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Review

Fumaric Acid Production: A Biorefinery Perspective

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Abstract: The increasing scarcity of fossil raw materials, together with the need to develop new processes and technology based on renewable sources, and the need to dispose of an increasing amount of biomass-derived waste, have boosted the concept of biorefineries. Both 1G and 2G biorefineries are focused on the obtention of biofuels, chemicals, materials, food and feed from biomass, a renewable resource. Fumaric acid, and most compounds involved in the Kreb cycle, are considered key platform chemicals, not only for being acidulants and additives in the food industry, but also for their prospective use as monomers. This review is focused on the biotechnological processes based on fungi, mainly of the *Rhizopus* genus, whose main product is fumaric acid, on the process conditions, the bioreactors and modes of operation and on the purification of the acid once it is produced.

Keywords: biorefinery; fumaric acid; waste valorization; bioprocess; bioreactor

1. Introduction

In these first years of the XXI century, increasing concerns on the availability of fossil resources for material and energy supply are surging due to the reduction of reserves, with an increasing technological challenge to obtain such resources (a good number of petroleum fields are working on secondary recovery conditions, while several tertiary recovery technologies are under development, like enhanced oil recovery and unconventional oil and gas recovery by using microbial surfactants and nanoparticles, for example) [1,2]. Apart from the political and economic strains that primary and secondary recovery resources (thus, relatively cheap resources) are creating in an insatiable world, this search for the last petroleum fields and the last drop of each of these fields comes hand in hand with the search for renewable and alternative energy and material resources. In this aspect, air and water movements can be utilized to the advantage of Humanity to create enough electricity [3]. On the other hand, biomass could be thought of as a renewable and almost endless source for the material needs of Humanity, as well as fuel for combustion engines (more than 100,000 million tons are created each year through photosynthesis) [4]. The confluence of the petroleum concept of refinery and the excess of renewable resources due to biomass have created the idea of a biorefinery as a complex net of processes of various natures (mechanical, thermal, chemical and biochemical) applied to biomass to create a plethora of energy vectors, chemicals, food and feed to foster Humanity. Due to the renewable nature of biomass, the possibility to develop sustainable processes is within our reach, but this could only happen if all activities are analyzed beforehand to ensure a low environmental impact, an acceptable economic feasibility and the adequate covering of social needs. To this end, global analyses like Life Cycle Assessment (LCA) and Life Cycle Costing seem to be essential management tools, while process development, integration, intensification and computer-aided design will be the engineering tools

of utmost importance in this new sustainability age [5,6]. As the petrochemical industry is based on simple platform chemicals such as ethylene or propylene, biorefineries should be based on their own range of chemical intermediates. The US Department of Energy (DOE) identified, in 2010 and 2014, several of them, mainly polyols, such as sorbitol, and monosaccharides, such as glycerol and glucose, but also several dicarboxylic acids, such as succinic and fumaric acid, and hydroxyacids, such as lactic acid [7]. For the polymer industry, bio-based classical monomers, like bio-ethylene, are an opportunity to resume its activity using biomass-based classical monomers [8], and dicarboxylic acids and hydroxyacids are an opportunity to develop new biodegradable polymers that reduce the impact on the environment, while ensuring a good technical performance and economic feasibility. This has been proved for lactic and succinic acids and it is under development for fumaric and itaconic acids [9,10].

Fumaric acid or (E)-2-butanodioic acid is a *trans*-C4 dicarboxilic acid, while maleic acid is its *cis*-counterpart. This acid presents a high boiling point due to its being a trans-acid, an aspect that permits a close intermolecular interaction. This same feature results in low water solubility, as compared to maleic acid. Some important physical constants of the acid are reported elsewhere [11,12].

Nowadays, fumaric acid is produced through petrochemical routes via isomerization of maleic acid that, in turn, is obtained by the hydrolysis of maleic anhydride. Worldwide, this latter compound was produced in excess of 3,400,000 tons in 2014 (estimated CAGR- or Compound Annual Growth Rate- from 2017 to 2021 is fixed at 6.8%), while fumaric acid global production was 225,200 tons in 2012, with an estimated production of about 300,000 tons for 2018 and about 346 ktons by 2020, at an estimated CAGR of 5.9% from 2014 to 2020 [13]. In both cases, as can be seen, moderate growth is forecast in the years to come, mainly due to the food industry increasing the demand for natural acidulants and flavoring agents for foods and beverages, and, in general, to the increasing demands from end use industries.

Figure 1 compiles global consumption of fumaric acid in 2016 considering its main application fields and the global regions and countries, in terms of tons consumed per year [14].

The most usual application, due to its relatively low price (FOB price: 1280–1700 USD/ton), is as an acidulant, preservative and flavoring agent in food and feed. Being a relatively strong acid, its buffering capacity at pH 3.0 is high, so only a small amount is needed to preserve acidity in foods near 4.5. On the other hand, this acid is a precursor for the production of other acids, like L-aspartic (2013 global production: 37,400 tones; 2021 CAGR 2021 *circa* 6%) and L-malic acid (2016 global production: 162,100 tones; CAGR2020 of 5.16%), that are also used in beverages, health drinks, and cosmetics [15,16].

While the second application by volume is in the paper and pulp industries, as an acid sizing agent, a progressive change to alkaline processing of wood to paper in North America and Europe is slightly reducing the importance of fumaric acid in this regard. Nonetheless, rosin Diels-Alder adducts with fumaric acid and maleic anhydride are common tackifiers in the paper industry and, regarding the rosin market, it is interesting to point out that China accounts for more than 90% of high-quality rosin global production (gum rosin).

Very close, in terms of tonnage, to this application is the consumption of fumaric acid to produce unsaturated polyester resins (UPEs). As in the previous case, the presence of an intermediate double C=C bond together with the two carboxylic acid moieties in the first and fourth carbon atoms permit the addition and condensation of polymerization reactions in the case of fumaric acid, giving rise to a plethora of possibilities. On the other hand, its trans-nature creates polymers that trend to be very compact due to multiple intermolecular interactions, so their mechanical and thermal properties are far better than those of the maleic acid-anhydride counterpart (albeit these latter ones are preferred for applications needing low-cost polymers) [11]. Together with succinic and itaconic acid, fumaric acid is a promising bio-derived C4 monomer for the production of UPEs, a family of polymers with wide applications as coatings, insulating materials, drug delivery systems and biomedical applications

(tissue engineering) [17,18]. Based on a similar reactivity, fumaric acid is used to modify natural polymers, like chitosan, to render films for the preservation of foods [19].

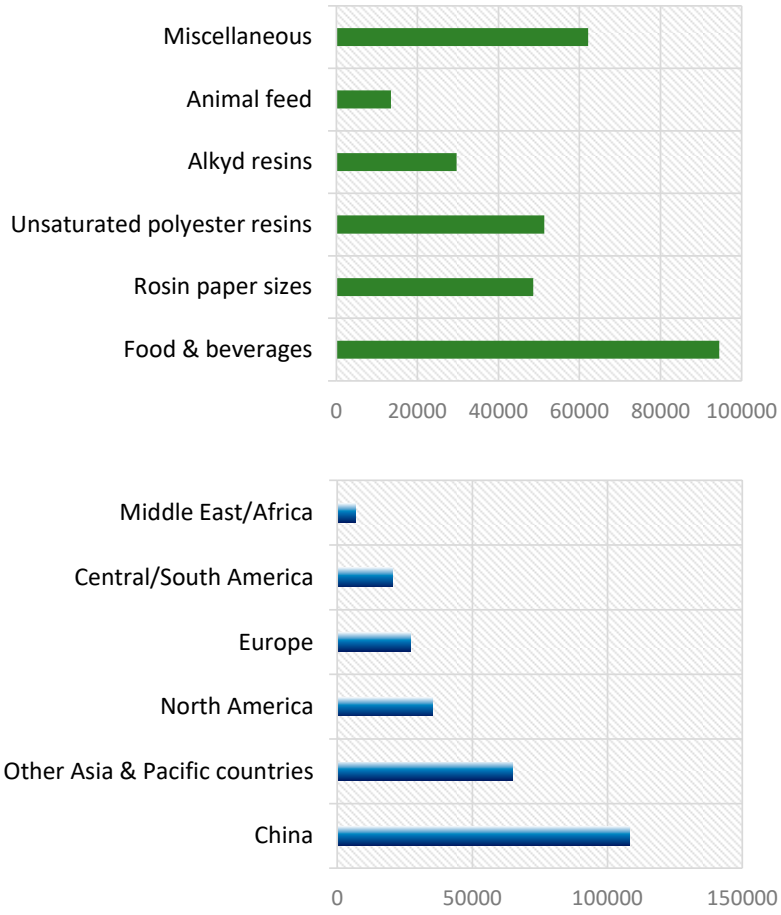

Figure 1. Fumaric acid consumption regarding its applications (in the upper graph) and countries/world regions (in the lower graph) [14].

