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# Integration of agro wastes as an alternative substrate for microalgae cultivation: A review

P. Scarponi [*], L. Forti

*Department of Life Sciences, University of Modena and Reggio Emilia, Via G. Campi 103, Modena 41125, Italy*

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ABSTRACT

The identification of alternative substrates is essential to reduce cultivation complexity and production costs in biotechnological processes. In the context of bioenergy applications, microalgae have emerged as promising feedstocks; however, the biochemical composition of the biomass is strongly influenced by the cultivation substrate. Consequently, ongoing research efforts focus on optimizing cultivation strategies to improve both biomass yield and product quality. A notable knowledge gap remains regarding the use of agro-waste as an alternative substrate for microalgae cultivation. Within a circular economy framework, agro-waste represents a valuable resource for enhancing biomass productivity and sustainability. Nevertheless, its practical application is challenged by seasonal availability, variability in agricultural practices and processing methods, and the influence of abiotic and biotic factors that can alter chemical composition and limit substrate consistency. This review examines the generation, availability, and compositional characteristics of major agro-wastes streams and evaluates their application in biotechnological processes aimed at bioenergy production. Particular emphasis is placed on microalgae cultivation—either as a standalone process or integrated with other biological treatments—using agro-waste-derived substrates. Reported data indicate that substrates such as vegetable peels, wine lees digestate, and piggery manure digestate can significantly enhance microalgal biomass production, increasing yields from 0.96 g/L up to 3.16 g/L, while also promoting lipid accumulation exceeding 20%. The strong influence of substrate composition on microalgae lipid profiles is highlighted, particularly the reduction of linolenic acid content. The potential integration of agro-waste-derived microalgal biomass into biorefinery platforms, including biodiesel production compliant with the European standard EN14214, is discussed.

## 1. Introduction

### *1.1. Agro-waste production*

The global population is projected to reach 9 billion by 2050 and approximately 11 billion by 2100. Consequently, the demand for food—particularly crops, meat, and their derivatives—is expected to increase significantly. Waste generated during food production is defined “agro-wastes” and arise from various activities, including crop cultivation, horticulture, dairy farming, market gardening, livestock breeding, and forestry production [1–3]. This population-driven growth has a cascading effect, resulting in a substantial increase in agro-industrial waste generation [3]. Conventional disposal practice for these wastes causes significant environmental impacts, such as water pollution, soil degradation and the emission of greenhouse gases [4]. For instance, the open burning of rice residues in India contributes to severe air pollution, while simultaneously releasing large quantities of greenhouse gases into the atmosphere [2].

Traditionally, agro-waste has been disposed of in landfills or through improper dumping, leading to increased concentrations of bacterial and fungal pathogens and the release of toxic compounds into the environment [5]. Despite these challenges, agro-waste contains a wide range of organic and inorganic compounds that can be valorised to produce biomass, energy, and biofuels (Fig. 1) [5–7]. Therefore, integrating agro-wastes into a circular economy framework represents a promising strategy for sustainable biomass production and the generation of high-value by-products. This approach aligns with several United Nations Sustainable Development Goals (SDGs), including “Affordable and clean Energy”, “Responsible consumption and production” and “Life on land” by supporting climate change mitigation and fostering the development of sustainable industries [8].

An example of national agro-waste production was reported by Cioffi

* Corresponding author.
*E-mail address:* p.scarponi@unimore.it (P. Scarponi).

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et al. [9]. Italy's agro-industrial sector is characterized by diverse, high-quality, and locally-rooted production systems that are difficult to replicate elsewhere. The Italian agricultural landscape extends across the entire country and is predominantly composed of small, family-owned farms operating within highly seasonal production cycles. Annually, Italy generates approximately 1.4 million tons of field residues, accounting for about 2.8% of total agro-waste production. In the Italian agro-industrial sector - as in other countries - the definition of "waste" is particularly important due to the wide variety of residues generated during crop cultivation, harvesting, and processing.

### 1.2. Agro-waste integration in the circular economy approach: microalgae cultivation

Agricultural activities generally produce four main categories of waste: (i) crop residues (e.g., seed pods, leaf litter, husks, weeds, stems, and straws); (ii) agro-industrial wastes (e.g., molasses, peels, pulps, bagasse, and oils-seed cakes); (iii) livestock waste (e.g., dung, urine, bedding materials, and droppings); and (iv) fruit and vegetable wastes [3,10]. Each category is characterized by a distinct chemical composition, providing opportunities for integration into circular economy framework through physicochemical and/or biotechnological processes (Fig. 2).

Several studies have focused on the integration of agro-waste into anaerobic digestion systems to enhance the biogas production, particularly through agro-waste co-digestion strategies [11–13]. In parallel, recent research has highlighted the potential of agro-waste valorization via fermentation processes employing various microorganisms, such as bacteria, microalgae, and yeast to increase the yield of value-added by-products within circular economy applications [14,15].

Among these approaches, microalgae cultivation has been extensively investigated for the phytoremediation of wastewater [7,16] and for the treatment of anaerobic digestion effluents [17,18]. However, despite its promising potential for microalgae biomass production and biodiesel generation [6,19], the direct application of microalgae for agro-waste treatment remains relatively underexplored.

### 1.3. Review purpose and methodological transparency

On these bases, the aim of this review is to provide an overview of the chemical characterization of agro-wastes and to assess their potential application as alternative substrates for microalgae cultivation. The use of novel and alternative substrates in microalgae cultivation may significantly reduce production cost, enhance the phytoremediation of agro-wastes, and stimulate macromolecular accumulation or biomass production suitable for biorefinery applications. In particular, in the context of third-generation biofuel production, this review evaluates the influence of agro-waste substrates on microalgal lipid accumulation and fatty acid composition.

This review also identifies existing knowledge gaps and research limitations related to the application of specific agro-wastes generated in different countries, thereby encouraging the development of alternative agro-waste treatment strategies and the broader adoption of circular economy approaches.

The integrative review was conducted using two major scientific databases: Scopus® (Elsevier Properties S.A., USA) and ScienceDirect® (Elsevier Properties S.A., USA). The analysis focused primarily on peer-reviewed research articles published within the last decade (2015–2025), which accounted for more than 95% of the selected literature. The search strategy employed targeted keywords, including 'microalgae,' the names of specific microalgal strains, agro-waste,' and the names of individual agro-waste types.

