



Biotreatment Potential and Microbial Communities in Aerobic Bioreactor Systems Treating Agro-Industrial Wastewaters

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Abstract: The thriving agro-industry sector accounts for an essential part of the global gross domestic product, as the need for food and feed production is rising. However, the industrial processing of agricultural products requires the use of water at all stages, which consequently leads to the production of vast amounts of effluents with diverse characteristics, which contain a significantly elevated organic content. This fact reinforces the need for action to control and minimize the environmental impact of the produced wastewater, and activated sludge systems constitute a highly reliable solution for its treatment. The current review offers novel insights on the efficiency of aerobic biosystems in the treatment of agro-industrial wastewaters and their ecology, with an additional focus on the biotechnological potential of the activated sludge of such wastewater treatment plants.

Keywords: aerobic wastewater treatment systems; agro-industrial effluents; activated sludge; microbial ecology; nutrients removal efficiency



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1. Introduction

For more than a century, activated sludge in batch setups was used to treat sewage samples under continuous cycles of operation. Since then, the activated sludge process has rapidly expanded across the whole world, as industrialization and urbanization have entailed the need for the treatment of produced wastewaters. Nowadays, in the European Union (E.U.), the treatment of municipal wastewater complies with Directive 91/271/EEC, which, through the setting of rules regarding treatment efficiency and discharge, aims to protect the receiving water bodies from the vast amounts of wastewater produced.

The process involves the mixing and aeration of the activated sludge; the oxidation of organic carbon and inorganic nitrogen, wherein additional biomass is produced; the separation of the liquid and solid phases in order to control the concentration of the total suspended solids (TSS); the recirculation and retention of the biomass and possible disposal of the excess activated sludge [1]. During this step, the removal of phosphorus nutrients is also feasible, whereas the implementation of an anoxic stage results in the reduction and removal of the oxidized nitrogen compounds.

Apart from municipal wastewater, activated sludge systems have gained ground in the treatment of a range of agro-industrial effluents, which, among others, include winery, brewery, dairy, citrus, olive mill, vinasse, cassava, pepper, caper, and even coffee processing wastewater [2–5]. Several full-scale treatment plants have been installed and operated under moderate organic loading rates and aerobic or oxic–anoxic conditions to effectively remove nutrients from agro-industrial effluents [2], mainly carbon and nitrogen but even phosphorus [6].

The present review provides new insights on the efficiency of aerobic wastewater treatment plants processing agro-industrial wastewaters and their ecology, with additional reference to the biotechnological potential of activated sludge in such biosystems.

2. Agricultural By-Products and Wastewaters

The agriculture and food sectors globally face significant challenges in the 21st century. Food waste occupies an increasing section of waste treatment facilities and landfills. Today, remaining residues and losses throughout the food supply chain are drawing attention due to the vast waste of valuable resources, constituting a complex environmental problem. In low-income countries, food waste causes serious socio-economic consequences, while, on the other hand, consumer attitudes and the mass consumption of goods and products cause the production of huge amounts of household waste in middle and high-income countries [7].

Food waste can be classified into animal and agricultural origins. In the first, the main sources of waste are those from the dairy, meat, and fishery industries. In the latter, it is possible to classify a variety of residues according to the source, which may include cereals, roots and tubers, oil seeds and legumes, fruits, and vegetables. However, spoiled foodstuffs, along with heterogeneity, make them difficult to exploit, while a comprehensive characterization process is required in order to determine their composition. Nevertheless, both animal and agricultural wastes are often characterized by high organic matter [8].

As reported by Leite et al. [9], in the European Union alone in 2018, more than 21 million tons of waste were generated, which derived from the agriculture, fishery, and forestry sectors. Ravindran et al. [10] pointed out that one third of the food produced worldwide which is intended for human consumption is wasted every year, corresponding to losses of about 1.3 billion tons, while 40 to 50% (520–650 million tons) of global food waste per year derives from fruits, vegetables, and roots. In the E.U., food waste is estimated to reach 89 million tons per year, about half of which is generated during production, while the total production of agricultural residues (crop residues or parts of plants that are not consumed as food) amounts to 367 million tons per year, although some of these residues are commonly used at the farm level as bedding and fodder [11].

Recently, the Food and Agriculture Organization (FAO) in a report under the title "The Future of Food and Agriculture—Alternative Pathways to 2050" stated that the planet's population is expected to increase to about 10 billion by 2050, with an expected increase in agricultural demand, in a scenario of moderate economic growth, of about 50% compared to 2013. Furthermore, an increase in income in low- and middle-income countries would hasten the dietary transition to a greater consumption of meat, fruits, and vegetables over cereals, necessitating subsequent production changes that are expected to put additional strain on natural resources. In fact, although investment in agriculture and available technological tools and innovations boost productivity, final output growth is less profound, as food losses account for a significant proportion of agricultural production; therefore, tackling the crucial parameters of food loss would in itself reduce the need to increase production, which is already hampered by the degradation of natural resources and the loss of biodiversity, as well as the cross-border spread of pests and diseases of plants and animals which are highly resistant to applied antimicrobials [12].

