipid content and further used this preliminary information to treat pulp and paper industry effluent. Data showed considerable reduction in phenol (99.60%), BOD (77.36%) and COD (94.22%) as well as color (89%), alongside elevated total lipid yield (8.56 g/L). GC–MS profiling revealed high quantity of mono unsaturated fatty acids, MUFA (45.43%); polyunsaturated fatty acids, PUFA (15.91%) that further improved biodiesel quality like OS, CN, cold filter plugging point (CFPP) at low temperature as per ASTM D6751 and EN14214 guidelines [105]. Deeba et al., (2018) also demonstrated that the oleaginous yeast Cryptococcus psychrotolerans ITRFD completely degraded a range of aromatic hydrocarbons including phenol (1 g/L), anthracene (0.50 g/L), naphthalene (0.75 g/L) and pyrene (0.50 g/L) with 46.54%, 44.97%, 46.38%, 44.16% lipid content respectively [106]. In a study, Trichosporon oleaginosus was used to metabolize 4-hydroxybenzoic acid (pHBA), resorcinol, and phenol.

Fig. 3. Metabolic pathways involved in bio-mitigation process of dyes and phenolic constituents linked to fatty acid synthesis. Abbreviations: NADH dichlorophenol indophenol (NADH-DCIP), Dihydroxyacetone phosphate (DHAP), Isocitrate dehydrogenase (ICDH), ATP-citrate lyase (ACL), acetyl-CoA carboxylase (ACC), phosphatidic acid phosphatase (PAP), diacylglycerol (DAG), diacylglycerol acyltransferase (DGAT), triacylglycerol (TAG).
Likewise, in glucose, similar growth rate and biomass yield was obtained with 4-hydroxybenzoic acid (pHBA) and resorcinol but increased lag phase observed in presence of phenol. Among all components, lipid-genesis only induced when resorcinol utilized as a sole carbon source (~69.5% lipid accumulation) [107].

Detailed metabolic pathway for aromatics degradation in oleaginous yeast has already been described by Patel et al., (2017). In brief, oleaginous yeast metabolism of PAs comprises of three main stages: 1) aromatic ring activation 2) deamoratization 3) transforming ring cleavage products into the TCA cycle’s intermediary metabolites. In the first step, phenol hydrolyses to form catechol by phenol hydrolase. Further, catechol undergoes cleavage either at ortho or meta position via catechol 1,2-dioxygenase and catechol 2,3-dioxygenase, respectively. The end products including succinate, acetyl CoA (ortho pathway) and formate, acetaldehyde, pyruvate (meta pathway) enter the TCA cycle and subsequent metabolism into lipids takes place (Fig. 3) [104].

3. Promising oleaginous yeast species with their pros and cons

To enhanced the industrial applicability and economic viability of the process, robust oleaginous yeasts are required that can tolerate extreme conditions like low and high pH, broad temperature range, adaptability to assimilate diverse wastes as carbon source, compromised sterility, as well as genetic accessibility [108]. In accordance to the scientific literature, over 160 yeast species (with lipid contents ≥ 20 % w/w) have been reported till date [109,110]. Y. lipolytica, T. oleaginosus, R. glutinis, L. starkeyi, R. toruloides, are some oleaginous yeast species gaining scientific interest due to their growth performance, substrate suitability, high attainable lipid content, and flexibility in genetic manipulation [109].

Y. lipolytica, can be isolated from wide variety of environments including protein or fat rich substrates (such as cheese, etc.) and previously named as Saccharomyces lipolytica, Endomyces lipolytica, and C. lipolytica. Its capability to utilize hydrophobic substrates (such as n-alkanes) enhanced its industrial relevance to produce SCO, citric acid, carotenoids, lipases, mannitol, erythritol and other microbial metabolites. Despite these advantages, limited capability for xylose metabolism as a sole carbon source (due to presence of cryptic xylose metabolism pathway) is a major drawback allied with Y. lipolytica [111]. However, Y. lipolytica’s genetic accessibility make its an industrially relevant versatile microbe thereso, ~70% of the genetically engineered oleaginous yeasts strains belong to this species [109]. Genome-wide CRISPR-Cas9 knockout library has recently established which being used for both strain engineering and functional genomics purposes. Advanced engineering in Y. lipolytica can also be achieved by combining multi-omic data with genome-scale models [111].

T. oleaginosus, also known as Cutaneotrichosporon oleaginosus, has being studied for decades primarily isolated in 1978 from drains and floors of a dairy farm. Its fast growth and lipid production (lipid profles) and inhibitor tolerance ability (which facilitates use of non-sterile fermentation condition) suggested a suitable organism for SCO, single cell protein (SCP) production from whey permeate [109]. Despite these advantages, genome-scale libraries and genome-scale model have not been currently existing for T. oleaginosus [111].

R. toruloides, identified oleaginous in 1944 is recognized for its industrial potential in producing, carotenoids, lipid and enzymes. Likewise, T. oleaginosus, R. toruloides DSM-444 has ability to metabolize lignin derived aromatic monomers [111]. Advancement in genetic tools has further increased its status in industrial biotechnology for instance, techniques like adaptive laboratory evolution, mutagenesis, and metabolic engineering applied to enhance its inhibitor tolerance ability. As a result, the evolved strain Y4 is specifically popular amongst yeast lipid researchers [109]. R. toruloides’s genome scale-model has also been reported that delivers pivotal information required for rational genome engineering to create resilient strains for biofuel production. For instance, an AMT based genome-wide insertional screen in R. toruloides ATCC 10657 and IFO 0880 abetted 150 genes identification, vital for lipid biosynthesis and curation of the genome annotation [111].

L. starkeyi, species from Lipomycetaceae (family of strong lipid producer) is discovered in 1946 and regarded as high ”biotechnological value” yeast due to having adaptability towards multiple carbon source co-utilization (xylose with glucose, acetate or cellobiose), a rare phenomenon amid other oleaginous yeasts. This quality makes L. starkeyi very fascinating for incessant processing via consuming mixed substrates like different waste waters and lignocellulosic hydrolysates [109]. Thought, genome-scale libraries and genome-scale model have not been currently existing for L. starkeyi [111].