A systematic screening process was applied to ensure the inclusion of studies reporting both the chemical characterization of agro-wastes and corresponding outcomes related to microalgal biomass production and composition. Only studies involving the direct and consistent application of agro-waste substrates in microalgae cultivation were considered. Articles lacking sufficient methodological detail or quantitative results

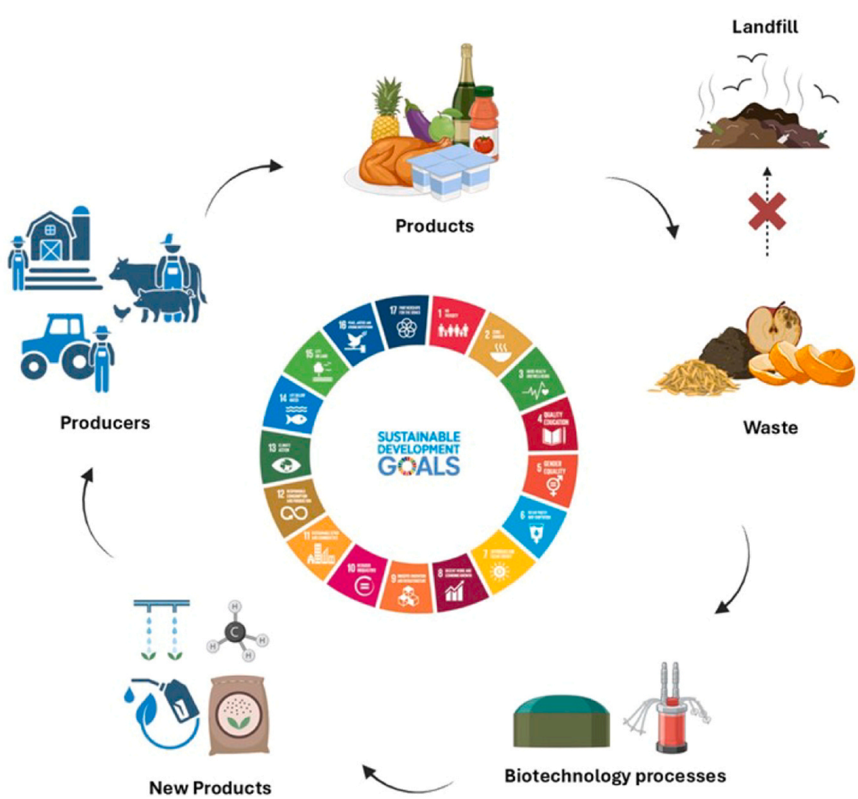


**Fig. 1.** Schematic circular economy approach in the of agro-waste integration using biotechnology approaches.

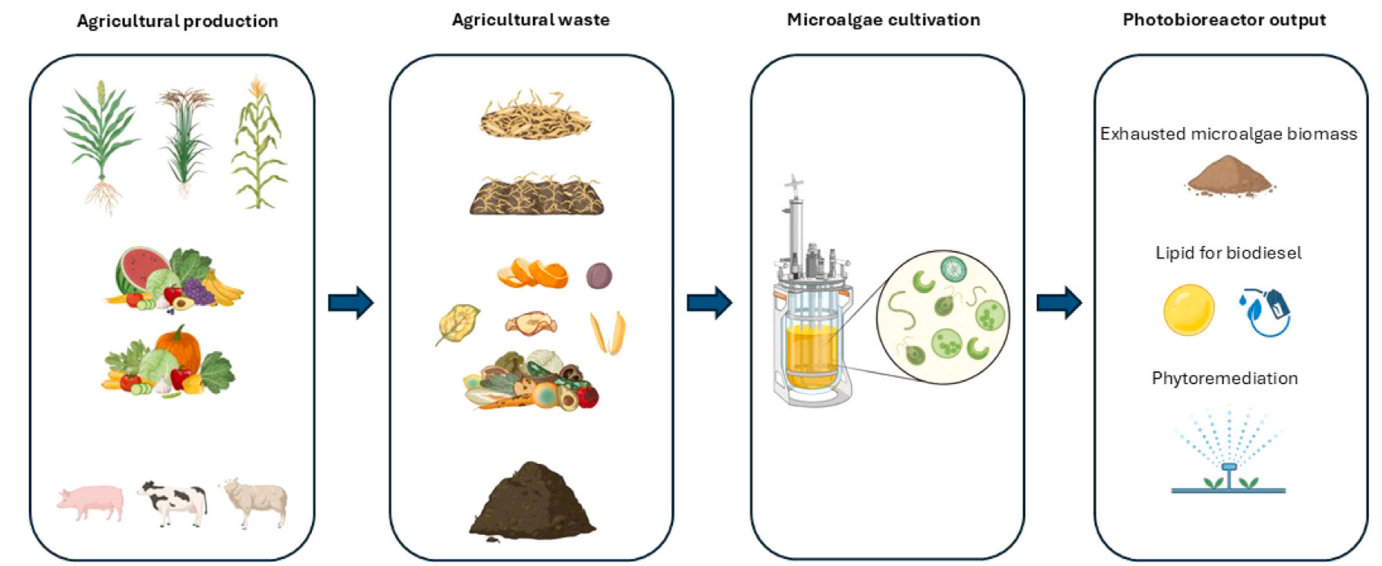


**Fig. 2.** Agro-waste integration as alternative substrate for microalgae cultivation.

relevant to the objectives of this review were excluded.

Overall, this integrative review aims to synthesize the current state-of-the-art on the use of agro-industrial residues as alternative substrates for microalgae cultivation, highlighting their potential within circular economy and biorefinery frameworks and their alignment with the United Nations Sustainable Development Goals.

## 2. Crop residues

Crop residues play a significant role in enhancing soil fertility and can be utilized as raw materials for biochar production. This application improves soil carbon and nitrogen content, as well as water retention capacity, thereby benefiting crop cultivation [20,21]. According to Seo et al. [22], crop residues represent promising feedstock for the production of biochar and a wide range of biofuels, including syngas, biogas, hydrogen, furan-based fuels, bioethanol, bio-oil, and biodiesel. Biochar derived from crop residues can be applied in agriculture as a substitute for coal and as a multifunctional component in biofuel production, serving as a pH-buffering agent, support matrix, adsorbent, and catalyst.

However, the chemical composition of crop residues strongly influences their suitability for specific applications. For instance, residue composition can affect soil pH- typically ranging from 5 to 6.5 - as well as carbon, nitrogen, and phosphorous contents [23]. The chemical specificity and structural characteristics of different plant types directly impact biochar yield and quality. Seo et al. [22] reported that acid washing of rice straw biochar reduced its yield from 37% to 33%. Similarly, hydrothermal carbonization was negatively affected by the formation of carbonaceous microspheres, which clogged biochar pores and required additional downstream processing. Comparative analysis of biochars showed differences in nutrient release efficiency, with rice straw outperforming millet straw, sorghum stalk, and corn stalks. Interestingly, despite higher carbon, nitrogen, and phosphorous contents (Table 1), corn stalk-derived biochar did not exhibit a strong fertilization effect [23].

Crop residues can also be utilized in anaerobic co-digestion processes with animal waste to produce biomethane, thereby overcoming limitations associated with dry digesters, such as low methane yields and system instability. However, pretreatment is often required to enhance the biodegradability of lignocellulosic materials. Biotechnological pretreatment strategies include enzymatic treatments and pre-fermentation using fungi or microbial consortia to degrade hemicellulose, cellulose, and lignin [26,28].

Anaerobic digestion only partially converts agro-waste into biogas, leaving residual biomass and metabolic intermediates in the resulting digestate. Due to its chemical composition, digestate requires further treatment, as high levels of soluble chemical oxygen demand, ammonia, and potentially hazardous substances (e.g. heavy metals) limit its environmental release. Nonetheless, digestate can be employed as an alternative substrate for microalgae cultivation, promoting biomass production while enabling phytoremediation (Fig. 3) [17,29].