Another application in the polymer industry that involves a high amount of fumaric acid is the production of alkyd resins, mainly in the Asia & Pacific region. These thermoplastic polymers are obtained by the condensation of polyols and polyacids (or their anhydrides) and subsequently modified with long fatty acids and oils, being one of the main monomers, in conjunction with phthalic anhydride, isophthalic acid, maleic anhydride, and many others. The main applications of these resins are in the coating industry [20].

In the last years, a growing concern about Climate Change has become evident; methane is a gas with a heating power more than four times that of CO_2 and an immense annual production, though much inferior. This fact, together with the need to care for the health and well-being of cattle and poultry, has introduced additives like fumaric acid in feed formulations [11,21]. Fumaric acid acts as a hydrogen sink in the rumen of cattle, thus reducing reductive conditions and avoiding reactions that end up in methane built-up, and increasing feed efficiency [22]. Curiously, Fumaric acid effects on pH in the rumen are not clear after several studies [23].

Finally, fumaric acid, and in particular, its esters (methyl, ethyl, propylfumarates), are finding very important applications: in dermatology (psoriasis), as anticarcinogenic and neuroprotecting agents, in multiple sclerosis and to mitigate inflammatory cardiac conditions, among other biomedical applications. The timely review of Das et al. compiles and discusses all these applications, while a profound review on psoriasis was recently written by Smith [24,25].

2. Production Processes

Fumaric acid is produced via biotechnological processes at a mass yield around 88% starting from glucose. However, with the advent of crude oil as the main carbon source in the industry at the end of World War II, fermentative processes were abandoned in favor of petrochemical routes through maleic anhydride and maleic acid, with a mass yield of about 112% [21]. In view of increasing concerns about oil availability, higher demands due to the Asia-Pacific region economic growth and increasing prices due to the implementation of enhanced recovery technologies, petrochemical prices are increasing, creating an adequate framework to consider once again biomass-based processes. On the other hand, environmental concerns and the need to dispose of huge quantities of biomass derived from human activities open the door to recover bioprocesses as the one that end up in the production of fumaric acid. Moreover, new knowledge on genetic engineering plus advanced process integration and intensification approaches should result in better yields from low-cost biomass resources and, what could be more important, better productivities.

Although petrochemical routes are not sustainable by nature, as they are based on a non-renewable raw material, they can be considered as benchmarks or references from the economic point of view. Nowadays, fumaric acid is produced by thermal or catalytic irreversible isomerization from maleic acid at 150 °C, which, in turn, is obtained from its anhydride by heating in presence of water. Maleic anhydride, on its part, is obtained from n-butane or from benzene, in both cases via catalytic oxidative processes [11,21]. The most modern process starts from n-butane, using vanadyl pyrophosphate $(VO)_2P_2O_5$ as a catalyst [26].

A possibility that can be developed from petrochemical or biomass resources is the dehydration of 1-butanol, an alcohol that is one of the products of the ABE fermentation [27] or that can be derived from propylene. The produced butenes are further oxidized to maleic anhydride. To this purpose, the same $(VO)_2P_2O_5$ is used as a complex catalyst, with mixed acid and redox sites [28].

2.1. Enzymatic Processes

Production via catalytic routes needs relatively high temperatures that result in inefficient conversion due to the formation of byproducts [21]. Biocatalysis approaches using enzymes reduce the thermal effect on isomerization of *cis*- to *trans*-groups and *vice versa*, as they work at very moderate temperatures [29]. Several microorganisms of the genus *Arthrobacter*, *Pseudomonas*, *Alcaligenes* and *Proteus* are able to synthetize maleate *cis-trans* isomerase at activities from 80 to 200 UI·L^{-1}. However, the enzymes from *Pseudomonas alcaligenes* and *Arthrobacter* sp. strain TPU 5446 are the most promising. The highest activity for the *Arthrobacter* enzyme is obtained with 0.5–1% maleic acid and 1% yeast extract. For *Pseudomonas alcaligenes*, the most interesting strain is XD-1.

As solvents, aromatics can be used with *Pseudomonas* species. If toluene is employed, up to 30 g·L^{-1} fumaric acid is produced, together with high amounts of L-malic acid (10.6 g·L^{-1}). A moderate temperature of 45 °C favors fumaric acid built-up, up to 7 g·L^{-1}·h^{-1} in 6 h. Further processing yields higher amounts of L-malic acid, reducing the concentration of fumaric acid.

To increase the yield of fumaric acid when using whole-cells, the thermal inactivation of fumarase was analyzed, as this enzyme transforms fumaric acid into L-malic acid. The relevant enzyme for the isomerization, cis-trans maleate isomerase, is more robust. In addition, thermal treatments enhance mass transfer across the cell membrane. Under these conditions, fumaric acid production from maleic acid by whole-cells gives a high yield of the target acid: 95% [30].

2.2. Fermentative Pathways

Before the advent of petrochemical processes, fumaric acid was produced in the USA by fermentative processes with the fungus *Rhizopus arrhizus* [11,21]. These processes have several stages: sporulation, mycelium or inoculum built-up and production. A scheme of the operations is presented in the next figure, depending on the base used for neutralization:

Once obtained, fumaric acid should be purified. This step depends on the basic agent used during neutralization: if Na_2CO_3 is used, the broth is filtered and acidified with H_2SO_4 to recover the fumaric acid needles; however, if $CaCO_3$ is employed, heating is needed before acidification to dissolve calcium fumarate [11,21].

Focusing on the fermentation step itself, there are several biochemical pathways that yield fumaric acid. One of them is pyruvate reductive carboxylation, being the pyruvate produced from monosaccharides, usually glucose, through the common glycolysis metabolism: The Embden–Meyerhof–Parnas pathway [9,11]. Another classical route in which fumaric acid is involved is the Krebs cycle [31]. This cycle includes reactions in which the oxalacetate interacts with Acetyl-CoA to form citric acid. In turn, citric acid undergoes two decarboxylation steps and other transformations to generate, again, oxalacetates: fumaric acid is one of the intermediates in such reactions [31,32]. The reductive pathway of the Krebs cycle is the main source of fumaric acid and involves fixation of CO_2 from a C3 acid to a C4 acid (pyruvate to oxalacetate). All steps can be observed in Figure 2.

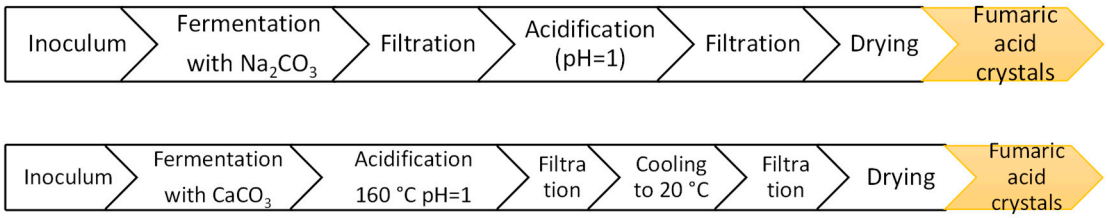

Figure 2. Operations during the fermentation process to fumaric acid depending on the basic neutralization reagent (Na_2CO_3 or $CaCO_3$).

C4 acids, as fumaric acids, tend to accumulate when nitrogen is lacking, though processes that involve microorganism growth, CO_2 fixation and glucose metabolism keep working [21]. Moreover, glycolysis is also linked to aminoacid metabolism, and this fact has resulted in the enhancement of fumaric acid production by mutant strains of *Rhizopus oryzae*. In any case, the only fumaric acid that is excreted by the fungi is produced in the reductive pathway.