R. glutinis, another species belongs to the genus Rhodotorula is a robust producer of lipid, enzyme, and carotenoid. Now-a-days, this species is gaining more attention among yeast lipid researchers than before and frequently considered as biocontrol agent due to its antagonistic traits, hence allowing non-sterile cultivation for lipid production [109]. Table 2 enlisted lipid producing abilities of these yeast in different types of waste effluent. Additionally, Table 3 provides comprehensive information about the opportunities and challenges associated with oleaginous yeast mediated bioremediation process of various recalcitrant waste streams.

4. Fatty acid profile and assessment of fuel properties

The previous sections have clearly highlighted the oleaginous yeast potential towards tolerance to poor quality water and their use in decontaminating wastewater effluents synchronized with high value lipid accumulation and concomitant biodiesel production. However, biodiesel must meet the criteria established by international standards including EN 14214 (Europe), ASTM 6751–3 (USA), and Bureau of Indian Standard (IS 15607–05) [136], The available literature provides an assessment of fuel properties of biodiesel derived from microbial lipids as well as shows the impact of fatty acid composition on those properties [136,137]. Nevertheless, there has been a lack of thorough assessment of the fuel properties of biodiesel obtained from various yeast strains grown in distinct wastewater conditions within a single, integrated framework. To assess the impact of wastewater medium on qualitative biodiesel production (as biodiesel properties are directly influenced by the chemical composition of lipids that vary with feedstock and yeast species) this section reviews the fatty acid profile and allied biodiesel properties obtained from 10 recalcitrant waste effluents. The fatty acid as well as biodiesel compositional data were obtained from numerous sources and represented in Table 4 & 5 respectively.

Lipid composition in varied effluents shows predominance of C16:0 (palmitic acid), C18:1 (oleic acid), C18:2 (linoleic acid), C18:3 (stearic acid) fatty acids that is similar to the profile attained from animal/vegetables oil. Above mentioned depictions also provide valuable insights into the different biodiesel types. For instance, all the 10 effluent composition were dominated by C18:1 fatty acid (oleic acid), commonly known to improves biodiesel quality in terms of high CN as per EN 14214, ASTM 6751–3 guidelines (Table 4). CN is a dimensionless parameter used to access ignition quality of diesel fuel and, to ensure efficient and smooth combustion in engines it should be >47 (ASTM D6751) and 51 (EN 14214) [139]. Increased CN in most cases confirms good ignition, smooth engine run, cold start behaviour, and complete combustion, thus minimized CO (carbon monoxide) as well as particulate emissions. Moreover, CN also influences significant biodiesel properties including heating value and density in which slight change can affect engine output [140]. Higher PUF content in S. No. 2, 3, 4, 5, 7, 9 results a decline and escalation in CN and NOx emissions respectively as well as drop lubricity leading gum formation in engines [141]. On the other side, higher unsaturation (due to low melting point) improves cold flow plugging properties (CFPP), which is a measure of crystallization formation or gelling in biodiesel at low temperature that severely affect
engine performance by clogging fuel line [142]. In present case, all conditions showed inadequate values of CFPP except one, No.10; –7.7431, indicating that this biodiesel can be used in cold climate. Fatty acid profile also reveals presence of C14 and C16 SFA in a considerable amount, key player in determining oxidative stability and kinematic viscosity (KV). The oxidative stability of the biodiesel, found most stable PUFA [143]. KV (control fuel flow in the engine) is an important fuel property and exceeding either the higher and lower limits cause engine performance. Hence, optimization of SFA to UFA ratio is necessary to control the fuel properties [144]. As per literature, carbon chain length and double bonds numbers are two key parameters of fatty acids that directly affect fuel properties. Ideally, a good quality biodiesel should contain maximum C\textsubscript{18:1} and C\textsubscript{18:3} with other methyl esters as low as possible or maintain a ratio of 5:4:1 (C\textsubscript{16:0}, C\textsubscript{18:1}, C\textsubscript{18:3}) is also recommended [141]. SFA protect biodiesel to undergo auto-oxidation, UFA are important for low temperature performance. Hence, optimization of SFA to UFA ratio is necessary to control the fuel properties [144]. The biodiesel obtained from the different yeasts post cultivation on waste effluents have some deficiencies concerning its properties associated with KV, density and CFPP. To overcome these challenges biodiesel blending with petroleum diesel fuel is a desirable choice. Further research is needed to optimize the conditions to gain desired fatty acid profile that can enhance the quality of waste derived microbial biodiesel.

5. Omics technologies integrated with process advancement

Biological toxicity and recalcitrant nature allied with the aforesaid waste effluents may rely on engineered microbial strains or microbial consortia to boost their performance. Thus, to better comprehend the mechanisms involved in the degradation of those waste or to identify new promising microbial candidates, various omic techniques (genomics, transcriptomics, proteomics, metabolomics) have been studying on a global scale. These system biology-based approaches can provide a detailed knowledge about biological processes together with identification of key microbial pathways involved. Omics techniques combine traditional molecular as well as advanced biological techniques accomplished with high resolution, high-throughput instrumentation that ease the several processes allied with collection of mass quantities of genes, cellular data, and protein, also helps in optimizing the architecture of discovery and methods validation in science (Fig. 4) [145].

### Table 2

Produced lipid from diverse oleaginous yeasts in various waste effluents. TN: total nitrogen; TP: total phosphorus; TDS: total dissolved solids; TOC: total organic carbon; MLSS: mixed liquor suspended solids; MLVSS: mixed liquor volatile suspended solids; TRS: total reducing sugar; Ni: nickel.