Co-digestion of crop residues with animal manure enhances methane yield by balancing substrate composition. However, this process is sensitive to of protein and lipid contents, which can affect hydrolysis

**Table 1**
Chemical composition and production of crop residues.

| | C (%) | N (%) | P (%) | Hemicellulose (%) | Cellulose (%) | Lignin | Total residues per year | Ref. |
|---|---|---|---|---|---|---|---|---|
| Rice straw | 40.13 – 40.74 | 0.45 – 0.79 | 0.31 – 1.97 | 21.64 | 31.12 | 22.34 | 209.2 (Kt) | [23–26] |
| Millet straw | 35.27 | 0.34 | 0.3 | n.d. | n.d. | n.d. | 8491–8550 (t) | [23,26] |
| Sorghum stalks | 33.11 | 0.27 | 0.42 | 19.4 | 35.4 | 10.3 | n.d. | [23,25] |
| Corn stalks | 43.8–47.6 | 0.55 – 1.00 | 0.58 – 0.95 | n.d. | n.d. | n.d. | 3144.1–10107 (Kt) | [23,24, 26] |
| Wheat straw | 42.1 | 0.60 | 0.45 | 18 | 36 | 16 | 0.7 – 22,218 (Kt) | [24–26] |
| Cotton stalk | 45.83 | 1.12 | 1.40 | n.d. | n.d. | n.d. | n.d. | [24] |
| Rape stalk | 42.93–45.3 | 0.5–0.77 | 0.78 | n.d. | n.d. | n.d. | n.d. | [24,27] |
| Miscanthus | 46.2 | 0.5 | n.d. | n.d. | n.d. | n.d. | n.d. | [27] |
| Pea, Alfaalfa, potato, red clover, mustard, sugar beet | 38.9–46.4 | 1.0–2.6 | n.d. | n.d. | n.d. | n.d. | n.d. | |

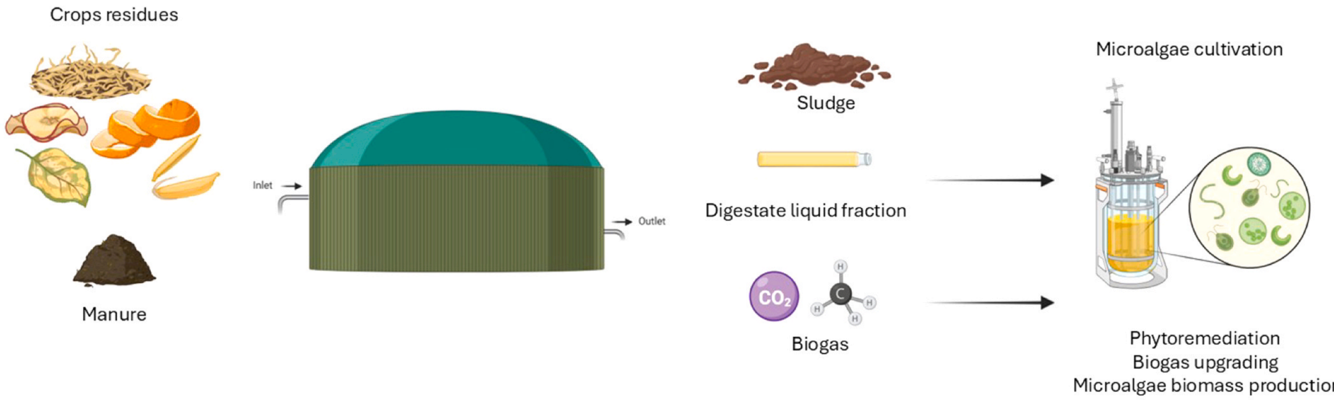


**Fig. 3.** Coupled biological treatment of crop residues and manure through anaerobic digestion and microalgae cultivation.

rates or induce inhibitory effects under certain conditions [30].

## 3. Agro-industrial wastes

Agro-industrial wastes commonly include molasses, peels, pulps, bagasse, and oilseed cakes. Among these, molasses is a major byproduct of sugarcane and sugar beet processing and is widely employed in biotechnological applications as a cost-effective and highly fermentable carbon source (Table 2) [31]. Although molasses is typically associated with sugarcane and sugar beet industries, it can also be derived from other plant materials. For instance, pomegranate molasses - a traditional Middle Eastern syrup - is produced by concentrating pomegranate juice through boiling [32].

The high sugar content of molasses make it an economical substrate for fermentation processes. However, untreated waste molasses is often discarded, raiseng serious environmental concerns due to its acidic pH, high chemical and biochemical oxygen demand, and dark coloration [33]. Despite these drawbacks, molasses has demonstrated considerable potential in the production of bioethanol and amino acids using yeast. Based fermentation systems, nevertheless, the presence of inhibitory compounds generally necessitates substantial dilution prior to its use [33].

As substrate for microalgae cultivation, molasses represents an affordable carbon source, with sugars accounting for approximately 48% of its composition. In addition, molasses contains nitrogenous compounds, vitamins, and trace elements that can support microalgal biomass growth and the accumulation of high-value secondary metabolites [33,39]. Microalgal responses to sugars such as glucose, fructose, sucrose, and xylose are strain-dependent (Table 3). Certain carbon sources can enhance the intracellular acetyl-CoA and malonyl-CoA pools, thereby promoting lipid accumulation [40]. However, excessive sugar supplementation may induce “glucose bleaching”, characterized by a reduction in chlorophyll content and impaired photosynthetic activity [41].

At metabolic level, glucose and fructose are converted into glyceraldehydes-3-phosphate, a key intermediate in both the pentose phosphate pathway and the Embden-Meyerhof-Parnas (EMP) pathway. In contrast, disaccharides such as sucrose and lactose are more difficult for many microalgal species to metabolize. Since sucrose is the dominant sugar in molasses, hydrolysis is often required as a pretreatment step to improve its bioavailability for microalgal fermentation. Nonetheless, some microalgal strains, such as *Coalastrella* sp., are capable of directly utilizing disaccharides. Consequently, current research increasingly focuses on identifying such strains or developing cost-effective pretreatment strategies to improve molasses integration into fermentation processes [42].

In addition to liquid byproducts, agro-industrial wastes include a substantial fraction of solid residues, such as de-oiled cakes generated from sesame, cottonseed, peanut, and mustard processing. These residues are commonly used in animal feed; however, their high nitrogen, ammonia, and phosphorus contents have prompted interest in their application as substrates for microalgae cultivation.

Reports on the use of hydrolyzed solid agro-industrial residues remain limited. Sadukha et al. [47] reported that *Chlorella variabilis* exhibited growth inhibition when cultivated on mustard or sesame hydrolysates (0.30 g/L and 0.48 g/L, respectively), whereas cottonseed and groundnut hydrolysates significantly enhanced biomass production and lipid accumulation (1.23 g/L, 1.01 g/L; 18% and 12%, respectively).