In the synthesis of fumaric acid, there are three main enzymes: pyruvate carboxylase (in cytoplasm), malate dehydrogenase and fumarase (both in the cytosol and in the mitochondria). The first one catalyzes the carboxylation from pyruvate to oxalacetate with the aid of ATP and CO_2, while maleate dehydrogenase induces the transformation of oxalacetate in malic acid, and fumarase isomerizes this latter acid to fumaric acid (but not the reverse reaction). This latter enzyme is particularly active under nitrogen stress conditions. The main pathways are shown in Figure 3.

Fermentation to fumaric acid is a process in which the type of microorganism employed, the conditions in which the operation is carried out, and the bioreactor selected are of utmost importance. In the next sections, these aspects will be commented on in depth.

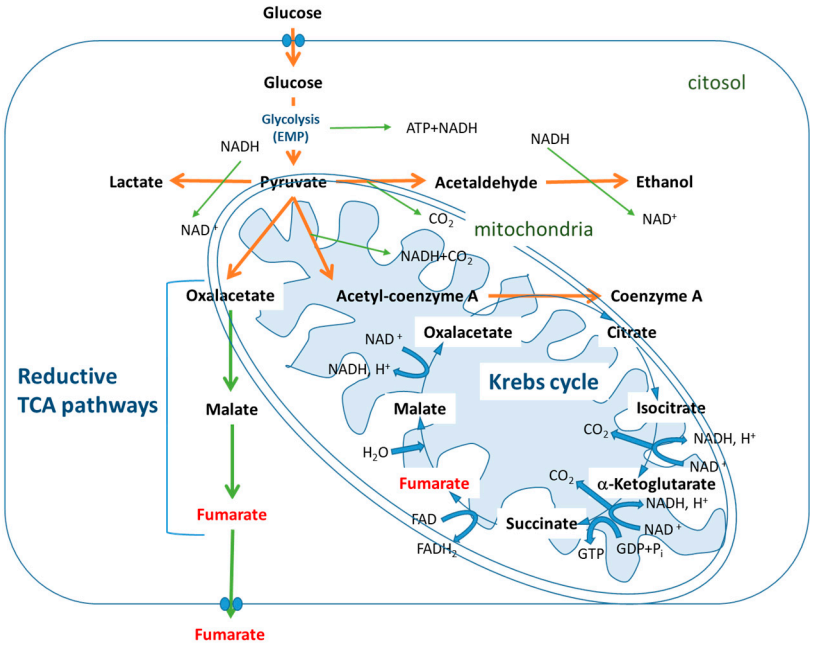

Figure 3. Pathways from glucose to fumaric acid (adapted from [31]).

3. Microorganisms

Filamentous fungi are the most employed microorganisms in fumaric acid production, in particular the genus *Rhizopus*, since their production yield is adequate and fungi of this genus provide the possibility of both aerobic or anaerobic fermentation, highlighting *R. arrhizus* and *R. oryzae* as the best producers.

Species from the genera *Mucor*, *Cunninghamella*, *Cirinella* and *Aspergillus* have also been studied as suitable fumaric acid producers [33].

Many other species could be used through genetic engineering strategies, introducing certain genes responsible for fumaric acid production, such as fumarase and fumarate dehydrogenase enzymes, across the huge number of possible host microorganisms, such as *E. coli* [34] or *Lactobacillus* species [21]. The host selection could be performed depending on the process specifications, for example, the used raw material.

However, sometimes fumaric acid natural producers are not able to achieve required yields or concentrations to implement a viable industrial process. In these cases, genetic improvement techniques can be used, obtaining microorganisms able to reach better production yields.

To improve *Rhizopus* species, several techniques have been used, e.g., random mutagenesis techniques, such as high energy irradiations (laser or UV) or the use of mutagens (N^+ or nitrosoguanidine).

Directed evolution is a more advanced technique than random mutagenesis, combining the promotion of variability in nucleic acids, through mutagenesis, for example, with a strong evolution pressure, such as the use of certain carbon sources.

3.1. Mutagenesis

Several studies have used random mutagenesis to improve different *R. oryzae* strains. The most common technique is high-energy radiation, applying two radiation stages, one with UV radiation, and the other with γ-rays, while selection is based on the diameter of the colony. *R. oryzae* RUR709 was developed from *R. oryzae* KTC 6946; this new mutant strain provides an increase of 88.81% in final fumaric acid concentration and 45.45% in productivity [35].

The application of the radiation influenced mutations, so a study about exposure time and irradiation power was done on *R. oryzae* ATCC 20344, optimizing the mutagenesis process which provided increases of 22.83% in final fumaric acid concentration [36].

These irradiation techniques, widely used, could be complemented with the addition of certain mutagens, such as nitrosoguanidine, combined with a selection pressure consisting of the presence of alkyl alcohol, to assure the survival of the cells without the alcohol dehydrogenase gene; strain MU-UN-8 was obtained, yielding fumaric acid concentrations 21.15% higher than the wild type strain: *R. oryzae* ME-F01 [37].

Nitrosoguanidine is not the only mutagen employed, N^+ has successfully been used on *R. oryzae* ME-F12, obtaining increases of 28.22% in the final concentration [38].

In addition to random mutagenesis techniques, PCR mutagenesis has been applied, overexpressing pyc (pyruvate carboxylase) and pepc (fosfoenolpiruvate carboxylase) genes, using plasmids from PCR (pyc plasmids) and generated by *E. coli* (pepc plasmids), introduced to *R. oryzae* 99,880, obtaining 26% higher production when the pyc gene was overexpressed; however, the overexpression of the pepc gene decreased fumaric acid production [39].

3.2. Directed Evolution

Directed evolution is a technique widely used for generating different mutants from *Rhizopus* species to make them able to use different raw materials for producing fumaric acid.

Xylose is an alternative carbon source for *R. arrhizus*, which is not able to consume it properly, while this sugar is widely used in several biotechnological studies as suitable raw material, shaped similar to lignocellulosic biomass.

For using this lignocellulosic biomass as a raw material, directed evolution is used on *R. arrhizus* RH-7-13, consisting of growing spores, and pellets later, on different mediums with increasing concentrations of xylose, obtaining a final increase of 190% in final fumaric acid concentration compared with the initial strain [40].

Developing high performance production strains is another use for directed evolution techniques, combining random mutagenesis using UV radiation with high-throughput screening techniques: a strain able to produce 160% more fumaric acid than the wild type strain has been chosen [41].

4. Production Conditions

As mentioned previously, fumaric acid production can be carried out by many microorganisms, but optimization of the process depends on several variables, including operational conditions, broth composition and raw materials employed.

4.1. pH

pH is the most important condition in the production of acids by fermentation, and, in general, in biotechnological processes, due to its regulatory effect on microorganism activities.

In the last years, some publications have studied fumaric acid production at low values of pH [42,43], performing exhaustive research about low pH internal effects on *Rhizopus oryzae* cells, observing the changes in the composition of the fatty acids on the membranes and how the ATP concentration decreases [42], or the alteration of metabolism in response to acid stress [43].

At a 5-L bioreactor scale, using Na_2CO_3 as a neutralizing agent, fumaric acid production could be possible at pH 3 using a small quantity of citrate in the medium, which reduces the acid stress and increases the ATP concentration and fumaric acid production [42]. Under the same conditions, a metabolic study has revealed that carbon flux at low pH is altered due to the necessity of the cells to ensure cellular functions, such as essential amino acids and fatty acid production. To avoid the high demand of unsaturated fatty acids, the addition of 1% linolenic acid to the medium helps the cell to perform its basic functions and increases fumaric acid production [43].

pH is not the only factor that matters in fumaric acid production. The neutralizing agents usually employed are sodium and calcium carbonates, bicarbonates and hydroxides. After several decades of research, $CaCO_3$ is known to be the best agent, thus indicating that calcium is an ion that could result in enhancement of fumaric acid production [11]. This is probably due to the low solubility of calcium fumarate, that switches all equilibria in metabolism to the production of this compound, thus lowering the amount of other acids and alcohols. However, calcium fumarate needs high amounts of acid and thermal energy to be solubilized, and biomass cannot be reused as inoculum for further productive cycles.

Moreover, $CaCO_3$ particle size plays an important role. The use of micro-(CCMPs) and nanoparticles (CCNPs) resulted in high neutralization rates and high titers of fumaric acid (approx. 67 $g \cdot L^{-1}$), while nanoparticles increased FA productivity by 57%, up to 0.74 $g \cdot L^{-1} \cdot h^{-1}$, and permitted activity at lower viscosity [44]. In this same work, microwave heating proved to reduce the heating time needed for FA solubilization from 28 to 10 min.