<table>
<thead>
<tr>
<th>Oleaginous yeast strains/consortium</th>
<th>Type of substrate</th>
<th>Characteristics</th>
<th>Biomass (g/L)</th>
<th>Lipid concentration (g/L)</th>
<th>Lipid content (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Y. lipolytica</strong></td>
<td>Industrial saturated fats (stearin)</td>
<td>C14:0, 10%; C18:0, 52%; C12:0, 10%; cis-9-C18:1, 2%; C16:0, 25%</td>
<td>12</td>
<td>6.48</td>
<td>54</td>
<td>[112]</td>
</tr>
<tr>
<td>Volatile fatty acids (acetic acid)</td>
<td>–</td>
<td>4.21</td>
<td>0.56</td>
<td>13.30</td>
<td>[113]</td>
<td></td>
</tr>
<tr>
<td>Propionic acid</td>
<td>–</td>
<td>3.53</td>
<td>0.31</td>
<td>8.90</td>
<td>[69]</td>
<td></td>
</tr>
<tr>
<td><strong>T. cutaneum</strong></td>
<td>Saline water with glucose (3.5% NaCl)</td>
<td>NaCl, 3.5%; glucose</td>
<td>–</td>
<td>31.7</td>
<td>–</td>
<td>[140]</td>
</tr>
<tr>
<td>Saline water with phenol</td>
<td>–</td>
<td>23.6</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T. fermentans</strong></td>
<td>Refined soybean oil wastewater</td>
<td>COD, 20,000 mg/L; NH\textsubscript{4}-N, 50 mg/L; oil content, 8000 mg/L; TP, 100 mg/L; pH,10</td>
<td>7.9</td>
<td>3.4</td>
<td>43</td>
<td>[54]</td>
</tr>
<tr>
<td><strong>R. glutinis</strong></td>
<td>Effluent from palm oil mill</td>
<td>Oil and grease, 280 mg/L; pH, 4.5; reducing sugar, 12 g/L; COD 48 g/L; TN, 262 mg/L</td>
<td>4.15</td>
<td>0.87</td>
<td>20.97</td>
<td>[114]</td>
</tr>
<tr>
<td><strong>R. glutinis</strong> + Chlorera sp.</td>
<td>Sewage effluent containing heavy metals</td>
<td>L; COD 34,680 mg/L; pH, 5.3; nitrate, 32 mg/L</td>
<td>4.6</td>
<td>2.8</td>
<td>60.86</td>
<td>[115]</td>
</tr>
<tr>
<td><strong>R. glutinis</strong> IOS AI</td>
<td>Sewage wastewater</td>
<td>COD, 355 mg/L; nitrate, 77 mg/L; phosphate, 2.8 mg/L; heavy metals</td>
<td>12.3</td>
<td>5.0</td>
<td>40.66</td>
<td>[90]</td>
</tr>
<tr>
<td><strong>R. glutinis</strong></td>
<td>Pulp and paper wastewater</td>
<td>COD, 1478 mg/L; pH, 2.25; ammonium, 3.95 mg/L; sulfate, 1400 mg/L</td>
<td>19.3</td>
<td>2.9</td>
<td>15</td>
<td>[116]</td>
</tr>
<tr>
<td><strong>R. kratschmolae HimPA1</strong></td>
<td>Clarified butter waste medium</td>
<td>Lactose, 2–14%; fat, 32–70%; moisture, 8–30%; protein 12–39%</td>
<td>1.52</td>
<td>0.22</td>
<td>14.64</td>
<td>[113]</td>
</tr>
<tr>
<td><strong>R. kratschmolae HimPA1</strong> L. starkeyi HL</td>
<td>Clarity of fishmeal</td>
<td>COD, 675 mg/L; BOD, 402 mg/L; TDS, 435 mg/L; TN, 187 mg/L; pH, 7.89; lignin, 376 mg/L; phenol, 58 mg/L</td>
<td>13.87</td>
<td>8.56</td>
<td>61.71</td>
<td>[105]</td>
</tr>
<tr>
<td>L. starkeyi</td>
<td>Fishmeal wastewater</td>
<td>COD, 78.4 g/L; BOD, 55 g/L; TOC, 23.5 g/L; TN, 4.3 g/L; pH, 6.5; salinity, 12.4 g/L; soluble protein, 9.5 g/L</td>
<td>17.6</td>
<td>2.7</td>
<td>15.3</td>
<td>[118]</td>
</tr>
<tr>
<td><strong>L. starkeyi</strong></td>
<td>Wastewater sludge with Cd\textsuperscript{2+}</td>
<td>MLSS, 5.6–8 g/L; MLVSS, 2.8–4.3 g/L; TP, 0.1 mg/L; TN, 8.5 mg/L; pH, 6.6–6.9; metals, Cr, Cu, Zn, Ni, Cd, Pb</td>
<td>–</td>
<td>–</td>
<td>41</td>
<td>[92]</td>
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<tr>
<td>Olive mill waste</td>
<td>Sewage sludge</td>
<td>2.7</td>
<td>0.48</td>
<td>17.8</td>
<td>[24]</td>
<td></td>
</tr>
<tr>
<td><strong>R. toruloides</strong> 9564 T</td>
<td>Phoen containing wastewater</td>
<td>9.27</td>
<td>3.3</td>
<td>35.6</td>
<td>[119]</td>
<td></td>
</tr>
<tr>
<td>C. visinianii (MTCC 232)</td>
<td>Paper mill sludge</td>
<td>Carbohydrate, 47%; cellulose, 4.1%; lignin, 2.3%; TRS, 38%; TN, 526 mg/L; Total protein, 521 mg/L; TP, 8.45 mg/L</td>
<td>14.6</td>
<td>7.8</td>
<td>53.40</td>
<td>[121]</td>
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<tr>
<td><strong>C. curvatus</strong></td>
<td>Waste cooking oil</td>
<td>–</td>
<td>18.62</td>
<td>13.06</td>
<td>70.13</td>
<td>[58]</td>
</tr>
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</table>

References

[140] K. Sartaj et al.
Industrial oily wastewater

Opportunities
Amongst various oleaginous yeast genera (Trichosporon, Cryptococcus, Wickerhamomyces) Yarrowia is the most desirable genus for SCO production using wastewater containing hydrophobic substrates (as discussed in aforementioned sections). High cell surface hydrophobicity in yeast allow hydrophobic pollutants to access the cell surface easily whereas, large cell size and high lipid accumulation as compared to bacteria provide an additional benefit in extraction and post processing of microbial lipids. Ability to produce biosurfactants/emulsifiers are also some key features that empower oleaginous yeast for hydrocarbon biodegradation [122].