These findings highlight the strain-specific responses of microalgae to different agro-industrial substrates and underscore the potential of agro-waste valorization to enhance biomass productivity and biochemical composition. Further studies are required to optimize species-specific cultivation strategies using selected substrates. Such approaches may also improve agricultural wastewater management and enable small-scale producers to convert waste streams into valuable resources, thereby supporting circular economy processes at the local scale.

## 4. Livestock manure

According to the agricultural industry standard NYT 1168–2006, livestock manure consists of a mixture of poultry and livestock feces, bedding materials, urine, flushing water, and rainwater [48]. The

**Table 2**
Characterization of molasses.

| | Cane molasses | Beet molasses | Grape molasses | Apple molasses | Pomegranate molasses | Cherry molasses |
|---|---|---|---|---|---|---|
| Dry matter (%) | 76.8 ± 1.0 | 77.6 ± 3.2 | 77.99 ± 0.66 | n.d. | 68.50 – 74.50 | 72.60 – 72.79 |
| Crude Protein (%) | 6.65 ± 1.79 | 13.5 ± 1.4 | n.d. | 3.05 – 4.01 | 0.95 | 42.87 – 50.83 |
| Total sugar (%) | 62.3 ± 4.7 | 62.1 ± 3.9 | 60.70 ± 0.14 | 64.04 – 76.53 | 16.57 | 55–80 |
| Sucrose (%) | 48.8 ± 6.4 | 60.9 ± 4.4 | n.d. | n.d. | n.d. | n.d. |
| Glucose (%) | 5.29 ± 2.69 | 0.28 ± 0.48 | 29.73 ± 0.28 | n.d. | 64.80 | n.d. |
| Fructose (%) | 8.07 ± 2.83 | 0.29 ± 0.30 | 30.96 ± 0.15 | n.d. | 71.50 | n.d. |
| | [34] | | [35] | [36] | [37] | [38] |

**Table 3**
Microalgae cultivation on different sugars as substrates under mixotrophic conditions.

| Microalgae strain | Sugar | Concentration | Experimental set up | Inhibition | Dry weight (g/L) | Lipid (%) | Reference |
|---|---|---|---|---|---|---|---|
| *Chlorella* sp. Y8–1 | Fructose | 1 g/L | Volume: 0.8 L | No | 0.30 | 5 | [40] |
| | Glucose | | Batch test: 12 days | No | 0.40 | 12 | |
| | Sucrose | | | No | 0.45 | 24 | |
| | Xylose | | | Yes | 0.20 | 7 | |
| *Chlorella* PCH05<br>*Chlorella* LB1H12<br>*Chlorella* LB1H10 | Xylose | 20 mM | Volume: 12 well microtiter plates containing 3.5 mL<br>Batch test: 17 days | No | 0.2 – 0.45 | 5–10 | [43] |
| *Coelastrella* sp. KKU-P1 | Glucose | 5 g/L (28 mM) | Volume: 250 mL | No | 2 – 3 | n.d. | [42] |
| | Fructose | 5 g/L (28 mM) | Batch test: 14 days | No | 3 – 4 | | |
| | Sucrose | 5 g/L (15 mM) | | No | 1–2 | | |
| | Lactose | 5 g/L (15 mM) | | Yes | > 1 | | |
| | Arabinose | 5 g/L (33 mM) | | Yes | > 1 | | |
| | Xylose | 5 g/L (33 mM) | | Yes | > 1 | | |
| | Real molasses | Total sugar: 60.43%<br>$CO_2$: 0.04% | | No | 4.28 | 15 | |
| *Desmodesmus* sp. | Xylose | 3 g/L | Volume: 1 L | No | 1.17 | 10.5 | [44] |
| | Hemicellulose hydrolysate | Diluted to had xylose content of 3 g/L | Batch test: 6 days | No | 1.28 | 10.1 | |
| Freshwater *Chlorella* sp. | Glucose | 2 g/L | Volume: 500 mL<br>Batch test: 7 days | No | 1.5 | 12–18 | [45] |
| Marine *Chlorella* sp. | | | | | 1.5 | 22–26 | |
| *Nannochloropsis* sp. | | | | | 1.2 | 25–30 | |
| *Chetoceros* sp. | | | | | 0.4 | 20–25 | |
| Marine *Chlorella* sp. | | | Volume: 500 mL | No | 3.76–4.48 | 15.6–25.4 | |
| *Nannochloropsis* sp. | | | Fed-batch (10 days), glucose fed every 2 days, glucose concentration maintained at 2 g/L | | 3.64–5.87 | 19.3–25.3 | |
| *Chlorella kessleri* | Glucose | 2 g/L | Volume: 200 mL | No | 1.0 | 25 | [46] |
| | | 4 g/L | Batch test: | | 1.7 | 28 | |
| | | 6 g/L | | | 2.5 | 32.7 | |
| | | 8 g/L | | | 3.0 | 32.0 | |
| | | 10 g/L | | | 3.6 | | |
| | | 12 g/L | | | 4.0 | | |
| | | 15 g/L | | | 4.4 | | |

chemical composition of animal manure highlights its strong potential for integration into circular economy models, particularly for energy and resource recovery. The increasing scale of animal husbandry - especially in goat, sheep, cattle, swine, and poultry farming - has led to a substantial rise in manure generation worldwide [49]. However, manure production and composition variy significantly depending on geographic region, livestock species, feed composition, and management practices (Table 4).

Biotechnological applications of livestock manure include its conversion into organic fertilizers through microbial fermentation. Fungi and yeast have been employed to treat manure over periods of 7–12 days, producing high-quality organic fertilizers suitable for fields application at rates of approximately 4 t/ha [57]. In addition, livestock manure is widely used as a feedstock for composting, vermicomposting, and emerging microalgae-based recovery systems [58].

Livestock manure is also a well-established substrate for anaerobic digestion. Haque et al. [59] reported biogas yields of 0.250 $m^3/kg_{VS}$, 0.301 $m^3/kg_{VS}$, and 0.222 $m^3/kg_{VS}$, from cattle, goat, and poultry manure, respectively, with corresponding methane yields of 0.163 $m^3/kg_{VS}$, 0.195 $m^3/kg_{VS}$ and 0.144 $m^3/kg_{VS}$. Variations in biogas production are influenced by factors such as bedding materials, animal diet, and growth stage. Moreover, higher fiber and nitrogen contents may inhibit anaerobic digestion, making manure particularly suitable for co-digestion with substrates characterized by low nitrogen concentrations [60,61].