Recently, the European Commission (E.C.) presented the action plan for the circular economy, which sets a coordinated strategy for a climate-neutral, resource-efficient, and competitive economy, as annual waste production until 2050 is expected to increase by 70%, given that "50% of total greenhouse gas emissions and more than 90% of biodiversity loss and pressure from water scarcity are due to resource extraction and processing" [13]. Moreover, regarding the economic aspect of this issue, the FAO stated in a report published in 2016 [14] that food losses worldwide correspond to economic losses of 490 billion US dollars per year, while the amount of water used corresponds to about 1/4 of all fresh water resources used in agriculture worldwide. These, in addition to the economic impact, are responsible for an estimated 8% of global greenhouse gas emissions [14], as well as 23% of the global use of fertilizers [15].

3. Quantities, Composition, and General Valorization Aspects of Agro-Industrial Wastewaters

Agro-industrial waste consists of agricultural and industrial residues, with the byproducts of agricultural production being further distinguished into on-site residues (produced on the field) and those deriving from treatment processes [16]. Usually, these residues, which are agricultural by-products, are used in the manufacture of animal feed, as soil conditioners and fertilizers, and even in some construction applications, although significant quantities remain unexploited due to the heterogeneity of their characteristics. Their composition, which is also reflected in the wastewater produced as a consequence of the processing procedure, usually comprises mainly cellulose in a percentage approaching 60% [17], followed by hemicellulose, lignin, and minerals [18]. In the food industry, significant amounts of waste are generated in semi-solid and liquid forms due to the industrial processing of juices, potato products, and confectionery, which also mainly consist of cellulosic and hemicellulosic compounds [19].

Agro-industrial wastewaters are produced throughout the whole multistep processing procedure due to the washing, sterilization, centrifugation, distillation, sanitation, chemical treatment, and cleaning that take place, depending on the initial raw material to be processed [20–24], and are expected to contain high biological oxygen demand (BOD) and chemical oxygen demand (COD) concentrations, as well as suspended solids (SS) (Table 1). In any case, physicochemical and biological processes are applied until final disposal becomes necessary. Ideally, prior to disposal, important components of the wastewater could be recovered, while the reuse of the treated effluent in agriculture could also be considered. Components of the produced wastewater, such as sugars, organic acids, aromatic compounds, pigments, and proteins, may be immediately recovered or upgraded by biotransformation, although the presence of detergents, pesticide residues, antibiotics, and essential oils complicates this process [25,26].

Agro-Industrial Wastewater	pН	BOD (g/L)	COD (g/L)	TS (g/L)	SS (g/L)	VSS (g/L)	TN/TP (g/L)	Phenols (g/L)	Oil and Grease/ Total Carbo- hydrates (g/L)	Reference
Brewery	5.1–10	2.0-5.0	3.6-49.5	1.7–38.9	0.7–5.7	38.9	0.07–0.17/ 0.07–0.06			[27–30]
Caper processing	6.8–7	1.5-4.0	2.2–8.4	-	-	-	0.12-0.35			[4]
Cassava processing	4.02-4.6	1.4–12.3	6.0–38.2	6.6–60.5	-	5.1–49.4	0.19-0.4/0.08-0.24		0.6/-	[31–34]
Cheese whey	3.92-6.6	90.1	45.0–91.6	47.6–73.9	9.4	8.3–59.9	0.15–3.2 (TKN)/0.12–0.70	0.27		[35–39]
Citrus processing	3.21–3.8	4.7–6.6	2.3–32.1	16.5	1.2	15.1	0.15/0.013		E.O. 0.04–1.0	[32,40-43]
Coffee- processing	3.50-4.4	4.3–37.9	7.6–45.9	3.8–19.5	2.9–8.6	1.9–8.2	0.27-0.7/0.01-0.04	0.05–0.28		[37,44–47]
Olive mill	4.0-5.7	10.2	36.7–299.0	38.9–94.9	24.7–42.8	23.2-83.2	0.1-0.6/0.06-0.2	0.54–11.0	-	[35,36,38, 39,48–50]
Palm oil mill	4.11-4.8	0.32–15.6	25.5-86.2	20.0-60.1	12.3–35.3	17.5-50.2	0.5-1.1/0.09-0.35	0.26-0.46	2.0-42.8	[31,51–54]
Pepper processing	4.4	1.4	1.9				0.05 (TKN) 0.02(NH4 ⁺)/0.01			[5]
Potato processing	4.6-7.1	0.9–5.0	1.5–37.0	4.8-42.0	2.0–3.8	3.5–4.4	0.03-0.62/0.04-0.1		-	[55–59]
Soyamilk	4.1		7.3	4.7		4.1	0.3		0.8	[31]
Vinasse	3.6-4.5	14.4-54.8	44.7–131.0	52.2-64.9	4.5-29.0	48.5-49.8	0.2-1.6 /0.1-0.6	0.35–0.7	-/25.1-47.30	[37,60-62]
Winery	3.6–4.9	0.15–8.0	0.5–15.9		0.08–2.4	0.07–1.6	0.06-0.2/ 0.01-0.055	0.03–0.2		[48,63-67]

Table 1. Physicochemical composition of the key agro-industrial effluents treated in aerobic wastewater treatment systems.