4.2. Morphology

The control of the fungal morphology must be exhaustively studied because different morphologies direct metabolism to different products [45]. These morphologies are the result of different operating conditions, including final pH, nitrogen amount, agitation and inoculum mass. *Rhizopus* species can grow in three different morphologies: filamentous mycelium, pellet and clumps.

Filamentous mycelium is known to be the most productive morphology [46] but presents certain problems in industrial implementation because the formation of the mycelium dramatically increases the broth viscosity, causing operational problems [45]. A possible strategy could be to reduce the biomass amount while promoting this morphology and increasing specific productivity of fumaric acid, reducing the nitrogen source and, thus, creating a nitrogen stress situation [47]. For fumaric acid production, stress to cells seems to improve production, as observed when the phosphorus concentration in the broth is reduced [48].

The clump morphology must be avoided, because this kind of cell growth consists of only one big clump where the inner biomass is totally isolated from the medium, with a lack of nutrients and oxygen at the clump core that drives metabolism to ethanol and other fermentative by-products, while promoting the death of the inner biomass [45].

The pellet morphology is preferred for this industrial process, avoiding a high viscosity increase, while the pellet's size must be optimized to permit convenient mass transfer and avoid the existing problems in clumps.

To control fungal morphology, many conditions have been optimized, such as initial inoculum spore concentration or different nitrogen sources [45] or even the use of certain solid supports to develop the desired morphology [49].

The initial spore concentration in the inoculum affects the final morphology, resulting in dispersed mycelia at higher spore concentrations (10^6 spores$\cdot L^{-1}$) and creating small pellets at low spore concentrations (10^2 spores$\cdot L^{-1}$) [45].

Different nitrogen sources could impact the obtained morphology; ammonium sulphate is proven to have a key role in *R. arrhizus* metabolism, improving it at high concentrations of ammonium sulphate [46].

Soybean meal hydrolysate presents fine particles of insoluble proteins, which reduce the lag phase duration, so cell growth is faster, providing a very productive dispersed mycelium (50.2 $g \cdot L^{-1}$), proving to be an effective nitrogen source to replace the industrial nitrogen sources [45].

The use of solid supports to develop morphology could be a solution for controlling morphology; the use of micro-nanoparticles gives a surface where spores can germinate and biomass can grow on the surface, preventing the problems of clumps and big pellet morphologies, and giving a direct particle size. These micro-nanoparticles could be used to supply certain micronutrients such as Fe^{2+}, Zn^{2+} and Mn^{2+} ions [49].

Fe_3O_4 seems to be the best support for the cell growth of *Rhizopus oryzae* 1526 and fumaric acid production, obtaining a 95.7% spore germination at 1000 mg/mL; on the other hand, MnO_2 is not recommended for supporting biomass, resulting in less than 10% germination [49].

Solid particle size seems to be a critical parameter. The use of nanoparticles of different materials, as support, causes several variations in morphology and fumaric acid production, as they considerably increase cation concentrations that induce stress-tolerance mechanisms, inhibiting growth and fumaric acid production. Thus, microparticles are best to support biomass growth [49].

4.3. Alternative Substrates

The composition of the culture medium depends on the microorganism requirements. In general, complex mediums are used when starting a process development, and these media are later complemented, simplified and optimized according to research and development [50].

Glucose is the most used carbon source, as it is readily metabolized by fungi. Its price is relatively low: 520–650 € per ton. Because cellulose is a glucose macropolymer and the main component of lignocellulosic biomass (125 USD/dry ton [51]; 40–50% *w*/*w*), its saccharification by enzymatic means should result in the production of enormous amounts of glucose, but biomass pretreatment, and enzyme production and adequate use, i.e., immobilization, recycling, process intensification are nowadays still under development to obtain economically competitive products in second-generation biorefinery facilities [52].

Apart from glucose, several substrates could be used in the search for new renewable raw materials to develop eco-friendly processes: carbon sources such as starch, xylose from lignocellulosic biomass, glycerol, etc. Metabolic profiling can aid to the identification and proper feeding of supplements that enhance the production of the acid, such as valine, leucine, sodium citrate, lactic acid, soybean oil or palmitic acid [50].

Alternative nitrogen sources have been analyzed in depth too, to find an alternative to classic nitrogen sources (ammonium salts, yeast extract, etc.). These alternative renewable nitrogen sources are treated biomass wastes such as corn step liquor or soybean meal hydrolysate [45].

New renewable raw materials are very interesting from economic and environmental points of view, but they must be complemented and optimized to overcome their limitations, sometimes eliminating compounds, as phenolics and terpenes, with antimicrobial activity (via detoxifying processes). Complementation can be simple, such as the adjustment of the carbon-nitrogen ratio, observing how at lower ratios, the fumarase enzyme increases its activity and, with little complementation with urea, by-product formation decreases [47].

Optimization of one synthetic production medium has been achieved through the study of the metabolic profiles [50], analyzing intracellular key metabolites in corresponding pathways, obtaining a method to complement the medium during the production process, and applying this research to renewable raw materials [53].

It has been observed that complementing a synthetic medium with palmitic acid and soybean oil added at 32 h and sodium citrate, lactic acid, leucine and valine added at 48 h could increase fumaric acid production by 14% and decrease the production of by-products [50].

4.3.1. Xylose

Xylose is the main component, together with cellulose and lignin, of agrowaste and wood lignocellulosic biomass. It is present, together with mannose, in the hemicellulose fraction in corn stover, sugarcane bagasse, wheat straw, and several wood species (for example, *Pinus radiata* or *Eucaliptus*), being one of the most abundant monosaccharides on Earth (the lignocellulose content in biomass ranges from 12 to 27%) [54]. Being such an abundant simple sugar, it is crucial to find novel processes based on this carbon source.

Wild type *Rhizopus* species have several problems growing in a medium rich in xylose, so it is necessary to choose certain mutant strains from *Rhizopus* species or find new microorganisms, modified or not, able to grow and produce fumaric acid from xylose.

Concerning *Rhizopus* species, *Rhizopus arrhizus* RH 7-13-9# has been chosen by growing it in a medium rich in xylose. It has proved to be a good fumaric acid producer from xylose (73% yield) coming from hemicellulose [55]. Also, fermentation conditions have been optimized and it has been observed that a co-fermentation process, combining glucose and xylose, is able to reach higher fumaric acid concentrations (46.68 $g \cdot L^{-1}$) than only xylose fermentation [56].

R. oryzae has also been studied in depth; transcriptome analysis has revealed that *R. oryzae* has different responses in the presence of glucose or xylose, suffering oxidative stress in xylose fermentation. This stress increases the carbon demand, making the growth and fumaric acid production more difficult [57].

The pulp and paper industry is the most important industry based on lignocellulosic materials; it produces an enormous amount of lignocellulosic wastes (11 million tons in the European Union [58]). After proper treatment, submerged fermentation has been studied, obtaining 23.47 $g \cdot L^{-1}$ with an optimum particle size [59]. Solid state fermentation has been performed too, directly over the solid waste, reaching 41.65 g of fumaric acid per kg of dry solid; electronic microscopy revealed proper fungal growth interacting with the solid [59].

Lignocellulosic raw materials must be pretreated for their use in submerged fermentation processes, but solid-state fermentation does not need those pretreatments; nevertheless, there is another possibility: simultaneous saccharification and fermentation processes (SSF). These processes reach higher yields than separate saccharification and fermentation processes (SHF); using corncob previously hydrolyzed and *R. oryzae*, simultaneous saccharification and fermentation improves biotechnological fumaric acid production (up to 41.32 $g \cdot L^{-1}$), compared to separate saccharification and fermentation (19.13 $g \cdot L^{-1}$) [60].

Another way to use xylose as a carbon source from lignocellulosic materials involves the use of microorganisms other than *Rhizopus* species, such as *Aspergillus* species [33,61], which are known to be optimal itaconic acid producers and can produce fumaric acid too, optimizing the culture conditions, as itaconic acid is a secondary metabolite, while fumaric acid is a primary one [61].

4.3.2. Glycerol

Glycerol is the main by-product from the biodiesel production process: 10% of oil biomass turns into glycerol, which is considered a C3 building block in the biorefinery framework. Due to the great growth of the biodiesel industry in recent years, the production of glycerol has increased exponentially, being regarded as a waste in the absence of an adequate local-regional market [62].