Fiber-rich livestock manure also shows potential as a feedstock for bioethanol production following acid hydrolysis. Lung et al. [62] demonstrated that acid hydrolysis sugar-rich substrates containing xylose, arabinose, glucose, and galactose, suitable for fermentation. Specifically, acid hydrolysis of dairy manure, feedlot cattle manure, cow manure, and horse manure generated substrates containing 53–114% pentoses relative to hemicellulose, up to 79% sugars per hemicellulose

**Table 4**
Livestock manure production and characteristics in different countries.

| Geographical area | Waste type | Production | Characteristics | Reference |
|---|---|---|---|---|
| Australia | Cattle In feedlots | 27 kg/day | Moisture content 20–78% | [50] |
| | Sheep | 5–12 kg/t | | |
| | Pig | 108 kg/year | | |
| Bangladesh | Buffaloes and cattle | 17 kg/head/day | Total solid 30% | [51] |
| | Sheep and goats | 0.8 kg/head/day | Total solid 25% | |
| | Poultry | 0.045 kg/head/day | Total solid 29% | |
| China | Pigs | $1.02 \times 10^{12}$ kg | | [52] |
| Korea<br>USA<br>Japan | Cattle Manure | 53 kg/head/day | sCOD (g/kg) 22.5<br>TS (g/kg) 200–310<br>VS (g/kg) 150–235.3<br>TN (g/kg) 9.1 | [53–56] |
| | Pig Manure | 4.5 kg/head/day | sCOD (g/kg) 39.4<br>TS (g/kg) 50–70.6<br>VS (g/kg) 45–56.5<br>TN (g/kg) 6.1 | |
| | Chicken Manure | 0.3 kg/head/day | sCOD (g/kg) 32–97<br>TS /g/kg) 132–171<br>VS (g/kg) 55–75<br>TN (g/kg) 4 | |

Note: sCOD: soluble chemical oxygen demand; TS: total solid; VS: volatile solid; TN: total nitrogen.

fraction, 15.9–25.4 g/L glucose, 8.6–9.5 g/L xylose, and overall sugar contents up to 78.7%. Despite these potential, these alternative substrates remain underexplored for bioethanol fermentation. Available studies by Lee et al. [63], Vancov et al. [64], You et al. [65], and Yan et al. [66] reported bioethanol concentration ranging from 7.3 to 18.9 $g_{ethanol}$/L, 10.6 $g_{ethanol}$/L, and 9 $g_{ethanol}$/L using *Saccharomyces cerevisiae*, *Zymomonas mobilis* ZMT2, and *Pichia stipites*, respectively.

More recently, biological treatment strategies have investigated livestock manure as a substrate for microalgae cultivation. Livestock manure and urine (LUM) are rich in ammonia, phosphorous, and organic compounds which can stimulate microalgae growth. In South Korea, LUM generation has been estimated at approximately 176,000 $m^3_{LUM}$ per day [67]. Conventional disposal methods, such as direct land application, raise environmental concerns due to ammonia volatilization, nitrogen leaching, and risk of over-fertilization.

Microalgae-based LUM treatment systems can be implemented under two main configurations: (i) direct microalgae cultivation on raw LUM, or (ii) integration of microalgae cultivation within existing LUM treatment facilities, coupled with biological treatment processes. Biomass produced from LUM-based microalgae cultivation can be valorized for biofuel production, including biodiesel and bioethanol. However, due to the potential accumulation of contaminants such as heavy metals, xenobiotics, and pathogenic microorganism (e.g., parasites, viruses, fungi, and bacteria), this biomass is unsuitable for nutraceutical or animal feed applications.

From an economic perspective, the use of LUM as a growth medium significantly reduces microalgae biomass production costs. Lee et al. [67] reported cost reductions from 14.6 to 13.8 USD/kg using conventional photobioreactors and from 5.4 to 5.1 USD/kg using submerged membrane photobioreactors, compared with cultivation in synthetic BG-11 medium. Nevertheless, studies on the direct cultivation of microalgae on untreated LUM remain limited [68,69]. An example of the direct utilization was reported by Noor et al. [70], who cultivated *Chlorella vulgaris* under mixotrophic conditions using poultry waste, sewage sludge, and livestock manure. Compared with phototrophic controls (87.5 mg/L biomass and 10.58% lipid content), treated cultures showed increased biomass production (128.57 mg/L, 136.7 mg/L and 107.32 mg/L, respectively) and lipid accumulation (18.51%, 25.5% and 10.32%, respectively). Most studies, however, focus on microalgae cultivation using digestate obtained after anaerobic digestion. Digestate, enriched in ammonia, phosphorus and soluble chemical oxygen demand (sCOD), provides an optimal growth medium for microalgae. Several authors, including Chang et al. [71], Cicci et al. [7], and López-Sánchez et al. [72], have reported effective digestate phytodepuration accompanied by enhanced biomass production and the generation of high-value secondary products. Consequently, coupled biological system integrating anaerobic digestion and microalgae cultivation represent a promising strategy to improve biomass productivity, reduce treatment costs, and strengthen circular economy implementation within existing waste management infrastructures [73].

## 5. Fruit and vegetable wastes

The Food and Agriculture Organization of the United Nations (FAO) estimated that approximately 14% of food produced globally was lost at the post-harvest stage in 2019 [74]. This issue has significant economic and environmental implications, resulting in income losses for farmers, increased costs for consumers, and adverse environmental impacts, including elevated greenhouse gas emissions and inefficient use of land and water resources. Food waste represents a substantial reservoir of nutrients and bioactive compounds that can be recovered and valorized, transforming waste streams into alternative raw materials and supporting circular economy framework [73].

Globally, about 1.3 billion tons of food are lost annually during production and processing. In Europe, nearly 50% of total food waste consists of fruit and vegetable residues [75], primarily derived from roots and tubers, which account for approximately 45–50% of this fraction [74]. Food waste generation is strongly influenced by regional production systems and consumption patterns. On a global scale, the annual distribution of food waste comprises cereals (19%), roots and tubers (20%), fruits and vegetables (44%), and oilseeds and pulses (3%) [73]. Food processing activities further contribute to fruit and vegetable waste, which mainly includes peels, cores, seeds, stalks, and pulp, accounting for approximately 30% of the total processed biomasses [76].

Fruit and vegetable wastes can be integrated into circular economy models as alternative raw materials, in line with the United Nations "Sustainable Development 2030" agenda [8,73]. These residues can be reused in animal feed, nutritional products, and biorefinery processes through technologies such as anaerobic digestion, fermentation, incineration, pyrolysis, and gasification. In particular, their integration into biotechnological processes has enabled the production of value-added products, including biogas [17], bioethanol [77], biodiesel [78], exopolysaccharides [19], and polyhydroxyalkanoates [79].

In addition to energy and bioproduct generation, fruit and vegetable wastes can be pre-treated to recover high-value bioactive compounds, such as phenolic acids, anthocyanins, carotenoids, flavonoids, and betalains. These compounds exhibit antioxidant, antimicrobial, anti-browning, adsorbent, and indicator properties [80]. Their recovery increasingly relies on innovative green extraction technologies. Studies by Rodríguez García et al. [81] and 'Aqilah et al. [80] demonstrated that techniques such as supercritical fluid extraction, pressurized liquid extraction, pulsed electric field treatment, and ultrasound-assisted extraction provide higher extraction yields, reduced processing times, lower solvent consumption, and improved overall efficiency. These advantages facilitate the incorporation of recovered extracts into pharmaceutical, cosmetic, and food industry applications. Nevertheless, such extraction processes generate residual biomass that requires further treatment to achieve complete circularity.