Indeed, the processing of agricultural products is responsible for the generation of vast amounts of wastewater all around the world. According to Martinez-Burgos et al. [2], 0.085 million m³ of wastewater was produced from cassava processing; 1.65 million m³ was produced from vinasse processing; and palm oil processing wastewater amounted to

0.256 million m³; whereas milk and cheese whey processing formed 143 and 158 billion m³ of wastewater worldwide. Zema et al. [24] reported the production of 500 million m³ of wastewater from the processing of citrus fruits, while this vast volume of processed effluent is expected to further increase and reach 750 million m³. Interestingly, Brazil is the leading country regarding the processing of citrus fruits (12.11 million MT processed, 80% oranges), covering almost 50% of global citrus processing, followed by the United States of America, which processes 20% of citrus fruits. Moreover, 2.45 and 1.65 billion m³ of effluents were produced due to slaughterhouse activity [2]. The produced effluents from each process are characterized by intense heterogeneity, even for raw materials of the same origin [2]. Due to the environmental conditions, the type of process, the technology, the seasonality, and the variety of plants (when plants are used as raw materials), the COD in the effluents of the winery and coffee processing industries may range between 0.5 and 16 g/L and 7 to 48 g/L, respectively; for palm oil effluent, from 25 to 86 g/L; and for cheese whey wastewater, from 45 to 91 g/L; whereas the COD of olive mill wastewater lies between 37and 300 g/L and that of vinasse wastewater between 44 and 131 g/L. Similarly, cassava and citrus wastewaters present similar concentrations of organics, as the effluent COD of the processed material can range from 6 to 38 and 2 to 98 g/L, respectively. The remarkably high organic fraction of these effluents may be attributed to the presence of carbohydrates and proteins, as well as fats and grease. Carbohydrate concentrations in vinasse wastewater and palm oil effluent can reach 47 g/L and 43 g/L, respectively. Furthermore, the strongly acidic pH of the majority of agro-industrial wastewaters (average pH value of 4.5), as well as total and volatile suspended solids, contribute to the uniqueness of these effluents in terms of treatment and exploitation for the recovery of value-added products (Table 1).

In general, the criteria and possible approach strategies for developing valorization processes are based on a number of key pillars, which are described in detail by Castro-Muñoz et al. [68]. In brief, the following are required:

- i. Case-by-case analysis of the production process and the characteristics of agroindustrial waste and residues, taking into account organic load, seasonality, chemical stability, and volume of produced wastewater
- ii. Selection of one or more objectives, such as recycling of compounds and/or water, and recovery of molecules and components, as well as energy production.
- iii. Assessment of possible technological and economic advantages in order to select appropriate disposal and exploitation alternatives.
- Identification of possible biotechnological approaches to achieve the initial goal.

4. Main Bioreactor Types, Nutrient Removal Processes, and Factors Affecting Systems Performance under Aerobic Conditions

Various aerobic bioreactor systems performing nutrient removal have been employed to treat agro-industrial wastewaters. The majority are membrane bioreactor (MBR) and sequencing batch reactor (SBR) systems due to their high efficiency and/or simplicity in operation. For instance, Tatoulis et al. [69] reported the high removal efficiency (80.3–96.5%) of an SBR system treating table-olive processing wastewater. Moreover, Vergine et al. [70] treated winery and vegetable canning factory wastewater in a microfiltration module, achieving removal efficiencies steadily above 90%. Most of these focus on the removal of carbonaceous compounds, although nitrification–denitrification schemes are also used to remove both the carbon and nitrogen load of the effluents [71].

The main factors affecting the performance of aerobic wastewater treatment systems are the oxygen supply, the temperature, the organic loading rate (OLR), the hydraulic retention time (HRT), the solids retention time (SRT), and the nature and composition of the wastewater [1,72]. In nitrification–denitrification treatment systems, the concentration of easily biodegradable organic matter is also crucial for enhancing nitrogen removal efficiency.

The initial microbial population and its adaptation to the applied operating conditions are the driving forces of the activated sludge processes [73,74]. A specialized microbial com-

munity often proliferates based on the specific composition of the treated agro-industrial effluent [75].

Conventional activated sludge systems are effective in removing macronutrients and solids during the treatment of agro-industrial wastewaters. However, micropollutants are often detected in the effluents of such bioreactor systems, a fact that induces toxicity to certain organisms. Nowadays, the use of membrane reactors, e.g., at nanofiltration scale, can improve micropollutants' removal efficiency during the biological treatment of such effluents [76]. Recently, the application of advanced oxidation processes in the effluents of aerobic treatment systems has improved the quality of the treated wastewater and minimized toxicity [77].