Pure glycerol is a very useful raw material for several industries such as cosmetics or automotive industries. However, glycerol obtained from first-generation and the most common biodiesel industry is crude glycerol, with a low triol concentration, so it has to be purified to be useful for industrial purposes. Therefore, crude glycerol could become a waste to be disposed of, as its purification can be economically unfeasible depending on local/regional needs [62].

Due to the high costs of the crude glycerol purification process, the idea of using it as raw material in a biotechnological process seems appealing. It can be transformed by several bioprocesses to high and medium added-value products, such as fumaric or succinic acids, or diols.

Glycerol, however, is not a suitable carbon source for all microorganisms [63], and even microorganisms that use glycerol could need the addition of another carbon source, so co-fermentation processes with glycerol as a supplement have been studied [62].

Using *R. arrhizus* RH-07-13, which is a well-known fumaric acid producer able to consume glycerol, the production of fumaric acid in a co-fermentation process combining glucose and crude glycerol yielded 22.81 $g \cdot L^{-1}$ of the acid, increasing the productivity in comparison to using only crude glycerol. In this case, a better conformation in smaller pellets was achieved using crude glycerol [62].

However, co-fermentation using crude glycerol provides a lower productivity of fumaric acid compared with only glucose fermentation, but the use of crude glycerol derived from the biodiesel industry increases the cost-effectiveness of the process and contributes to solve the problem of the treatment of crude glycerol as a waste [62].

As stated above, not all microorganism are able to consume glycerol. For this reason, some studies have used genetic engineering to solve this problem. Modifying a well-known succinic acid producer from a glycerol *E. coli* strain by deleting three fumarases, fumaric acid production was achieved, obtaining acetate as the main by-product. This acetate production was minimized by overexpressing PEP carboxylase, finally obtaining 41.5 $g \cdot L^{-1}$ of fumaric acid in a fed-batch process [64].

Fungal species such as *R. oryzae*, an optimum fumaric acid producer, could be modified to use glycerol as a carbon source through adaptive evolution, obtaining the G80 strain, where novel mechanisms for fumaric acid overproduction from glycerol have been observed [63]. In this work, up to 25.5 $g \cdot L^{-1}$ of the acid was obtained in an optimized fed-batch process.

4.3.3. Apple Wastes

The apple juice and cider industry produces about 12 MM tons of residues per year [65] in the typical apple juice making process, pressing the apples and later separation of liquid from solids; these solids are the apple pomace, which represents between 20% and 35% of the fruit weight. After the pressing stage, the juice must be clarified and, in this stage, another important waste in juice industry, apple pomace ultrafiltration sludge, is produced [66].

Apple pomace and apple pomace ultrafiltration sludge are ideal raw materials for the fermentation process, due to their high sugar content and the fact that they are wastes of the juice industry, so the raw material cost is reduced.

To develop a typical submerged fermentation process, apple pomace ultrafiltration sludge has been used with *R. oryzae* 1526. By optimizing the fermentation conditions and manipulating the content in sugars and solids in apple pomace ultrafiltration sludge, 25.2 $g \cdot L^{-1}$ of fumaric acid was produced; the morphology was also optimized to use small pellets that reached the highest productivity [66].

On the other hand, direct use of apple pomace requires a process of solid state fermentation, carried out in plastic trays, obtained with *R. oryzae* 1526, after 14 days of fermentation; 52 g of acid per kg of solid was used. Through electron microscopy analysis, it was observed that the fungus utilized the apple pomace to drive its hyphal growth [66].

4.3.4. Brewery Wastewater

The brewing industry could be described as a high pollutant industry according to its water consumption, using between 4 and 7 L of water for each liter of beer produced. After the brewing process, all this used water must be biologically treated to reduce its high organic content, due to spent hops, yeasts and grains. This decontamination process causes great costs to breweries [67].

These brewery wastewaters have been used as substrates of a submerged fermentation process. With *R. oryzae* 1526 as a biocatalyst and appropriate fermentation conditions, 31.3 $g \cdot L^{-1}$ were obtained, which indicates that brewery wastewaters are a promising and cheap raw material for fumaric acid production [67].

4.3.5. Food Wastes

The high production of urban residues and trash is a real problem in our society. All this trash must be disposed of and kept safely in dumps, which represent an environmental risk, mainly due to the increasing amount of trash produced. According to the FAO, and including food residues that were mentioned previously, such as apple pomace (very rich in free glucose and fructose), and other fruit residues (like citric waste), up to 1.3 MM tons of food waste is dumped every year, i.e., 1/3 of all food produced [68].

One important fraction of trash is food waste, this fraction could be solid or liquid, and has a high content of water, proteins and carbohydrates. Its use as a substrate in fermentation processes, submerged or solid state, represents a promising advancement in urban waste treatment [60,69].

Adopting the proper treatment to improve food wastes as raw material and using *R. arrhizus* RH-07–13, 32.68 $g \cdot L^{-1}$ were achieved from the liquid fraction of food wastes, with a higher yield than that obtained from glucose [69].

However, solid-state fermentation does not provide results as good as those from the liquid fraction (submerged fermentations). The solid food waste fraction requires complementation with glucose to obtain an adequate fermentation process, yielding 31.65 $g \cdot L^{-1}$ of fumaric acid. This fact indicates that the solid fraction is suitable as a nitrogen and micronutrient source but not as a complex substrate; on the other hand, the liquid fraction is a perfect raw material to develop the fumaric acid production process [69].

5. Bioreactors

While the most usual bioreactor for fumaric acid production is the batch bioreactor (results presented in Tables 1 and 2), some results, in Table 3, are presented for other configurations: bubble reactors, biofilm bioreactors, fluidized beds and fixed beds for immobilized microorganisms. Productivities of batch reactors are in the range of 0.18–0.7 g L^{-1} h^{-1}, with titers up to 121 g L^{-1}, and similar values are reached for other bioreactors. Coupling separation operations to bioreactors results in an enormous enhancement in productivity, i.e., 4.25 g L^{-1} h^{-1}.

Considering the raw material, glucose renders the best results in terms of productivity, yield and titer, but glycerol, xylose and hydrolyzed molasses also lead to promising results.

5.1. Stirred Tanks

Stirred tanks are widely used in research and industry due to their ease of control and handling during operation and the fact that they have low operation costs at the industrial scale, providing the process with great capacity and reliability [70].

The laboratory studies carried out with this kind of bioreactor have used volumes between 2 and 7 L, using glucose as a main substrate for *Rhizopus* species. When working with medium to high viscosity broths, Rushton turbines permit an adequate agitation and mixing that result in an enhanced oxygen mass transfer that is key to the production of chemicals by fungi. In the case of fumaric acid, high concentrations of oxygen in the broth yield high titers of the acid, but low productivities, while the situation is the inverse at low DO values. A combination of high and low DO, in this order, permits high titers of fumaric acid and high glucose conversions [71].

Concentrations and yields reached are not very remarkable (30–40 $g \cdot L^{-1}$), which shows slow production rates and a high substrate consumption for production [42]. The most important research using this type of bioreactor is a Dupont patent where 121 $g \cdot L^{-1}$ were produced, achieving promising yields to develop an industrial process that is economically profitable [72].

5.2. Bubble Column

Bubble column reactors can enhance the oxygen supply to the biomass and can reach a very good agitation rate, optimizing the air flow. In these reactors, a high air flow results in high gas phase hold-up and enhance oxygen mass transfer, with small gas bubbles being mixed with the liquid phase by bigger and helicoidally moving bubbles. Under these conditions, for fixed gas phase speeds, bubble columns (and airlift bioreactors) yield higher titers and productivities of fumaric acid than in stirred batch reactors [21].

With a 10-L bubble column containing *R. oryzae*, lower initial concentrations of glucose were required to reach very similar results as obtained in a stirrer tank reactor, improving the yield of the process and even doubling the productivity [73].

These results suggest that, with optimal morphology, agitation and oxygen supply are very important conditions to be optimized to develop a suitable industrial production process.

5.3. Immobilized Biomass

Filamentous fungi are known to be the best fumaric acid producers, mainly *Rhizopus* species. However, fungal growth presents many operational problems, such as obtaining an appropriate morphology or having high viscosity in the medium due to the development of the mycelium.

To avoid these operational problems, immobilized biomass reactors are a good solution because immobilization provides a very permanent morphology, protects microorganisms from pH or product inhibition, helps to purify final products, and enables production for longer periods of time [74].