Challenges
Industrial oily wastewater contains substantial amounts of hydrophobic organic matter and phosphate, among other components. The short chain length fatty wastes are easy to degrade however long chain lengths usually found more saturated (with elevated melting points) and remain solid at 26°C – 28°C the typical culture temperature of Y. lipolytica thus, hinders oxygen transfer to liquid medium. Being an obligate aerobic, Y. lipolytica’s cultivation demands efficient aeration systems. To resolve this, high agitation rates are employed frequently but it negatively affects lipid biosynthesis (as highest lipid accumulation was achieved in low (5-15%) dissolved oxygen conditions and in highly aerated cultures). In contrary, elevated oxygen transfer rate causes excessive foam generation, necessitating the addition of antifoam agents in substantial amounts. This ultimately raises production cost and decrease overall productivity [122,123].

Besides, elevated phosphorus content in oily wastewater tends oleaginous yeast like Trichosporon fermentans, Y. lipolytica to form pseudohyphae (long branched chain of yeast cells). These pseudohyphaes leads poor cell dispersion where cells become clumpy and aggregates into a large particle. As a result, nutrients uptake by cells hinders due to reduced contact rate between cells and organic substances including oil and oxygen. Furthermore, pseudomycelium morphology has an adverse impact on lipid accumulation and demonstrate poor settling performance which is not favourable for rapid sedimentation as well as separation of yeast [124].

Wastewater from mining

Opportunities
Yeasts are considered better bio-sorbents (because of high growth rate and cell wall structure) for heavy metal ions removal from wastewater as compared to microalgae, fungi, and bacteria. Various metal binding groups, like phosphate, hydroxyl, sulfhydral, sulfate, imidazole, amine are present in cell wall polymers of yeast which enhances its capability to bind heavy metal ions [125]. Indeed, large cell size provides additional advantage to yeast to physically intertwine the pseudosymbiotic mycelia to form flocs. Besides, facilitating oxygen diffusion, this net structured yeast floc also eliminates the necessity of excessive dissolution of oxygen in water. Thus, using yeast notably provide highly efficient oxygen supply and minimize energy consumption via reducing the supplied air flow. Furthermore, to remove these contaminants, yeast have property being used whether they are dead (metabolically inactive/passive) or alive (metabolically active) [126,127].

Challenges
Treatment of multiple elements containing real effluents is a challenging task. As most studies (in case of oleaginous yeasts) have been conducted in synthetic wastewater that contain only one heavy metal element and therefore, these studies are far from a commercial context. Till date, oleaginous yeast-based bioremediation is limited at polishing step (performed post chemical treatment of raw effluent for process expenses [130].

Wastewater containing hydrophobic substrates (as discussed in aforementioned sections). High cell surface hydrophobicity in yeast allow hydrophobic pollutants to access the cell surface easily whereas, large cell size and high lipid accumulation as compared to bacteria provide an additional benefit in extraction and post processing of microbial lipids. Ability to produce biosurfactants/emulsifiers are also some key features that empower oleaginous yeast for hydrocarbon biodegradation [122].

Challenges
High glucose requirement and necessity of a longer culture time due to slow utilization of yeast are some existing weaknesses. Moreover, most of oleaginous yeasts may limit their ability for practical treatment under extreme environmental conditions like oxygen limitations etc [133].

Seafood industry effluents

Opportunities
In comparative studies, osmotolerant yeast cultures outperformed bacterial systems in treating wastewater with elevated organic and salinity levels, conditions that typically hinder bacterial growth and bioremediation performance. Yeast cells exhibited a higher uptake of specific nutrients compared to bacterial cells, resulting in elevated rates of phosphorus and nitrogen removal from the wastewater. Additionally, yeast exhibited the potential to enhance membrane performance (notably reducing the rate of membrane clogging) in bio-membrane reactor when treating highly saline wastewater and potentially improve the treatment system’s economics via reducing operating and maintenance costs [66,127].

Challenges
Saline wastewater treatment using oleaginous yeasts have received limited research attention. Furthermore, available data indicate that the size and fractal dimension of flocs diminish as salt concentrations increase, leading to poor settling ability of microbial floc. High salt content characterized by high effluent solids, poor flocculation, and severe depletion in substrate utilization rate [134,135].

Table 3
Opportunities and challenges allied with oleaginous yeast derived bio-mitigation process of diverse recalcitrant waste streams.