5. Biotreatment of Agro-Industrial Wastewaters in Aerobic Bioreactor Systems

Aerobic biological treatment systems, including nitrification–denitrification plants, are commonly applied for the biotreatment of domestic wastewater and a range of agroindustrial wastewaters, due to their simplicity in operation, low cost of installation, high efficiency, and ability to biologically remove nitrogen through nitrification–denitrification. During the activated sludge process, organic matter is oxidized using air, mainly to carbon dioxide and water, and the microbial flocs formed are separated in a sedimentation tank [78]. Despite the fact that effluents of high organic content could be subjected to anaerobic digestion, the inability to biologically remove nitrogen in an efficient and simple manner and the high cost of installation often make the activated sludge process attractive for the biotreatment of certain agro-industrial effluents, especially those in which the COD concentration is low or moderate, or those that can be co-processed with municipal wastewater or washings. Even though the anaerobic treatment of agro-industrial wastewaters has the benefit of the production of biogas, this is balanced by the high HRT required, increasing the volume of the required digesters and resulting in specific space requirements, as well as the instability of the process, which provides no assurance of stable and satisfactory energy production [79]. Moreover, aerobic treatment enables the effective removal of nutrients, which is considered a strong benefit of the process, as high quality effluents are produced, capable of satisfying the stricter standards for disposal, which are not met in the case of anaerobic treatment systems [80].

Thus, there are several examples of using aerobic biological treatment systems for the depuration of agro-industrial effluents. For instance, activated sludge was immobilized on polyurethane particles in an aerobic bench-scale bioreactor for the treatment of winery wastewater under a maximum organic loading rate of 8.8 kg COD/m³·d and a hydraulic retention time of 0.8 d. Even at an OLR of 3 kg COD/m³·d, the ability of the aerobic immobilized cell bioreactor to remove COD was high, recording a COD removal efficiency of 87% [81]. Moreover, Roveroto et al. [82] treated brewery wastewater in a fixed-bed batch reactor, which operated under an intermittent aeration of 3 h aeration in 4 h cycle and a hydraulic retention time (HRT) of 0.83 d. The COD and BOD of the raw brewery wastewater ranged between 2 and 10 g/L and 1.2 to 3.6 g/L, respectively, while the total nitrogen reached up to 0.08 g/L. The highest removal efficiency, 92%, was recorded in the bioreactor when the influent COD was 2.7 g/L and the COD/N ratio was 107. Under these conditions, the nitrification efficiency was 88% and the total nitrogen (TN) removal was 85%.

Antiloro et al. [40] investigated the biotreatment of citrus processing wastewater with a high organic content and essential oils concentration, i.e., between 20 and 30 g/L and 0.6 to 1.0 g/L, respectively, in an aerated lagoon system, reporting COD removal efficiencies from 59 to 97% and the establishment of a microbial community capable of coping with the increased concentration of essential oils. In addition, two aerobic granular sludge bench-scale SBRs operating under a sludge retention time (SRT) of 10 d and organic loading rates (OLRs) ranging from 3 to 15 kg COD/m³·d were used for the biotreatment of a citrus processing effluent of 5.5 g/L COD. At a neutral pH, the biosystem could remove COD by 90% regardless of the organic loading rate applied, although the reactor's efficiency under

acidic conditions declined to 75% when the OLR exceeded 7 kg COD/m³·d. Furthermore, Zema et al. [83] treated citrus processing wastewater of 5.0g/L COD and an essential oils concentration of 0.5 g/L under aerobic conditions in a full-scale treatment plant, reporting reasonable COD and essential oils removal efficiencies.

Moore at al. [84] treated wastewater deriving from mixtures of fruits and vegetables in an aerobic pilot-scale ultrafiltration membrane bioreactor (MBR), for potential water reuse. Lettuce, beets, carrots, and cassava were processed to produce the first wastewater mixture, while potatoes, carrots, apples, onions, lettuce, beets, and bananas constituted the raw materials for the production of the second mixture of wastewater. The COD and total Kjeldahl nitrogen (TKN) content of the first mixture were 1.5 g/L and 0.01 g/L, respectively, whereas the respective concentrations in the second mixture were 7.1 g/L and 0.23 g/L. The HRT in the two experimental schemes examined varied from 24 to 52 h, whereas the OLR ranged from 0.82 to 2.7 kg COD/m³·d in the first and from 2.9 to 6.5 kg COD/m³·d in the second experimental setup. For both fruit- and vegetable-derived effluents treated in the MBR, high COD removal efficiencies of 97–98% were recorded, whereas the TKN removal efficiencies exceeded 91% for both wastewater mixtures. In this case, the activated sludge system, in combination with UV disinfection and the implementation of activated carbon for color removal, could produce an effluent of enhanced quality, which could be used in the agri-food sector.