Food and vegetables wastes from agricultural production and processing industries typically account for 20–40% of the total original biomass and contain organic compounds suitable for fermentation to produce high-value secondary products. Acidogenic fermentation has been explored for lactic acid production using *Bifidobacterium* [82], while high biological-value proteins have been produced using *Penicillum, Bacillus subtilis, Rhizopus oligoporus*, or *Fusarium flocciferum*. Additionally, carbohydrates and glycosidases have been obtained using *Aspergillus*, *Trichoderma*, and *Yarrowia* species [83]. Other studies have focused on β-carotene production using *Blakeslea trispora* [84] and bioethanol production using *Saccharomyces cerevisiae* [85,86]. Typically, yeasts, fungi, and bacteria are employed to ferment fruit and vegetable wastes under aerobic or anaerobic conditions.

In recent decades, increasing attention has been given to the use of fruit and vegetable wastes as substrates for microalgae cultivation (Fig. 4, Table 5), particularly for the production of lipids, pigments, and proteins.

Most studies have employed mixed fruit and vegetable waste streams. Experimental evidence highlights strain-specific microalgal responses to different substrates, largely driven by variations in substrate chemical composition, which directly influence biomass growth and biochemical profiles. For example, unbalanced media characterized by high sugar content and nitrogen depletion may inhibit microalgal growth. Scarponi et al. [19] demonstrated such strain-dependent response using the liquid fraction of watermelon waste: *Spirulina* and *Chlamydomonas* strains were inhibited, whereas *Chlorella* and *Scenedesmus* strains exhibited enhanced biomass production compared with control cultures. Substrate composition also affected lipid accumulation, fatty acid profiles, and macromolecular storage patterns.

These findings underscore the importance of systematically screening both microalgal strains and waste-derived substrates to identify optimal combinations. Such evaluations are essential for maximizing biomass productivity and tailoring biochemical composition, thereby strengthening the integration of fruit and vegetable waste

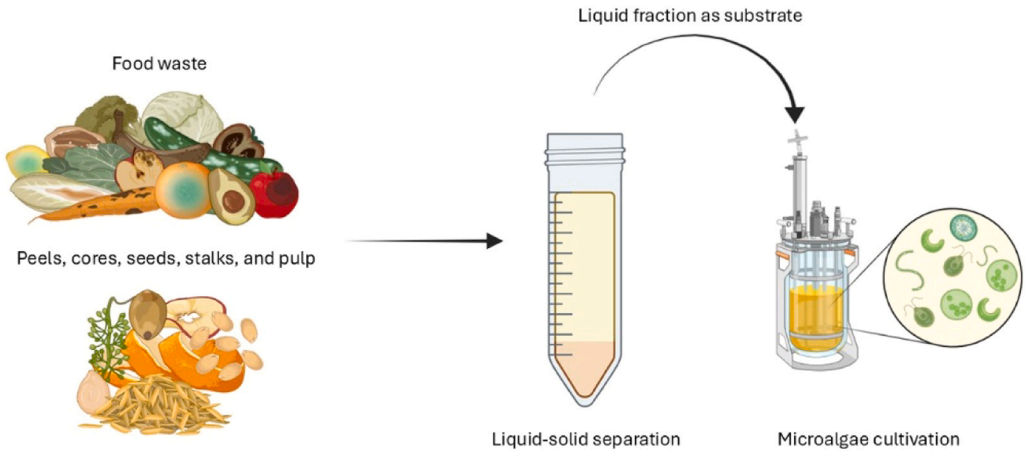


**Fig. 4.** Fruit and vegetable liquid fraction waste as an alternative substrate for microalgae cultivation.

valorization within circular economy and biorefinery frameworks.

## 6. Biofuel production

The application of microalgae in waste treatment is primarily associated with third-generation biofuel production. Techno-economic analyses have shown that the production of 1 kg of lipids from *Chlorella vulgaris* biomass costs approximately 480 USD under phototrophic cultivation, compared with only 7.5 USD under heterotrophic conditions. This substantial cost disparity is mainly attributable to the slower growth rates observed under phototrophic conditions, in contrast to the rapid biomass accumulation achieved when organic carbon sources are supplied. However, for microalgae-derived biodiesel to be commercially viable, a market price of approximately 2 USD/kg is required, with a target production cost of around 1 USD/kg. At present, the production cost of microalgal biodiesel remains relatively high, ranging between 4 and 5 USD/kg [18,94]. Consequently, the identification of alternative substrates capable of enhancing biomass productivity and lipid accumulation is critical to reducing overall production costs. In addition, to ensure suitability for biodiesel application, the final product must comply with the European standard EN 14214 (Table 6) [95]. One of the major challenges associated with microalgae-derived biodiesel is the high linolenic acid content ($> 12\%$) commonly observed in microalgae lipid profiles, which exceeds the permissible limit for biodiesel feedstocks. As a results, pretreatment or process optimization steps are often required to reduce linolenic acid levels prior transesterification [17].

Total lipid content and fatty acid composition play a pivotal role in determining the sustainability, fuel quality, downstream processing requirements, and marked value of microalgae-based biodiesel. Accordingly, lipid quantification, yield optimization, and compositional analysis are areas of intensive research. Biodiesel production is favored by a high proportion of saturated and monounsaturated fatty acids, which enhance oxidative stability and cold-flow properties [96]. As summarized in Table 7, comparison of lipid content and fatty acid composition across microalgal strains are challenging due to the heterogeneous nature of lipid profiles, which are strongly influenced by substrate type, strain selection, and cultivation conditions.

The substrate composition has a decisive impact on lipid biosynthesis pathways in microalgae, although the underlying metabolic mechanisms are not yet fully understood. In general, microalgal lipid synthesis proceed via two main pathways: (i) acetyl-CoA carboxylase activity, which catalyzes the conversion of acetyl-CoA to malonyl-CoA, and (ii) fatty acid synthase activity, responsible for fatty acid chain elongation [97]. Ma et al. [96] reported that hexose - the most abundant naturally occurring carbohydrates - stimulate both biomass growth and lipid accumulation through the pentose phosphate pathway, the Embden-Meyerhof-Parnas pathway, and the Entner-Doudoroff pathway. In addition, simple organic carbon sources such as glycerol and acetate are readily assimilated and converted into glycerol-3-hosphate and acetyl-CoA, respectively. In contrast, complex substrates such as cellulose, starch, and sucrose require enzymatic hydrolysis prior to they assimilation by microalgal cells. The supplementation of organic carbon sources generally promotes lipid accumulation, although the magnitude of this effect is highly strain-dependent and often modulated by stress conditions, including nutrient limitation [98].

Based on the EN14214 specifications and the lipid compositions reported in Table 7, the use of digestates or raw agro-wastes induced distinct, strain-specific metabolic responses in microalgae. In several cases, lipid yields and fatty acid profiles were favorable for biodiesel production. In particular, substrates such as sweet sorghum bagasse hydrolysate, carrot pomace, dairy wastewater, and watermelon liquid fraction waste promoted lipid profiles compatible with EN14214 requirements. Conversely, digestate-based cultivation sometimes resulted in linolenic acid concentration exceeding the EN14214 threshold of 12%.