<table>
<thead>
<tr>
<th>Industrial oily wastewater</th>
<th>Table 3 (continued)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opportunities</td>
<td>Industrial oily wastewater</td>
</tr>
<tr>
<td>Amidst various oleaginous yeast genera (Trichosporon, Cryptococcus, Wickerhamomyces) Yarrowia is the most desirable genus for SCO production using wastewater containing hydrophobic substrates (as discussed in aforementioned sections). High cell surface hydrophobicity in yeast allow hydrophobic pollutants to access the cell surface easily whereas, large cell size and high lipid accumulation as compared to bacteria provide an additional benefit in extraction and post processing of microbial lipids. Ability to produce biosurfactants/emulsifiers are also some key features that empower oleaginous yeast for hydrocarbon biodegradation [122].</td>
<td></td>
</tr>
<tr>
<td>Challenges</td>
<td>its derivatives, tolerance to pH alterations, osmotic pressure, growth inhibition, high content of dye, is critical. Lacking of potent oleaginous yeasts having capability to transform lipids from various aromatics, and unknown metabolic regulation mechanism presented in natural oleaginous yeast cells are the fundamental challenges. Demand for basic nutrients especially glucose (carbon and energy source for yeast growth, produce reducing power NADH/FADH2, and substrate for H2O2 production) in medium to treat textile wastewater results in escalation of overall process expenses [130].</td>
</tr>
<tr>
<td>Pulp &amp; paper industry wastewater Opportunities</td>
<td>Pulp &amp; paper industry wastewater</td>
</tr>
<tr>
<td>Due to higher C/N ratio of lignocellulosic wastes (found in pulp &amp; paper industry wastewater), oleaginous yeasts hold great potential for effective treatment and utilization of these wastes, contributing to improved environmental sustainability and resource management in the industry [131]. Rapid growth rate similar to bacteria, reduced susceptibility towards viral infection, high potential to control bacterial contamination, and ability to tolerate adverse environmental conditions makes yeast a resilient microorganism for pulp &amp; paper industry wastewater treatment [132].</td>
<td></td>
</tr>
<tr>
<td>Challenges</td>
<td>High glucose requirement and necessity of a longer culture time due to slow utilization of yeast are some existing weaknesses. Moreover, most of oleaginous yeasts may limit their ability for practical treatment under extreme environmental conditions like oxygen limitations etc [133].</td>
</tr>
<tr>
<td>Seafood industry effluents Opportunities</td>
<td>Seafood industry effluents</td>
</tr>
<tr>
<td>In comparative studies, osmotolerant yeast cultures outperformed bacterial systems in treating wastewater with elevated organic and salinity levels, conditions that typically hinder bacterial growth and bioremediation performance. Yeast cells exhibited a higher uptake of specific nutrients compared to bacterial cells, resulting in elevated rates of phosphorus and nitrogen removal from the wastewater. Additionally, yeast exhibited the potential to enhance membrane performance (notably reducing the rate of membrane clogging) in bio-membrane reactor when treating highly saline wastewater and potentially improve the treatment system’s economics via reducing operating and maintenance costs [66,127].</td>
<td></td>
</tr>
<tr>
<td>Challenges</td>
<td>Saline wastewater treatment using oleaginous yeasts have received limited research attention. Furthermore, available data indicate that the size and fractal dimension of flocs diminish as salt concentrations increase, leading to poor settling ability of microbial floc. High salt content characterized by high effluent solids, poor flocculation, and severe depletion in substrate utilization rate [134,135].</td>
</tr>
</tbody>
</table>

Core enzymes accountable for some inimitable functions such as toxic by-product utilization and unique substrates degradation. For instance, enzyme catalysis in textile industry effluent has been of great interest owing to their functional capability in broad pH range, temperature, saline concentration and pollutants [147]. Laccases, hydrolases, dehalogenases are prominently used enzymes in which laccase catalyse the oxidation of aromatic amines and phenolic compounds, hydrolases disrupt chemical bonds and convert larger molecule into smaller one. Dehalogenases degrade various halogenated compounds by cleaving C-X bonds (in which X represents a halogen compound) [147]. Considering enzymatic approach, metagenomics has been widely used to explore typical characteristic of microorganisms, diverse enzyme identification, identification of specific enzymes or microorganisms that catalyse or metabolize the contaminant and transform toxic forms into nontoxic or even convert them into new product like biodiesel.

For example, highly conserved enzyme families, lignin degrading proteins and unique glycoside hydrolases were discovered through metagenomic study of microbes present on sugarcane bagasse [145]. Several other lignin and dye degrading enzymes like manganese peroxidase, laccases and lipoxygenase, hydrolytic enzymes that play crucial role in treatment of hydrocarbon-rich waste effluent have also been identified through metagenomic [148]. Novel esterases, possessing tolerance to salt and multiple solvents have been discovered from environmental metagenomic libraries using sequence and function-based screening approaches. A new esterase Est16, thermally stable and active against a great variety of toxic substrates was recovered from diesel oil degrading metagenomic collections [149]. Besides, a novel esterase cloned from Soda Lake Dabusu sediment’s metagenome has
Meta-transcriptomics play a crucial role for studying polluted/stressful systems and concurrently provides a suitable approach to mine eukaryotic, prokaryotic gene pool encoding aldehyde dehydrogenase isolated from polluted soil and its functional activity. Moreover, limited ability of computational methods like sequence homology-based genomic techniques to unravel protein-substrate interactions and biochemical reactions towards novel protein-substrate interactions and biochemical reactions identified involved in bio-mitigation process demand new protein characterization method such as meta-transcriptomics and meta-proteomics.

5.2. Meta-transcriptomics

Meta-transcriptomics involves studying RNA-based sequence information of microbial communities in a complex ecosystem [148]. Unlike metagenomic analysis, it is a potent tool to identify the expression of discrete genes in an organism by yielding mRNA transcripts (expressed from DNA) which actively transcribing during waste metabolism. It can also provide a snapshot of gene expression changes during growth conditions on different substrates [151]. Meta-transcriptomics play a crucial role for studying polluted/stressful systems and concurrently provides a suitable approach to mine eukaryotic, prokaryotic gene pool for genes of biotechnological relevance [151]. Despite limited researches, a substantial effort was performed to study metal resistance genes acquired from the environment which proving meta-transcriptomics a worthwhile study method for heavy metals bioremediation. In line, Lehembre et al. (2013) prepared a huge meta-transcriptomic library (from heavy metal contaminated soil) where diverse sequences were screened for heavy metal tolerance [152].

been found highly cold adapted (retain 70% of its activity even at 0°C), salt tolerant (5 M NaCl) and withstand broad pH range [150]. Altogether, genome sequencing is a breakthrough technique for registering an organism’s genes for toxicants breakdown, but still necessitates further gene characterization method such as meta-transcriptomics and meta-proteomics.

5.3. Meta-proteomics

Study of total proteome expressed by the microorganisms within an ecosystem at a particular time period is known as meta-proteomics [148]. Transcript expression and gene content do not completely reveal gene’s functional activity. Moreover, limited ability of computational methods like sequence homology-based genomic techniques towards novel protein-substrate interactions and biochemical reactions identified involved in bio-mitigation process demand new protein