More than 10.5 million tons of coffee were exported by its producing countries in 2020 [85], a process that leads to the production of significant amounts of wastewater, since up to 45 kg of wastewater is generated during the pulping and washing of 1 kg of green coffee. Villa-Montoya et al. [3] treated coffee processing wastewater of a high organic content (COD of 7 to 15 g/L) and a TN concentration between 0.03 and 0.04 g/L in a sequencing batch reactor (SBR) under an OLR of 9 g COD/L.d, reporting that the intermittently aerated biological system achieved a COD removal efficiency of 92%. Coffee processing wastewater of a high COD concentration (17 g/L) was also treated in a constructed wetland system by Rossmann et al. [86], in order to achieve the efficient removal of nutrients and phenolic content. At an HRT of 11.8 d, the biosystem could remove total nitrogen (TN), total phosphorus, and total phenolic compounds by 69.1, 72.1, and 72.2%, respectively.

6. Biomass Valorization of Aerobic Biosystems Treating Agro-Industrial Wastewaters

Microorganisms are an important source of enzymes, as they grow rapidly in a short period of time. In addition, a wide variety of agro-industrial residues and wastes can be used as substrate, thus reducing overall production costs and the use of natural resources while value-added products are produced. Enzymes of microbial origin can find a variety of applications in industry, such as in the production of food and beverages, as well as in the manufacture of chemicals and pharmaceuticals. The properties and activities of an enzyme are considered to be directly dependent on the strain that is capable of inducing them, while their effectiveness in biotechnological applications is being constantly and increasingly evaluated. Therefore, there is a strong scientific interest and a wide scientific field in the search for new strains capable of producing high-activity enzymes at a reduced cost with potential uses in industry [87]. Moreover, aerobic bioreactor systems treating agroindustrial wastewater can be considered as microbial cell factories producing a wide range of industrial enzymes, such as cellullases, xylanases, glycosidases, lipases, and proteases.

For instance, Zerva et al. [4] assessed the hydrolytic potential of an immobilized cell bioreactor treating caper wastewater at an elevated salinity (3.12 to 101 g/L). The non-halotolerant microbiota of the immobilized cells at a salinity of up to 20 g/L were able to highly hydrolyse celluloses, hemicelluloses, starch, fats, and proteins. Increased endo-1,4- β -xylanase activity above 1785 U/g protein was recorded throughout the experimental period. Endo-1,4- β -D-glucanase activity of 250 U/g protein was also reported, even though it was highly affected by the elevated salinity. Regarding polygalacturonase, its activity exceeded 533 U/g protein and further increased to 959 U/g protein under the highest

salinity. Furthermore, β -1,4-D-glucosidase activity was above 510 U/g protein, while the increase in the organic loading rate and low salinity resulted in the elevation of α -1,4-D-glucosidase activity up to 905 U/g protein. Initial lipase activity was above 352 U/g protein but was affected by a salinity concentration of 1% w/v and decreased to 130 U/g protein. Moreover, Zerva et al. [5] treated pepper processing wastewater in an aerobic immobilized cell bioreactor and monitored the hydrolytic potential of bacteria isolated from the immobilized biomass of the biosystem, reporting a high endo-1,4- β -xylanase activity of 107,000, 72,000 and 70,000 U/g protein for three bacterial isolates belonging to the genera *Nocardia* and *Gordonia*. Bacterial isolates related to Aquincola, *Microbacterium, Planococcus, Sphigopyxis*, and *Xanthobacter* were also found to exert endo-1,4- β -xylanase activity from 29,700 to 37,400 U/mg protein.

In addition, several white-rot fungi can be used for the biotreatment of various agro-industrial enzymes and produce ligninolytic enzymes. For instance, a *Phanerochaete chrysosporium* strain was immobilized by Sharari et al. [88] on polyurethane foam for the treatment of bagasse wastewater and the simultaneous production of ligninolytic enzymes, reporting peroxidase activity of 260 U/L and laccase activity of 131 U/L, whereas xylanase activity of 74 U/L was also detected.

Moreover, Mafakher et al. [89] isolated lipase-and citric acid-producing yeasts from agro-industrial wastewater treatment plants. Among the 300 yeast isolates examined, 6 exhibited a high lipase activity, which were identified as *Yarrowia lypolitica* isolates.

7. Microbial Communities' Structure in Aerobic Biosystems Treating Agro-Industrial Wastewaters

The recent development and application of high-throughput sequencing techniques have led to a better understanding of microbial communities' structure and functions in bioengineering systems. In the last decade, the implementation of molecular methods, such as next generation sequencing techniques, has permitted the elucidation of the microbial ecology and biotechnological potential of certain aerobic bioreactor systems treating agroindustrial wastewaters (Table 2).

In that direction, by implementing high-throughput sequencing techniques, Fang et al. [90] stated the dominance of *Zoogloea* in the activated sludge of an SBR treating rice winery wastewater under an OLR of 2.4 g COD/L.d. Apart from the presence of *Zoogloea* species, *Rhodobacter* and *Rubrivax* were also detected in high abundances. The dominance of *Zoogloea* spp. in the activated sludge of this aerobic bioreactor system can find a biotechnological application potential, since this genus is considered an important PHA accumulating microorganism [91]. Bacteria of the genus *Amaricoccus*, *Zoogloea*, and *Azoarcus* were also identified in winery wastewater using FISH, whereas *Amaricoccus* species dominated the constructed clone library [92].