5.3.1. Stirred Tank with Immobilized Biomass

The simplest use of immobilized biomass is suspending the particles in a stirrer-tank reactor (slurry reactor). In this process, many natural solids used as supports where the immobilization of microorganisms has been studied, for example, eggshell [75] or loofah fiber [70]. Some synthetic supports have been tried as well: plastic or metal nets [76].

Table 1. Main results in batch (tank) bioreactors with overhead impellers.

Raw Material	Species	Broth Composition (ref)	Operational Conditions	Main Results
Glucose	*Rhizopus arrhizus*	130 $g \cdot L^{-1}$ glucose 80 $g \cdot L^{-1}$ Ca^{2+} 1.8 $g \cdot L^{-1}$ NH_4^+ 0.3 $g \cdot L^{-1}$ KH_2PO_4 0.4 $g \cdot L^{-1}$ $MgSO_4 \cdot 7\ H_2O$ 0.044 $g \cdot L^{-1}$ $ZnSO_4 \cdot 7\ H_2O$ 0.0075 $g \cdot L^{-1}$ $FeCl_3 \cdot 6\ H_2O$	Strain NRRL 1526 Changes in stirring speed V = 3 L T = 34 °C N = 200–800 rpm pH = 5–7 Q_{air} = 0.5 $L \cdot L^{-1} \cdot min^{-1}$ t = 6 day	Titer = 121 $g \cdot L^{-1}$ Productivity = 1.02 $g \cdot L^{-1} \cdot h^{-1}$ Yield = 0.37 $g_{prod} \cdot g_{sust}{}^{-1}$ Reference [72]
		Several C/N ratios (140–200 $gC \cdot gN^{-1}$) 130 $g \cdot L^{-1}$ glucose 0.4 $g \cdot L^{-1}$ Yeast extract 0.4 $g \cdot L^{-1}$ $MgSO_4 \cdot 7\ H_2O$ 0.044 $g \cdot L^{-1}$ $ZnSO_4 \cdot 7\ H_2O$ 0.01 $g \cdot L^{-1}$ Fe tartrate 100 $g \cdot L^{-1}$ $CaCO_3$ 15 mL CH_3OH 0.5 mL corn oil	Strain NRRL 1526 Phosphorus stress V = 3 L T = 32 °C N = 800 rpm pH = 5.5 Q_{air} = 1 $L \cdot L^{-1} \cdot min^{-1}$ t = 6–10 day	Titer = 40 $g \cdot L^{-1}$ Productivity = 0.46 $g \cdot L^{-1} \cdot h^{-1}$ Yield = 0.33 $g_{prod} \cdot g_{sust}{}^{-1}$ Reference [48]
	Rhizopus oryzae	10% glucose 0.1% KH_2PO_4 0.05% $MgSO_4 \cdot 7\ H_2O$ 0.002% $ZnSO_4 \cdot 7\ H_2O$ 2.0% $CaCO_3$ 0.5% corn steep liquor Varied nitrogen source	Strain RUR709 V = 5 L T = 35 °C N = 400 rpm Q_{air} = 1 $L \cdot L^{-1} \cdot min^{-1}$ t = 4 day	Titer = 32.1 $g \cdot L^{-1}$ Productivity = 0.32 $g \cdot L^{-1} \cdot h^{-1}$ Yield = 0.45 $g_{prod} \cdot g_{sust}{}^{-1}$ Reference [35]
	Rhizopus oryzae	100 $g \cdot L^{-1}$ glucose 0.2 $g \cdot L^{-1}$ urea 0.6 $g \cdot L^{-1}$ KH_2PO_4 0.5 $g \cdot L^{-1}$ $MgSO_4 \cdot 7\ H_2O$ 0.11 $g \cdot L^{-1}$ $ZnSO_4 \cdot 7\ H_2O$ 0.0088 $g \cdot L^{-1}$ $FeSO_4 \cdot 7\ H_2O$ 50 $g \cdot L^{-1}$ $CaCO_3$	Strain ME-F01 GMO (ME-UN-8) V = 5 L T = 35 °C N = 400 rpm pH = 5.5 Q_{air} = 0.5 $L \cdot L^{-1} \cdot min^{-1}$ t = 4 day	Titer = 52.7 $g \cdot L^{-1}$ Productivity = 0.54 $g \cdot L^{-1} \cdot h^{-1}$ Reference [37]
		100 $g \cdot L^{-1}$ glucose 2.0 $g \cdot L^{-1}$ urea 0.6 $g \cdot L^{-1}$ KH_2PO_4 0.5 $g \cdot L^{-1}$ $MgSO_4 \cdot 7\ H_2O$ 0.11 $g \cdot L^{-1}$ $ZnSO_4 \cdot 7\ H_2O$ 0.0088 $g \cdot L^{-1}$ $FeSO_4 \cdot 7\ H_2O$ 50 $g \cdot L^{-1}$ $CaCO_3$	Strain ME-F12 Oxygen control (stage 1: 80% OD; stage 2: 30% OD) V = 7 L T = 35 °C t = 5 day	Titer = 56.2 $g \cdot L^{-1}$ Productivity = 0.7 $g \cdot L^{-1} \cdot h^{-1}$ Yield = 0.54 $g_{prod} \cdot g_{sust}{}^{-1}$ Reference [71]

Table 1. *Cont.*

Glucose	*Rhizopus oryzae*	10 $g \cdot L^{-1}$ glucose 2.0 $g \cdot L^{-1}$ Urea 0.6 $g \cdot L^{-1}$ KH_2PO_4 0.25 $g \cdot L^{-1}$ $MgSO_4 \cdot 7\ H_2O$ 0.088 $g \cdot L^{-1}$ $FeSO_4 \cdot 7\ H_2O$	Strain ATCC 20344 O_2 and pH control V = 2 L T = 35 °C N = 600 rpm pH = 2.8–6.3 (pH_0 = 3.2) Q_{air} = 0.5 $L \cdot L^{-1} \cdot min^{-1}$ t = 7 day	Titer = 30.2 $g \cdot L^{-1}$ Productivity = 0.18 $g \cdot L^{-1} \cdot h^{-1}$ Yield = 0.28 $g_{prod} \cdot g_{sust}{}^{-1}$ Reference [21]
Manure	*Rhizopus oryzae*	100 $g \cdot L^{-1}$ glucose 0.6 $g \cdot L^{-1}$ KH_2PO_4 0.25 $g \cdot L^{-1}$ $MgSO_4 \cdot 7\ H_2O$ 0.088 $g \cdot L^{-1}$ $ZnSO_4 \cdot 7\ H_2O$ Fiber hydrolases	Strain ATCC 20344 pH control V = 1 L T = 30 °C N = 200 rpm pH = 5 Q_{air} = 1 $L \cdot L^{-1} \cdot min^{-1}$ t = 4 day	Titer = 31 $g \cdot L^{-1}$ Productivity = 0.31 $g \cdot L^{-1} \cdot h^{-1}$ Yield = 0.31 $g_{prod} \cdot g_{sust}{}^{-1}$ Reference [78]

Table 2. Main results in batch shaken flasks (orbital stirring).