Table 4

Fatty acids composition in oleaginous yeast cultivated under varied waste substrates.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Single oleaginous yeast/ Consortium</th>
<th>Type of wastewater</th>
<th>C14:0</th>
<th>C15:0</th>
<th>C16:1ω7c</th>
<th>C17:0</th>
<th>C18:0</th>
<th>C18:1ω9c</th>
<th>C18:2ω6c</th>
<th>C18:3ω3c</th>
<th>C20:0</th>
<th>C20:1ω9c</th>
<th>C22:0</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>R. glutinis TISTR 5159</td>
<td>Effluent from palm oil mill</td>
<td>1.04</td>
<td>20.37</td>
<td>0.83</td>
<td>1.38</td>
<td>10.33</td>
<td>47.88</td>
<td>7.31</td>
<td>0.85</td>
<td>–</td>
<td>–</td>
<td>1.50</td>
<td>[114]</td>
</tr>
<tr>
<td>2.</td>
<td>R. kratochviliensis HMPA 1</td>
<td>Clarified butter waste sediment</td>
<td>–</td>
<td>16.67</td>
<td>–</td>
<td>–</td>
<td>15.37</td>
<td>49.70</td>
<td>14.35</td>
<td>2.13</td>
<td>0.56</td>
<td>0.23</td>
<td>–</td>
<td>[117]</td>
</tr>
<tr>
<td>3.</td>
<td>C. curvatus</td>
<td>Waste cooking oil</td>
<td>–</td>
<td>3.11</td>
<td>–</td>
<td>–</td>
<td>2.16</td>
<td>58.06</td>
<td>20.18</td>
<td>–</td>
<td>6.73</td>
<td>–</td>
<td>–</td>
<td>[58]</td>
</tr>
<tr>
<td>4.</td>
<td>Apisiricrium porosum DSM27194</td>
<td>Volatile fatty acids (butyric acid, acetic acid, propionic acid)</td>
<td>0.75</td>
<td>22.08</td>
<td>–</td>
<td>–</td>
<td>12.98</td>
<td>37.18</td>
<td>23.89</td>
<td>0.54</td>
<td>0.68</td>
<td>–</td>
<td>–</td>
<td>[138]</td>
</tr>
<tr>
<td>5.</td>
<td>Y. lipolytica EBL13</td>
<td>Fish canning wastewater</td>
<td>2.37</td>
<td>26.53</td>
<td>3.53</td>
<td>1.21</td>
<td>5.51</td>
<td>31.99</td>
<td>22.15</td>
<td>0.15</td>
<td>0.62</td>
<td>0.55</td>
<td>0.65</td>
<td>[68]</td>
</tr>
<tr>
<td>6.</td>
<td>L. starkeyi HL</td>
<td>Fishmeal wastewater</td>
<td>11.15</td>
<td>45.99</td>
<td>0.87</td>
<td>–</td>
<td>9.37</td>
<td>8.99</td>
<td>4.20</td>
<td>–</td>
<td>–</td>
<td>11.05</td>
<td>–</td>
<td>[118]</td>
</tr>
<tr>
<td>7.</td>
<td>OYC.Y.BC.SH consortium</td>
<td>Textile wastewater</td>
<td>0.89</td>
<td>30.08</td>
<td>0.33</td>
<td>–</td>
<td>4.79</td>
<td>45.11</td>
<td>18.04</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>[11]</td>
</tr>
<tr>
<td>8.</td>
<td>R. glutinis ISO A1</td>
<td>Wastewater containing heavy metals</td>
<td>0.99</td>
<td>24.79</td>
<td>1.12</td>
<td>0.17</td>
<td>6.47</td>
<td>63.34</td>
<td>1.28</td>
<td>–</td>
<td>0.18</td>
<td>0.42</td>
<td>–</td>
<td>[90]</td>
</tr>
<tr>
<td>9.</td>
<td>R. kratochviliensis HMPA 1</td>
<td>Paper and pulp industry effluent</td>
<td>–</td>
<td>21.86</td>
<td>0.15</td>
<td>0.5</td>
<td>45.43</td>
<td>15.91</td>
<td>–</td>
<td>0.12</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>[105]</td>
</tr>
</tbody>
</table>

Table 5

Biodiesel properties (FAME) estimated from lipid profile shown in Table 4. D: density; SV: saponification value; LCFA: long chain saturated fatty acids; OS: oxidative stability; CFPP: cold flow plugging properties; IV: iodine value; HHV: high heating value; CN: cetane number; DU: degree of unsaturation; KV: kinematic viscosity.