These findings reinforce the strain-specific responses previously reported by Tan et al. [101] and Scarponi et al. [19]. To optimize biodiesel production from waste-derived microalgal biomass, comprehensive screening of both microalgal strains and waste substrates is essential. Such screening should aim to maximize lipid accumulation while tailoring fatty acid composition to meet EN14214 standards, thereby enhancing the sustainability and commercial feasibility of microalgae-based biodiesel.

## 7. Economic sustainability and future perspectives

As reported by Mata et al. [107], the microalgal biomass productivity-rather than lipid content alone- is the primary determinant of the economic sustainability of microalgae-based treatment processes. Maximizing areal productivity enable full utilization process equipment, thereby justifying capital investments and reducing the unit cost of the final product cost. Assuming a stable lipid content of 30% and maximized biomass productivity, Mata et al. [107] estimated selling price of approximately 1 EUR/kg for dry microalgal biomass and 2 EUR/L for microalgal oil. These values were directly linked toincreased areal productivity, which reduced the land area required for cultivation and enhanced overall process profitability.

Freshwater and nutrient inputs, including $CO_2$ and fertilizers, significantly affect biomass production cost. Biomass cultivated using freshwater and synthetic nutrients resulted in a production cost of 5.57 EUR/kg, whereas partial substitution with wastewater (5%) reduced the cost to 4.45 EUR/kg. However, increasing the wastewater fraction to 10%-20%) led to a decline in biomass productivity from 20 $g/m^2$/day to 9.19–12.97 $g/m^2$/day, resulting in higher production costs ranging

**Table 5**
Fruit and vegetable wastes used as culture media for microalgae cultivation and the production of biomass and high-value secondary products.

| Fruit and vegetable wastes | Microalgae strain | Biomass production | Secondary high value product production | Reference |
|---|---|---|---|---|
| Mango, Passion fruit, melon, watermelon, banana, pineapple, papaya, tomato, peppers, eggplant, chayote, potatoes, jerimum, kale, chard, lettice, cauliflower and onion bio-compost | *Lagerheimia longiseta* | 0.38 g/L | Carbohydrate: 11.98% Proteins: 55.76%Lipids: 19.38% | [87] |
| | *Monoraphidium contortum* | 0.25 g/L | Carbohydrate: 16.97% Proteins: 49.08%Lipids: 24.21% | |
| | *Scenedesmus quadricauda* | 0.51 g/L | Carbohydrate: 18.08% Proteins: 59.15%Lipids: 10.62% | |
| Banana, peels and pomace, papaya, pineapple, potato, cucumber, pumpkin and radish compost | Pretreatment using 5% *Bacillus flexus; Oscillatoria sancta* | 0.13-0.68 g/L | Carbohydrate: 0.11-0.45 mg/mLProteins: 2.02-2.08 mg/mLLipids: 1.03-1.08 mg/mL | [88] |
| Onion peels, garlic peels, brinjal waste and pumpkin wastes extract | *Asterarcys* sp. SPC | 0.96 g/L | Lipid: 20% | [89] |
| | *Scenedesmus* sp. KT-U | 0.96 g/L | Lipid: 20% | |
| Mango, banana, apple, tomato, plum, melon, orange, tangerine, grapes, strawberry, cherimoya, pear, pineapple, peach and cucumber centrifugated or homogenized | *Chlorella* sp. | 46.50-65.56 x10$^5$ cells/mL/day | Lipid: 9.20-15.60 mg/L | [90] |
| Banana peel and sweet lime peel | *MonoraphidiumI sp.* | 1.05 g/L | Lipid: 14000 RFU (relative fluorescence units) Carotenoids: 11 μg/mL | [91] |
| Watermelon liquid fraction | *Scenedesmus* sp. | 0.95 g/L | Lipid: 13.54% | [19] |
| | *Chlorella* sp. | 0.37 g/L | Lipid: 20.33 % | |
| Rotten apple | *Spirulina platensis* | 0.46-0.71 g/L | Protein: 53-58%Lipid: 6.31-14.62% | [92] |
| Orange, pumpkin, papaya peels | *Chlorella sorokiniana* | 3.16 g/L | Lipid: 1.55 g/LCarotenoids: 9.18 mg/L | [93] |

from 6.87–9.69 EUR/kg. These findings further confirm the critical role of biomass productivity in determining economic feasibility [108].

Economic assessments of waste-derived substrates for microalgae cultivation have highlighted substantial potential benefits. Wu et al. [109] reported that integrating food waste as a nutrient feedstock in microalgae cultivation systems can enhance circular economy practices in waste treatment while reducing raw material and unit production

**Table 6**
EN14214 limits for biodiesel production from microalgal lipids [95].

| Property | Lower limit | Upper limit |
|---|---|---|
| FAME content (%m/m) | 96.5 | - |
| Viscosity at 40°C (mm$^3$/s) | 3.5 | 5.0 |
| Density at 15 °C (kg/m$^3$) | 860 | 900 |
| Flash point (°C) | > 101 | - |
| Carbon residue remnant (%m/m) | - | 0.3 |
| Sulfur content (mg/kg) | - | 10 |
| Cetane number | 51.0 | - |
| Water content (mg/kg) | - | 500 |
| Sulfated ash content (%m/m) | - | 0.02 |
| Total contamination (mg/kg) | - | 24 |
| Oxidation stability, 110°C (hours) | 6 | - |
| Acid value (mg KOH/g) | - | 0.5 |
| Iodine value (%m/m) | - | 12 |
| Polyunsaturated (>=4 double bonds) methylester (%m/m) | - | 1 |
| Linolenic acid methylester (%m/m) | - | 12 |
| Methanol content (%m/m) | - | 0.2 |
| Monoglyceride content (%m/m) | - | 0.8 |
| Diglyceride content, triglyceride content, free glycerine and total glycerine (%m/m) | - | 0.2 |
| Phosphorous content (mg/kg) | - | 4 |
| Group I metals (Na+K) (mg/kg) and Group II metals (Ca+Mg) | - | 5 |

costs up to 38%. Specifically, food waste integration reduced medium costs to approximately 38–40 USD/m$^3$, compared with 71.4 USD/m$^3$ for conventional culture media. Similarly, microalgae cultivation coupled with anaerobic digestion, using digestate as a fertilizer, demonstrated favorable economic outcomes, producing approximately 4.3 kg/m$^3$ of microalgal biomass. Under these conditions, any biomass selling price exceeding 0.39 USD/kg was sufficient to offset the costs associated with wastewater anaerobic digestion.

The use of digestates from agro-industrial, abattoir, and piggery effluents generated microalgae biomass value of 0.24 USD/m$^3$, 0.83 USD/m$^3$, and 0.78 USD/m$^3$, respectively. If this biomass were sold at price of 0.92 USD/kg, 0.41 USD/kg, and 0.51 USD/kg, respecitvelly, the resulting economic credit could fully cover the costs of waste treatment [110]. Furthermore, Dias et al. [111] reported that waste integration reduced microalgae biomass production costs to as low as 1.68 EUR/kg while simultaneously increasing biomass productivity.