Moreover, in meat processing wastewater treated in an SBR, the activated sludge microbial community was dominated by the class *Alphaproteobacteria*, which are frequently identified in similar samples [93], where *Amaricoccus* spp. covered 11% of the microbial diversity in the SBR. Furthermore, the biotreatment of dairy wastewater in a full-scale aerobic SBR under an OLR of 2.5 kg COD/m³·d revealed the predominance of the genera *Proteiniphilum*, *Byssovorax*, *Acidobacterium*, and *Zoogloea*, which covered 35.9, 14.5, 10.1, and 8.3% of the total relative abundance [94], despite the fact that *Proteiniphilum* and *Byssovorax* bacteria are rarely reported as microbial constituents of activated sludge. The same authors also reported that *Thiothrix* and *Leptothrix* spp. were the main filamentous bacteria of the activated sludge system, with the presence of *Thauera* being involved with the formation of granular structures and the cohesion of the activated sludge due to the release of extracellular polymeric substances (EPS).

Other major inhabitants of activated sludge systems treating agricultural wastewater are members of the genera *Bacillus*, *Pseudomonas*, *Thauera*, *Xanthomonas*, *Spingobacterium*, and *Comamonas*, such as in aerobic biosystems treating olive mill [95], winery [96], and dairy [97] wastewaters.

In addition, Pires et al. [98] isolated bacterial and fungal strains from a coffee processing wastewater treatment plant. The bacterial isolates were mainly members of the phyla *Proteobacteria*, e.g., *Acetobacter*, *Serratia*, and *Enterobacter* spp.; *Actinobacteria*, e.g., *Corynebacterium* and *Arthrobacter*; and *Bacteroidetes*, e.g., *Chrysobacterium*. Regarding the fungal community structure, the majority of isolates were identified as yeasts of the order Sacharomycetales, such as *Wickerhamomyces*, *Torulaspora*, *Kazachstania*, *Saturnispora*, *Meyerozyma*, *Hanseniaspora*, and *Pichia* spp., which have been often detected in municipal wastewater treatment plants and in other biosystems treating agro-industrial wastewater, e.g., palm oil effluent [99,100]. Pires et al. [98] also detected filamentous fungi, such as *Alternaria alternata* and *Fusarium oxysporum*. Petruccioli et al. [101] also isolated yeasts from the activated sludge of an aerobic jet loop reactor treating winery wastewater, identifying microbiota such as *Saccharomyces*, *Candida*, and *Trichosporum*, reporting a link between the presence of *Saccharomyces* and the formation of biofilm.

Regarding the bacterial community structure in aerobic immobilized cell bioreactor systems, Zerva et al. [5] treated pepper processing wastewater under two different loading rates (0.31 and 0.70 g COD/L.d), revealing the composition of microbial communities in the aerobic immobilized biomass. The microbial community structure in the immobilized cell bioreactor was affected by the shift in the OLR, where the relative abundance of the genera *Pirellula*, *Nakamurella*, *Nitrospira*, and *Planctomyces* decreased from 54 to 19%. Moreover, the recorded total reads of the genera *Denitratisoma*, *Blastopirellula*, and *Holophaga* decreased from 3.8 to 0.9%. In the meantime, the OLR increase favored the presence of the genera *Gemmata*, *Nitrosospira*, and *Chitinophaga*. A thriving nitrifying population was also reported (near 10% of the total relative abundance at the OLR examined), even though bacteria of the genera of the genera *Nitrosospira* dominated the *Nitrospira* population after the OLR increase.

Zerva et al. [4] also examined the succession in microbial communities during the treatment of caper processing wastewater in an immobilized cell bioreactor under elevated salinity (from 0.3 to 10% w/v). A diverse ecology was revealed in the immobilized activated sludge at the lower salinities examined, while the increase in the salt concentration resulted in the restriction of the diversification of the microbial community. At low salinities, members of the genera *Pirellula, Amaricoccus, Planctomyces,* and *Arenibacter* accounted for 31.5 and 35.8% of total reads, respectively, while, at moderate salinities, representatives of the genera *Defluviimonas, Formosa, Muricauda, Arenibacter, Rhodobacter,* and *Roseovarius* were the predominant taxa. Remarkably, at the highest salinity examined, *Halomonas,* which represented 45% of the total relative abundance, followed by members of the genera *Roseovarius* (18%), *Idiomarina* (7%), and *Cyclobacterium* (5.7%) dominated hypersaline immobilized sludge.

Table 2. 1	Microbial	communities i	in aerobic	biosystems	treating	agro-industrial	effluents.