<table>
<thead>
<tr>
<th>BV (mm COH)</th>
<th>IV (g/L/100 g)</th>
<th>CN</th>
<th>DU (wt%)</th>
<th>LCFS (wt%)</th>
<th>CFPP (°C)</th>
<th>HHV (MJ/kg)</th>
<th>KV (mm/s)</th>
<th>D (g/cm³)</th>
<th>OS (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASTM D6751-02</td>
<td>–</td>
<td>–</td>
<td>≥47</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.9-6.0</td>
<td>0.86-0.89</td>
</tr>
<tr>
<td>EN 14,214</td>
<td>–</td>
<td>Max 120</td>
<td>≥51</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.5-5.0</td>
<td>0.86-0.90</td>
</tr>
<tr>
<td>(From Table- 3) 1.</td>
<td>185.75</td>
<td>59.45</td>
<td>62.31</td>
<td>65.03</td>
<td>9.45</td>
<td>13.21</td>
<td>36.17</td>
<td>1.28</td>
<td>0.79</td>
</tr>
<tr>
<td>2.</td>
<td>199.78</td>
<td>76.97</td>
<td>56.30</td>
<td>83.09</td>
<td>9.91</td>
<td>14.66</td>
<td>40.08</td>
<td>3.96</td>
<td>0.87</td>
</tr>
<tr>
<td>3.</td>
<td>187.18</td>
<td>93.60</td>
<td>54.41</td>
<td>–</td>
<td>8.12</td>
<td>9.04</td>
<td>37.51</td>
<td>3.75</td>
<td>0.83</td>
</tr>
<tr>
<td>4.</td>
<td>–</td>
<td>77.10</td>
<td>57.10</td>
<td>0.87</td>
<td>–</td>
<td>40.06</td>
<td>–</td>
<td>4.65</td>
<td>–</td>
</tr>
<tr>
<td>5.</td>
<td>204.9</td>
<td>76.40</td>
<td>55.75</td>
<td>83.54</td>
<td>9.22</td>
<td>39.48</td>
<td>3.13</td>
<td>1.12</td>
<td>–</td>
</tr>
<tr>
<td>6.</td>
<td>194.28</td>
<td>25.60</td>
<td>68.63</td>
<td>29.31</td>
<td>9.82</td>
<td>12.68</td>
<td>36.03</td>
<td>1.25</td>
<td>0.79</td>
</tr>
<tr>
<td>7.</td>
<td>203.49</td>
<td>73.57</td>
<td>56.57</td>
<td>81.52</td>
<td>5.40</td>
<td>0.49</td>
<td>39.15</td>
<td>1.35</td>
<td>0.86</td>
</tr>
<tr>
<td>8.</td>
<td>203.07</td>
<td>60.74</td>
<td>59.51</td>
<td>67.44</td>
<td>5.89</td>
<td>2.03</td>
<td>39.26</td>
<td>1.38</td>
<td>0.86</td>
</tr>
<tr>
<td>9.</td>
<td>163.83</td>
<td>120.02</td>
<td>49.01</td>
<td>122.68</td>
<td>2.56</td>
<td>–</td>
<td>40.91</td>
<td>4.62</td>
<td>0.87</td>
</tr>
</tbody>
</table>
techniques. Thus, incorporating proteomics is extremely vital for gaining in-depth knowledge of complex metabolic pathways involved in pollutant degradation. Proteomic based studies not only help in fulfilling the knowledge gap that exist between genomic annotation and functional characterisation but also provide deep understanding about protein changes in existence of different toxic waste. Furthermore, dynamic changes in a microbial community functions can also be monitored by proteomics [158]. Proteomic measurements including changes in protein abundance, turnover rate, post-translational modifications, subcellular localization, isoform expression and interactions are usually performed by mass spectrometry (MS) based techniques that are categorised into the discovery approach (used to identify changes in proteins) and target specific peptides (used to verify changes) [159]. Previously, metaproteomic was predominantly focused on metal contaminants like Cd, however, recent studies turned towards a broad range of pollutants degradation [160,161]. For example, metaproteomic analysis on soil microcosms conducted to investigate toluene [162], 2,4-dichlorophenoxy acetic acid (2,4-D) [163], and diesel fuel [164] degradation. Post toluene amendment, an upsurge in ABC transporters was observed presumably for toxic substances export [162]. Bastida et al., (2016) employed metaproteomic approach where they analyzed differential protein expressions to elucidate alteration in metabolic processes during compost-treated bioremediation of hydrocarbon-polluted soil. Results indicated that highly expressed catabolic enzymes (by uncultured microbes) like 2-hydroxymuconic semialdehyde, cis-dihydrodiol dehydrogenase, catechol 2,3-dioxygenase, and dehydrogenases catalyse first oxygenation step of aromatic rings and thus induce hydrocarbons biodegradation. This study has opened up a new avenues in microbial ecology field where both functional and phylogenetic relationship of contaminated soil, and the microbial key players involved in pollutant bioremediation can be analysed on the basis of their proteome [165]. Molecular mechanism of 2,4,5-trichlorophenoxyacetic acid degradation, a commonly used defoliant was also studied, which revealed many folds changes in reductive dehalogenation encoding enzyme and showed involvement of 60 protein groups in the mitigation process. In future, this information may be introduced in other microbes capable of 2,4,5-trichlorophenoxyacetic acid degradation [166]. Indeed, poor protein yield (due to environmental protein contamination, low protein purity because of humid interference, poor protein extraction efficiency), incomplete genome database are some major drawbacks of metaproteomic and unlike DNA or RNA no techniques like PCR exist to ‘replicate’ protein [167].

5.4. Metabolomics

Metabolomic analysis is used to find out vital biochemical tailbacks as points for improvements in microbes mediated waste degradation process. Likewise proteomics, metabolomics includes MS-based techniques like gas chromatography (GC) or liquid chromatography (LC) and these identify enzymatic reaction products with high resolving power, sensitivity and specificity [168]. For instance, using metabolite quantification and regression modelling, Wang et al., (2015) illustrated the inhibitory mechanism of phenol in S. cerevisiae. Their study revealed that proline and myo-inositol synthesis were main pathways associated with tolerant phenotypes. Further genes deletion exhibited augmented sensitivity whereas enhanced biosynthesis and external supplementation with either myo-inositol or proline increased tolerance [169]. Phenolic amides (e.g. coumaroyl and feruloyl amide) associated toxicity mechanism was also examined by using dynamic 

6. Economic aspects and sustainability of yeast biodiesel production

Feedstocks, fermentation system and energy consumption in the production steps are three fundamental pillars in biodiesel economics. Being heterotrophic, oleaginous yeast require carbon and nitrogen sources, considered as main operating cost of the process [1]. For example, one-ton yeast oil requires 4 to 5 tonnes sugars that usually obtained by sugarcane/corn and cost around USD 430 per tonne, (~40% of total production cost) [171]. Thus, economically feasible biodiesel can be achieved with low-cost medium utilization. Towards this direction, industrial wastes are showing potentials to replace sugarcane and corn derived sugars. For instance, Chen et al., (2018) exerted waste water sludge as a renewable feedstock for biodiesel production using oleaginous yeasts like \textit{R. toruloides}, \textit{T. oelaginous}, and \textit{L. starkeyi}. In this study, production cost per unit was estimated to USD 1.08/kg biodiesel (USD 0.94/L biodiesel) that further reduced by simplifying the cultivation process (by avoiding sterilization). Cultivation performed without sterilization reduced cost from USD 1.08/kg biodiesel to USD 0.91/kg biodiesel, that was on par with soybean oil derived biodiesel (0.92 USD/kg biodiesel). Oleaginous yeast mediated process was also found advantageous regarding high amount of sludge reduction (160.16 tonnes)
of residual sludge from every 260 tonnes) and higher biodiesel production [172]. The type of bioreactor also makes huge impact on the capital cost for example, single 250 m³ field-erected fermenter is estimated to around USD 3.6 million (90% of the equipment cost in yeast lipid production) [173,174]. Thus, low cost alternative reactor systems like airlifts and bubble columns has been testing towards yeast derived commodity oil commercialization [109]. Breakeven price for microbial oil could be 27% lower while culturing yeast in open pond system compared to stirred tank reactor [175]. Moreover, to make former process more economical, high productivities have been deemed necessary which accompanied by increased oxygen, agitation, even supply of oxygen rich air, necessitate high aeration hence rises operating and capital cost. With 0.8 g/L/h yeast biomass productivity, electricity cost in fermenters has been calculated 63% of the total utility cost towards biodiesel production [109,173]. In this case co-product evaluation (protein-carbohydrate rich spent biomass for animal feed) or production of multiple metabolites (succinic acid, citric acid) alongside lipid might give the biggest benefits in cost reduction [179]. Cultivation and other process steps require vast amount of water, thus yeast cultivation in seawater might prove as a long-term approach in cost deduction. However, complex organic matter pre-treatment and regular supply with constant composition is additional challenge in employment of such low-cost liquid wastes [71]. Requirement of toxic organic solvents (40 kg hexane per tonne of oil extracted) to extract lipid might be removed by achieving extracellular lipid secretion and by using whole cell biomass as product [109,176].