Raw Material	Species	Broth Composition	Operational Conditions	Main Results
Glucose	*Rhizopus nigricans*	50 $g \cdot L^{-1}$ glucose 2.0 $g \cdot L^{-1}$ $(NH_4)_2SO_4$ 0.5 $g \cdot L^{-1}$ KH_2PO_4 0.5 $g \cdot L^{-1}$ $MgSO_4 \cdot 7\ H_2O$ 0.01 $g \cdot L^{-1}$ $CaCl_2$ 0.01 $g \cdot L^{-1}$ $Fe_2(SO_4)_3$	Strain 45 Isocitrate lyase extraction V = 0.25 L T = 28 °C pH = 7.0 t = 3.5 day	Titer = 20 $g \cdot L^{-1}$ Productivity = 0.25 $g \cdot L^{-1} \cdot h^{-1}$ Yield = 0.66 $g_{prod} \cdot g_{sust}{}^{-1}$ Reference [79]
Glycerol	*Rhizopus arrhizus*	0–40 $g \cdot L^{-1}$ glucose 40–80 $g \cdot L^{-1}$ glycerol 0.3 $g \cdot L^{-1}$ peptone 1.55 $g \cdot L^{-1}$ KH_2PO_4 1.0 $g \cdot L^{-1}$ $MgSO_4 \cdot 7\ H_2O$ 0.0176 $g \cdot L^{-1}$ $ZnSO_4 \cdot 7\ H_2O$ 0.0005 $g \cdot L^{-1}$ $FeSO_4 \cdot 7\ H_2O$ 50 $g \cdot L^{-1}$ $CaCO_3$	Strain RH-07-13 Crude glicerol alone (80 $g \cdot L^{-1}$) or Crude glicerol-glucose mixture (40 $g \cdot L^{-1}$ each) V = 0.25 L T = 30 °C N = 200 rpm pH = 5.5 t = 8 day	Titer = 22.8 $g \cdot L^{-1}$ Productivity = 0.16 $g \cdot L^{-1} \cdot h^{-1}$ Yield = 0.35 $g_{prod} \cdot g_{sust}{}^{-1}$ Reference [62]
Hydrolyzed molasses	*Rhizopus oryzae*	107.1 $g \cdot L^{-1}$ sucrose 69.6 $g \cdot L^{-1}$ $CaCO_3$ 1.34 $g \cdot L^{-1}$ $(NH_4)_2SO_4$ 0.26 $g \cdot L^{-1}$ $MgSO_4 \cdot 7\ H_2O$ 0.53 $g \cdot L^{-1}$ KH_2PO_4 0.042 $g \cdot L^{-1}$ $ZnSO_4 \cdot 7\ H_2O$ 0.086 $g \cdot L^{-1}$ $Fe_2(SO_4)_3 . H_2O$ 0.01 $g \cdot L^{-1}$ $MnSO_4 \cdot H_2O$ 0–150 $mg \cdot L^{-1}$ $NiCl_2 \cdot H_2O$	Invertase for hydrolysis Ni^{2+} effects T = 28 °C T = 6 day	Titer = 68 $g \cdot L^{-1}$ Productivity = 0.48 $g \cdot L^{-1} \cdot h^{-1}$ Yield = 0.64 $g_{prod} \cdot g_{sust}{}^{-1}$ Reference [80]
Corn stover	*Rhizopus oryzae*	80 $g \cdot L^{-1}$ glu or xyl 0.2 $g \cdot L^{-1}$ urea 0.6 $g \cdot L^{-1}$ KH_2PO_4 0.5 $g \cdot L^{-1}$ $MgSO_4 \cdot 7\ H_2O$ 0.11 $g \cdot L^{-1}$ $ZnSO_4 \cdot 7\ H_2O$ 0.0088 $g \cdot L^{-1}$ $FeSO_4 \cdot 7\ H_2O$ 50 $g \cdot L^{-1}$ $CaCO_3$	Strain ATC 20344 Diluted H_2SO_4 acid CS pretreatment: Liquid phase rich in xylose Enzymatic Hydrolysis: Liquid phase rich in glucose V = 0.25 L T = 35 °C N = 200 rpm pH = 3.0 (pH_0 = 4.0) t = 3.5–4.5 day	Titer = 27.79 $g \cdot L^{-1}$ Productivity = 0.33 $g \cdot L^{-1} \cdot h^{-1}$ Yield = 0.35 $g_{prod} \cdot g_{sust}{}^{-1}$ Reference [81]
Xylose	*Rhizopus arrhizus*	80–100 $g \cdot L^{-1}$ xylose 0.4 $g \cdot L^{-1}$ urea 0.6 $g \cdot L^{-1}$ KH_2PO_4 0.5 $g \cdot L^{-1}$ $MgSO_4 \cdot 7\ H_2O$ 0.0018 $g \cdot L^{-1}$ $ZnSO_4 \cdot 7\ H_2O$ 0.0005 $g \cdot L^{-1}$ $FeSO_4 \cdot 7\ H_2O$ 45 $g \cdot L^{-1}$ $CaCO_3$	Strain RH-07-13 GMO Adapted to several xylose concentrations (50–100 $g \cdot L^{-1}$) V = 0.25 L T = 32 °C N = 220 rpm pH = 5.5 t = 7 day	Titer = 38.48 $g \cdot L^{-1}$ Productivity = 0.23 $g \cdot L^{-1} \cdot h^{-1}$ Yield = 0.43 $g_{prod} \cdot g_{sust}{}^{-1}$ Reference [40]

Table 2. *Cont.*

Hydrolyzed yucca bagasse and potato residue	*Rhizopus formosa*	Weight ratio 80:20 yucca bagasse: potato waste Weight C/N: 168 (C = cassava bagasse; N = KNO_3) 0.15 g·L^{-1} KH_2PO_4 0.25 g·L^{-1} $MgSO_4$·7 H_2O 0.04 g·L^{-1} $ZnSO_4$·7 H_2O 20 g·L^{-1} $CaCO_3$ 10 g·L^{-1} biotin 15 mL·L^{-1} CH_3OH	Strain MUCL 28422 Two stage raw material enzymatic hydrolysis T = 32 °C N = 200 rpm pH = 6.5	Titer = 21.28 g·L^{-1} Yield = 0.23 g_{prod}·$g_{sust}$$^{-1}$ Reference [38]

Table 3. Main results in batch special reactors.

Raw Material	Species	Broth Composition	Reactor Type and Operational Conditions	Main Results
Glucose	*Rhizopus oryzae*	95 g·L^{-1} glucose 0.3 g·L^{-1} urea 0.1 g·L^{-1} KH_2PO_4 0.05 g·L^{-1} $MgSO_4$·7 H_2O 0.01 g·L^{-1} $ZnSO_4$·7 H_2O	Bubble column Strain ATCC 20344 Effects of basic agents ($CaCO_3$, $Ca(OH)_2$ and $NaHCO_3$) V = 10 L T = 32 °C pH = 5.5 Q_{air} = 1.5 L·L^{-1}·min^{-1} t = 1.5 day	Titer = 37.2 g·L^{-1} Productivity = 1.03 g·L^{-1}·h^{-1} Yield = 0.53 g_{prod}·$g_{sust}$$^{-1}$ Reference [73]
	Rhizopus arrhizus	80 g·L^{-1} glucose 0.044 g·L^{-1} urea 0.6 g·L^{-1} KH_2PO_4 0.5 g·L^{-1} $MgSO_4$·7 H_2O 0.002 g·L^{-1} $ZnSO_4$·7 H_2O 60 g $CaCO_3$ 6 mL soybean oil	Batch slurry reactor GMO strain RH-7-139# Effects of support (stainless steel, loofah fiber, sponge) V = 5 L, 5 g L^{-1} support T = 30 °C Q_{air} = 2.0 L·L^{-1}·min^{-1} N = 300–500 rpm t = 120 h	Best N = 400 rpm Titer = 30.3 g·L^{-1} Productivity = 0.63 g·L^{-1}·h^{-1} Yield = 0.211 g_{prod}·$g_{sust}$$^{-1}$ Reference [70]
Hydrolyzed molasses	*Rhizopus oryzae*	100 g·L^{-1} glucose 2.0 g·L^{-1} urea 0.6 g·L^{-1} KH_2PO_4 0.25 g·L^{-1} $MgSO_4$·7 H_2O 0.088 g·L^{-1} $ZnSO_4$·7 H_2O Base $CaCO_3$	Rotating biofilm reactor Strain ATCC 20344 Compares RBC with batch reactor V = 2 L Biodisc surface: 750 cm^2 T = 35 °C pH = 5 Q_{air} = 1.0 L·L^{-1}·min^{-1} t = 20 h	Titer = 85.0 g·L^{-1} Productivity = 4.25 g·L^{-1}·h^{-1} Theoretical yield = 0.91 g_{prod}·$g_{sust}$$^{-1}$ Reference [74]
Corn stover	*Rhizopus arrhizus*	50 g·L^{-1} glucose molasses 0.25 g·L^{-1} $(NH_4)_2SO_4$ 0.4 g·L^{-1} yeast extract 0.45 g·L^{-1} corn oil 0.3 g·L^{-1} KH_2PO_4 0.4 g·L^{-1} $MgSO_4$·7 H_2O 0.044 g·L^{-1} $ZnSO_4$·7 H_2O 0.01 g·L^{-1} Fe tartrate	Fluidized bed reactor Strain NRRL 1526 Polyurethane immobilized microorganisms V = 0.25 L T = 32 °C pH = 6.0 Q_{air} = 3.0 L·L^{-1}·min^{-1} t = 1–3 day	Titer = 17.5 g·L^{-1} Productivity = 0.36 g·L^{-1}·h^{-1} Yield = 0.36 g_{prod}·$g_{sust}$$^{-1}$ Reference [77]

Using loofah fiber as a support, promising yields (0.21 g of fumaric acid per gram of glucose) and concentrations (30.3 g·L^{-1} in the broth) were obtained, proving the potential use of loofah fiber to immobilize biomass in the fermentative fumaric acid production process [70].