System	Effluent /Method	HRT (d)	OLR (g COD/ L.d)	COD (g/L)	NH4 ⁺ -N (g/L)	CODrem (%)	NH4 ⁺ - Nrem (%)	Microbial Community	Reference
Activated sludge reactor	Winery/ Isolates	2.1–4.4	0.4–5.9	0.8–12.8	0.001-2.0	90–98	100	Bacteria: Bacillus sp., Pseudomons paucimobilis, Pseudomonas sp., Agrobacterium radiobacter, Acinetobacter. Fungi: Saccharomyces cerevisiae, Candida sp., Candida humicola, Candida kefyr, Trichosporum capitatum, Geotrichum penicillatum.	[101]
Aerobic batch reactors	Rice winery/ Illumina	0.5	1.2, 2.4, 3.6	170	2.3 (as TN)	91.8–93.2	-	PHA-accumulating microorganisms: Zoogloea (5–41.1%), Rhodobacter (0.6–3.2%), Rubrivivax (0.1–2.6%), Leptofthrix (0.1–2.5%), Burkholderiaceae, Comamonas, Haliscomenobacter, Rhodobacteraceae, Amaricoccus and Plasticicumulans. Under OLR of 2.4 g COD/L.d PHA-accumulating microorganisms covered 29.6% of the relative abundance. Phylum level: Proteobacteria, Bacteroidetes, Verrucomicrobia, Patescibacteria, Acidpbacteria, Chloroflexi.	[90]

System	Effluent /Method	HRT (d)	OLR (g COD/ L.d)	COD (g/L)	NH4 ⁺ -N (g/L)	CODrem (%)	NH4 ⁺ - Nrem (%)	Microbial Community	Reference
Full-scale A/O	Dairy/ Clone library	7	-	0.4–2.9 (as BOD)	10.1	98 (as BOD)	87	Lactococcus, Veillonela, Atopobium, Olsenella, Zoogloea spp., Dechloromonas spp., Leptothrix spp.	[102]
Activated sludge system	Dairy/ Clone library	5	-	14.9	0.8	77 (as BOD)	88	Phylum: Proteobacteria (55.1%), Bacteroidetes (15.4%), Actinobacteria (10.9%), Firmicutes (9.5%), TM7 (4.5%) Species Aerobic effluent: Thuera terpenica, Aeromicrobium marinum, Pseudomonas sp., Dyella japonica, Roseobacter sp., Sphingobacterium thalpophilum, Xanthomonas axonopodis, Dyella koreensis, UBA318142, AF507866. Aerobic storage: Thauera terpenica, Pirellula sp., Roseobacter sp., Rhodobacter praeacuta, AY570630, Xanthomonas axonopodis, Tissierella praeacuta, AY438740, Thermononas hydrothermalis, Petrimonas sulfuriphila	[34]
SBR	Dairy/ Illumina	4	-	10.1	0.3	89	99	Phyla: Proteobacteria (73–75%) Bacteroidetes (15–18%) Verrucomicrobia (0.6–3%) Planctomycetes (0.5–3%), Actinobacteria (1.2–1.8%), Chloroflexi (0.7–1%). Class: Alphaproteobacteria (43–68%), Betaproteobacteria (5–21%), Deltaroteobacteria (1.5–9%), Gammaproteobacteria (1–7%). Sphingobacteriia (10–11%), Flavobacteriia (3–6%). Genus: Terrimonas (2.6–4.6%), Thauera (2.2–12%), Nannocystis (2–6%), Flavitalea (2.7–6%), Hyphomonas (2.7%).	[103]
Immobilized cell bioreactor	Pepper process- ing/ Illumina- Isolates	2.75	0.31–0.70	1.92	0.01	81.0-92.2	83.3–95.0	AOB: Nitrosospira (1.45–9.21%), NOB: Nitrospira (1.73–7.53%), Nitrobacter (0.04–0.13%), AOB abundance (2.00–9.77%), NOB abundance (71.77–7.66%), Pirellula, Nakamurella, Nitrospira, Planctomyces (54.03 to 19.10%), Gemmata, Nitrosospira, Chitinophaga (from 4.40 to 24.20%). Fungal taxa: Rhizopus, Paramicrosporidium, Candida, Acaulospora, Neobulgaria, (94.93 and 87.64%). Isolated microorganisms: Paracoccus laeviglucosivorans, P. lutimaris, Microbacterium lacus, Microbacterium aurum, Sphingopyxis soli, Pseudoxanthomonas japonensis and P. mexicana, Gordonia hongkongensis, G. terrae, Oleiharenicola sp.	[5]
Immobilized cell bioreactor	Capper process- ing/ Illumina	2.75– 13.75	0.22–0.59	0.8–8.5	0.05–0.4 (as TKN)	70	37–70	Periods I and II: Pirellula, Amaricoccus and Planctomyces, Arenibacter (31.50 and 35.76%). Period III: Amaricoccus (16.14%), Planctomyces (12.39%), Defluviimonas (10.04%), Formosa (6.13%), Arenibacter (5.96%). Periods IV and V: Defluviimonas, Formosa, Muricauda, Arenibacter, Rhodobacter, Roseovarius (56.57 and 55.61%). Period VI: Halomonas (45.16%), Roseovarius (18.12%), Idiomarina (7.01%), Cyclobacterium (5.68%)	[4]
Full scale SBRs	Dairy/ DGGE- Pyrosequenc	- cing	2.5	1.8	0.7 (as TN)	94	95 (as TN)	Bacteria: Proteiniphilum (35.9%), Byssovorax (14.5%), Acidobacterium (10.1%), Zoogloea (8.3%), Rhodomicrobium (3,8%), Roseomonas (3.8%), Comamonas (3.8%), Leptothrix (2.1%), Hydrogenophaga (1.7%), Ingella (1.7%), Thiothrix (1.1%), Genmatimonas (1.1%) DGGE: Candidatus Accumulibacter sp., Lysobacter brunescens, Thauera sp., Saprospiraceae, Xanthomonadaceae, Caldilineaceae, Micrococcineae (order Actinomycetales), Zoogloea caeni, Amaricoccus sp., Chiayiivirga flava, Candidatus Competibacter sp.	[94]