7. Practical applications and future trends

Despite the intensive research progress, commercial usage and treatment of real industrial wastewater is very scarce till date due to some major hurdles like nitrogen content, toxic impurities, low productivity as well as bioavailability [177]. On this road, inadequate scale-up strategies and research gaps are some areas which should be given more attention. Accompanying with developing robust strains and sustainable biodegradation techniques, future research should concentrate on profound knowledge on metabolic pathways and intermediate product formation so that enzyme of interest allied with biodegradation process could be overexpressed, purified and utilized. Further efforts should be made in optimizing pre-treatments, media formulation (mixing waste streams), cultivation techniques to address bioavailability (studying microbes in mono and co-culture form on varied pollutants), as well as toxicity in a dynamic method for the most optimal microbial valorization. Isolation of new strains from waste sources are equally important because they often possess the required tolerance and by studying tolerance mechanism, altering these traits into well-studied hosts becomes easier. Though, metagenomics has shown great potential in this direction, process inhibition and discrepancy of complex toxicants in real industrial wastewater is a foremost challenge. Genetic engineering to increase toxicity tolerance against eclectic pollutants usually found in industrial wastewater may be a potential area for future further research. Detailed study for understanding the mechanisms of microbes mediated bioremediation could be a step forward. Educating and making aware people about significance of microbial communities in environmental cleaning and bioenergy production as well as field trials for demonstrating the efficiency of bioremediation technology is also important. Further, to establish their full potential and evaluate their performance, microbial treatment processes need to be tested and upscaled using real industrial wastewater [77].

8. Critical analysis and recommendations

In addition to unfolding the toxicity impacts allied with recalcitrant waste streams, the present review concludes that oleaginous yeast stands as a promising candidate for bioremediation. Despite monoculture, yeast microbial community notably found better in effective treatment of wastewater which possesses very few auto-degradable characteristics (dye wastewater). However, most of researches concerning oleaginous yeast consortium has been conducted in synthetic wastewater on a lab scale that might not exemplify actual conditions. Indeed, available yeast metabolic pathways intricated in recalcitrant waste biodegradation shed light on the complex interplay of enzymatic reactions, transforming recalcitrant polymers into neutral lipids. Though, detailed insights into the potential promoting mechanism with respect to metagenomics and metaproteomics is rarely reported. Combined analysis of metagenomic/metaproteomic in microflora aids in identification of crucial genes where selectively-annotated proteins provide valuable information about their significant roles in recalcitrant waste degradation. Further, to provide deep insight into the biodegradation mechanism, key degrading enzymes can be identified with help of molecular techniques [178]. In depth multi-omics technologies further can delves relationships between gene expression, protein synthesis, and metabolite production. This analysis surely illuminates the dynamic processes underlying the biodegradation phenomenon and except these, more excellent benefits can be gained from these technologies, but various bottlenecks and challenges have yet to be resolved [179–182].

- Experiments need to carried out on real wastewater on mass scale in addition to in laboratories and studying the influence of various conditions such as nutrient availability, pH, temperature on the behavior of consortium system;
- Traditional process optimization (single factor, orthogonal experiments, Box-Behnken, Plackett-Burman design) often is black box and time consuming. Utilization of multi-omics technologies for process optimization is still at its nascent stage. Thus, more efforts should be given in this direction;
- For rapid and accurate capture of metabolic bottleneck from sea of data (omics analysis generates enormous amounts of data related to uptake and nutrients utilization, cell growth, product synthesis and efflux) machine learning would be an important direction for future research in process optimization;
- Concerning the engineering of novel consortium systems, additional studies are needed for improvised capabilities to metabolize complex substrates and accumulate lipids and gaining complete understanding of the associations between yeast, which have not been well described;
- Evolving authentic mathematical model that accurately represent the consortium behavior, supportive to determine operational conditions and process design;
- Generation of metagenomic data resources from extreme and relevant environmental niches is extremely necessary for discovery of novel genes encoding enzymes with enhanced degradation efficiency, selected genes should be subjected to bioinformatics and molecular dynamics to improve that further;
- To rebuild yeast metabolic pathway/network with application of various “omics” technologies or synthetic biology methods is still challenging;
- A comprehensive life cycle assessment (LCA) should be perform to evaluate the environmental, economic and social impacts of the entire process. This assessment will guide decision-making and help identify potential area for improvement;
- A closed-loop green production system can be built to ensure sustainable and eco-friendly operations.

9. Conclusion

Growing public concern towards toxicants removal has introduced bioremediation as an economically feasible and innovative technology. The ongoing pursuit for novel bioresources has been progressing for successful implementation of this technique. Thus, the present review critically analyze and advocates the significance of recalcitrant waste
streams as sustainable nutrient resources for oleaginous yeast and its further applicability for biodiesel production. The current review outcomes also recommend that oleaginous yeast-based systems are very promising tools for complex, interfering pollutant degradation in real industrial wastewater scenarios. Despite such a significant contribution in circular economy, underutilized and nonconventional waste streams valorisation have not reached its full potential and requires further research for commercialization of process in near future.

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.

Data availability

No data was used for the research described in the article.

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References

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