European case studies further illustrate the economic potential of microalgae cultivation. Hoque et al. [112] reported production costs of approximately 12–13 EUR/kg for *Scenedesmus almeriensis* in tubular photobioreactor in Spain at a scale of 200 t/year. In Italy, a 100 ha Green Wall Panel system produced *Tetraselmis suecica* at a cost of 5.1 EUR/kg, with the potential to decrease to 3.2 EUR/kg under optimal climatic conditions. These results are promising, particularly in light of market projections indicating that the global microalgae market is expected to grow at a compound annual growth rate of 7.29%, reaching a value of approximately 1.38 billion USD by 2032 (Future Business Insights, June 2025) [112].

Despite these encouraging trends, microalgal biomass production costs remain highly dependent on effluent quality and the stability of biomass productivity. While increasing biomass productivity often leads to higher energy consumption per unit of biomass, the associated cost increases can be economically sustainable only if technological advancements are implemented at industrial scale and a biorefinery approach is adopted. Such an approach integrates the production of biomass, energy, biofuels, and high-value bioproducts, thereby improving overall process efficiency and profitability.

To achieve these goal, ongoing research efforts are focused on the selection and development of novel microalgae strains, optimization of abiotic conditions ti enhance lipid accumulation, improvement of photobioreactor design to maximize solar energy conversion efficiency, optimization of $CO_2$ delivery, identification of alternative substrates to

**Table 7**
Total lipid content and fatty acid composition of microalgal biomass cultivated on different waste derived substrates.

| | | % | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Agro waste | Microalgae strain | Palmitic acid (C16:0) | Linoleic acid (C18:2n6) | Linolenic acid (C18:3n6 o n3) | Palmitoleic acid (C16:1) | Stearic acid (C18:0) | Oleic acid (C18:1n9c) | Arachidic acid (C20:4n6) | Total lipid | Reference |
| Orange, papaya and pumpkin peels | *Chlorella sorokiniana* | 47.9 | 12.76 | 0 | 7.54 | 1.8 | 4.98 | 0.4 | 0.15 | [93] |
| Watermelon | *Chlorella* sp. | 0 | 0 | 0 | 0.86 | 38.41 | 9.51 | 0 | 20.33 | [19] |
| | *Scenedesmus* sp. | 0 | 1.35 | 0 | 4.47 | 18.54 | 32.33 | 0 | 13.54 | |
| Piggery manure | *Desmodesmus* sp. | 2.58 | 0.08 | 0.77 | 1.42 | 11.05 | 1.59 | 0.16 | 23 | [99] |
| Digested piggery manure | | 1.63 | 51.65 | 13.89 | 1.13 | 2.06 | 9.15 | 0.08 | 28 | |
| Chicken manure | *Chlorella vulgaris* | 24.62 | 26.76 | 17.07 | 2.72 | 1.74 | 8.08 | 0.72 | | [100] |
| Food processing digestate | *Chlorella pyrenoidosa* | 15.6 | 17.2 | 28.7 | 11.5 | 4.5 | 9.8 | 0 | 10 | [101] |
| Swine wastewater digestate | *Chlorella pyrenoidosa* | 27.7 | 16.4 | 17.5 | 14.8 | 3.9 | 9.3 | 0 | 20 | |
| Food processing digestate | *Scenedesmus obliquus* | 11.8 | 18.5 | 15.9 | 5 | 10.1 | 26.2 | 0 | 15 | |
| Swine wastewater digestate | *Scenedesmus obliquus* | 21.3 | 14.6 | 9.2 | 2.1 | 13.5 | 18.4 | 0 | 20 | |
| Livestock manure | *Chlorella vulgaris* | 9.4 | 3.3 | 0 | 2.6 | 2.6 | 0 | 0 | 18.51 | [70] |
| Poultry waste | | 14.9 | 12.0 | 5.2 | 3.09 | 9.8 | 22.2 | 0.9 | 10.32 | |
| Sewage sludge | | 15.19 | 55.44 | 4.17 | 2.09 | 1.34 | 20.78 | 0 | 25.5 | |
| Red wine digestate | *Chlorella vulgaris* | 39.2 | 15.96 | 0.5 | 0.93 | 23.61 | 11.58 | 0 | 33.48 | [17] |
| White wine digestate | | 27.89 | 52.35 | 0.53 | 4.48 | 2.75 | 7.83 | 0 | 20 | |
| Sweet sorghum bagasses hydrolysate | *Chlorella vulgaris* | 25 | 5 | 3 | 0 | 5 | 60 | 0 | 42 | [102] |
| Food waste hydrolysate | *Scenedesmus obliquus* | 40.65 | 31.89 | 44.7 | 4 | 10.54 | 65.71 | 0 | 20 | [103] |
| Dairy wastewater | *Scenedesmus* sp. | 19.5–23.7 | 5.8–7.1 | 3.2–3.6 | 3.5–4.3 | 5.8–6.8 | 44.4–49.7 | 0 | 31.23–36.12 | [104] |
| Lipid-rich food waste | *Scenedesmus obliquus* | 43.54 | 34.27 | 45.38 | 6.16 | 11.06 | 69.64 | 0 | 25 | [105] |
| Carrot pomace | *Chlorella vulgaris* | 15 | 10 | 55 | 7 | 5 | 20 | 0 | 40 | [106] |

increase biomass and lipid productivity, genetic engineering to promote lipid biosynthesis and storage, and the development of environmentally friendly lipid extraction and biodiesel production technologies [113].

## 8. Conclusion

Agro-industrial waste represents a valuable resource within circular economy frameworks, offering promising substrates for the sustainable production of high-value compounds through biotechnological processes. Crop residues, agro-industrial byproducts, livestock manure, and fruit and vegetable wastes can serve as effective alternative substrates for microalgae cultivation, either with or without pretreatment. However, further research is required to optimize the integration of agro-wastes into microalgae-based cultivation systems, particularly to elucidate strain-specific responses to different substrates and to enable successful scale-up.

As highlighted in this review, agro-waste derived substrates can significantly enhance the feasibility of integrating microalgae into biorefinery systems by modulating the biochemical composition of the resulting biomass. In particular, microalgal lipid content and fatty acid profiles are strongly influenced by substrate composition, thereby affecting downstream applicability across multiple industrial sectors. In accordance with the European Standard EN14214 for biodiesel production, linolenic acid content must remain below 12% to ensure fuel quality. Experimental evidence from innovative agro-waste-based cultivation strategies demonstrates that appropriate strain–substrate combinations can yield lipid profiles compliant with this standard.

Beyond biofuel applications, the presence of mono- and polyunsaturated fatty acids, proteins, carbohydrates, and pigments in microalgal biomass enables its valorization in high-value markets, including the pharmaceutical, nutraceutical, and food industries. Despite significant progress in recent decades, additional research is necessary to further optimize agro-waste-based microalgae cultivation systems. Such efforts would facilitate the decentralization of biomass production and promote localized cultivation strategies within agricultural and food-processing sectors, ultimately strengthening the role of microalgae in the development of a sustainable and circular bioeconomy.

## CRediT authorship contribution statement

**P. Scarponi:** Writing – original draft, Investigation. **L. Forti:** Writing – review & editing.

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## 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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