Table 2. Cont.

System	Effluent /Method	HRT (d)	OLR (g COD/ L.d)	COD (g/L)	NH4 ⁺ -N (g/L)	CODrem (%)	NH4 ⁺ - Nrem (%)	Microbial Community	Reference
Full-scale WWTP	Coffee/ Isolates	-	-	13.3	0.1 (as TN)	-	-	Bacteria: Acetobacter indonesiensis, Chrysobacterium bovls, Corynebacterium flavescens, Serratia marcescens, Enterobacter sp., Corynebacterium callunae, Moxarela osloensis, Arthrobacter woluwenis Fungi: Wickerhamomyces anomalus, Torulaspora delbrueckii, Kazachstania gamospora, Saturnispora gosingensis, Meyerozyma caribbica, Kazachstania exigua, Hanseniaspora uvarum, Pichia fermentans Filamentous fungi: Fusarium oxysporum, Geotrichum silvicola, Geotrichum candidum, Alternaria alternata	[98]
Full scale SBR	Winery/FIS Clone library	H- 0.5	-	5–16	-	-	-	FISH: Amaricoccus spp., Defluviicoccus cluster I and II, Candidatus 'Alysiosphaera europaea', Zoogloea sp., Azoarcus sp., type 0803, type 1851, Haliscomenobacter hydrosis. Clone library: Amaricoccus kaplicensis, A. capsulatus, A. veronensis, A. tamworthnensis, Rhodocyclus tenuis, Geminococcus roseus, Tetracoccus cechii.	[92]
Jet loop reactor	Winery/ Isolates	0.8–1	-	3.1–27.2	0.02–0.06 (as TKN)	63–84	-	Bacteria: Acinetobacter sp., Bacillus sp., Pseudomonas sp., Sphingomonas paucimobilis, Agrobacerium radiobacter Fungi: Blastoschizomyces capitalis, Candida sp., Saccharomyces cerevisiae	[96]
SBR	Olive mill/ Isolates	30	-	75.1	0.6 (as TKN)	60	-	Phyla: Firmicutes (57.1%), Proteobacteria (35.2%), Actinobacteria (7.7%) Genera: Bacillus, Lysinibacillus, Brevibacillus, Paenibacillus, Roseomonas, Ochrobactrum, Pseudomonas, Klebsiella, Rhodococcus Species: Bacillus amyloliquefaciens (11 isolates), B. cereus (8 isolates), B. nealsonii (7 isolates), B. thioparans (4 isolates), B. thuringiensis (3 isolates), B. subilis (1 isolate), Lysinibacillus macroids (6 isolates), Brevibacillus laterosporus (6 isolates), Paenibacillus xylanilyticus (6 isolates), Klebsiella oxytoca (6 isolates), Pseudomonas aeruginosa (1 isolate), Kocuria rosea (1 isolate), Cellulosimicrobium cellulans (1 isolate)	[95]

Table 2. Cont.

8. Conclusions

Aerobic systems constitute a particularly reliable solution for the treatment of agroindustrial wastewater due to their stability and high nutrient removal efficiencies, particularly in cases where bioreactor systems operate under intermediate organic loading. Moreover, the ability to remove both carbon and nitrogen makes them attractive. In addition, activated sludge systems, in the case of bacterial communities, are dominated by members of the phylum *Proteobacteria*, while due to their nature, a significant diversity of yeasts is also observed. Most importantly, biological activated sludge systems that treat agro-industrial wastewater can be considered as a valuable source for the isolation of novel microbial strains, while the biotechnological potential of such wastewaters can be exploited through the production of hydrolytic enzymes and the recovery of addedvalue products. Future explorations of aerobic bioreactor systems, e.g., through the use of immobilized cell bioreactors and highly aerated reactors, could allow the biotreatment of agro-industrial wastewaters at higher organic loading rates, a modification that will expand the practical applicability of such biosystems in the treatment of highly polluted agro-industrial effluents.

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