5.3.2. Rotary Biofilm Contactor

The rotary biofilm contactor (RBC) or biodisc reactor is widely used in wastewater treatments due to its advantages such as a low required operation volume or its handling as a series of perfectly-stirred tank reactors. The immobilization is carried out by inoculating spores on the discs: a biofilm with a thickness smaller than 2 mm is developed. These bioreactors need a low turning speed to have a better contact between the biomass and the medium. High productivity rates have been achieved using a 2-L biodisc reactor coupled to an anion-exchange adsorption column that liberated hydroxyl ions, maintaining the pH at 4.5, thus avoiding high concentrations of byproducts due to metabolic switching. High productivities (4.25 g·L^{-1}·h^{-1}) and titers (85 g·L^{-1}) were obtained due to the combination

of production and adsorption (reactive adsorption), suggesting that fumaric acid inhibits its own production, and pH control is key to reach the best possible results [21,74].

5.3.3. Fluidized Bed

Using small polymer particles as support for the immobilization, fluidized beds could be used. This kind of reactor is very useful in catalytic industrial processes and in the oil & gas industry where is widely employed. However, the fluidized bed has proven as not very suitable for biotechnological processes, as low yields (35.56%) and final concentrations (12.3 $g \cdot L^{-1}$) are obtained at very high operational costs [77].

In Tables 1–3, the run operations in several bioreactors are described, indicating operational conditions, broth composition, and main results (regarding acid titer, productivity and yield of substrate for this product). In Figure 4, schemes of the most promising bioreactors are shown.

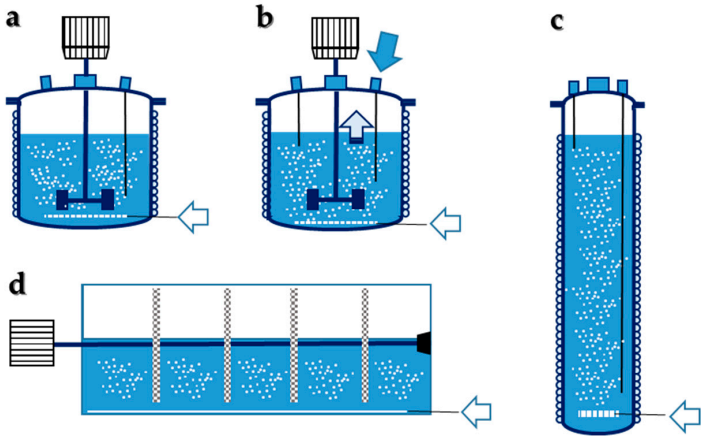

Figure 4. Different types of bioreactors for fumaric acid production: (**a**) Stirred tank -batch operation; (**b**) Stirred tank–fed-batch operation; (**c**) bubble-column reactor; (**d**) Rotary biofilm contactor.

5.4. Comparison

Different reactor types have been compared according to performance and cost effectiveness criteria; all types have been compared using the stirrer-tank reactor as a reference: (+) means a better result than the stirrer-tank reactor, (−) means a worse result and (=) means a similar result. In Tables 2–4, the run operations in several bioreactors are described, indicating operational conditions, medium composition, and main results (regarding acid titer, productivity and yield of substrate for this product).

Table 4. Reactors for fumaric acid bio-production as compared to the classical batch reactor: strengths and weaknesses.

Reactor	Final Concentration	Productivity	Yield	Production Costs
Bubble column	=	++	+	=
Biodisc	++	++++	++	−
Fluidized bed	−	−	=	−

As seen in the table, the best results are obtained when operating a stirrer-tank reactor, with or without immobilized biomass, and biodisc reactors, highlighting the results and procedures obtained from the Dupont patent, in terms of final concentration, and the results of the Rotating Disc Contactor (RBC) in terms of productivity.

When choosing the best possible reactor, one of the greatest problems, as stated before, is the high increase of the medium viscosity, which requires a great amount of energy to maintain correct homogenization and high mass transfer rates inside the reactor.

The reactors most adequate for this task at a lower energy input will probably have no direct mechanical agitation by impellers. Bubble columns, air lifts and fluidized beds, where a very acceptable turbulence level is provided, could permit an optimal control of viscosity and oxygen concentrations. To reduce the energy input even more, a good reactor is the rotary biodisc contactor (RBC), which provides an acceptable agitation level, a good gas-solid mass transfer, ensuring adequate oxygenation of the biomass, and a fast liquid-solid mass transfer.

Direct mechanical agitation not only involves high energy inputs but also high shear stress at the tips of the impellers (usually, Rushton turbines) commonly implemented in industrial bioreactors. This high shear stress results in hydrodynamic stress that is most deleterious to biomass integrity. Again, the absence of impellers and agitation by bubbles reduce this mechanical stress to the cells. This is also correct in the case of immobilizing the biomass on the disc of the RBC. In both cases, is possible to keep an optimal metabolic stage of the biomass.

Immobilized biomass has also several advantages like the separation between biomass and the medium, avoiding possible inhibitions and controlling the metabolic state of the biomass that can be altered to reach better yields by choosing the appropriate immobilization method, support and conditions.

To sum up, rotary biodisc contactors are very promising reactors to host fumaric acid production process using filamentous fungi because of their characteristics related to immobilization and low energetic requirements, opening the door for reaching high yields with an adequate selectivity and thus reducing the presence of by-products. Productivities can also be dramatically enhanced by coupling the fermentation process in RBC with separation processes like adsorption on ion-exchange resins. In particular, a wise use of processing time is most relevant to biorefinery processes, and high productivities are sought, reaching, for example, more than 22 g L^{-1} h^{-1} for the case of lactic acid [82] and *circa* 3 g L^{-1} h^{-1} for succinic acid [83]. Using productivity as the most relevant technical variable in a biorefinery process of this kind, RBC combined with adsorption results in a comparable productivity of 4.25 g L^{-1} h^{-1}, which compares well to the cases of lactic acid and succinic acid, rendering this fermentative process to fumaric acid the best one reported until now, with a productivity that is ten times higher than the average batch productivity [74]. The main variables and values of this process are presented in bold in Table 3.

6. Downstream Processing: Fumaric Acid Purification

After the production of fumaric acid in bioprocesses, its low solubility in water and its high polarity can be employed to separate it from other acids, salts, remaining carbohydrates and nitrogen compounds. The traditional purification process is quite complex and involves high operational costs (acidification, heating, filtration, drying) [11,21,84].

Extraction with amines, such as tridodecilamine (TDA), in the presence of ketones as diluents, provides a simple means for fumaric acid purification from watery broths, with maximum extraction productivities around 96% [85]. Low solubility of the acid permits its precipitation from the broth followed by a further polishing using adsorption with active carbon to yield a polymer-grade monomer [86]. In fact, adsorption can be applied to separate the acid from the broth, and, afterwards, acetone can desorb it with a high recovery yield (93%), while a subsequent water sweeping could enhance its purity to 98% [84]. This adsorption can be also performed using ion-exchange resins, like IRA-900; in fact, the use of intermittent in-situ removal of the acid from the broth coupling the bioprocess with the separation unit allowed for a 25% increase in fumaric acid yield and an even higher increase in productivity, as the acid is an inhibitor of its own production by fungi [87]. This mixing of operations in one operation unit (hybrid operation) to enhance final productivities and yields is commonly performed in the industry. In the case of fumaric acid, some more examples can be

encountered: for example, the reactive extraction of the acid with non-toxic solvents, with extraction efficiencies in excess of 92% [88], or the combination of membrane technologies, nanofiltration and bipolar electrodialysis, with reactive extraction, with a 90% efficiency [89]. In some cases, the separation of fumaric acid from other by-products, like acetic acid, presents a notable difficulty, and complex chromatographic techniques involving ion-exchange resins in simulated moving-beds schemes have been developed for the task, attaining high purity (99%) and recovery (99%) of the target acid [90].

7. Conclusions and Future Prospects

Nowadays, fossil resources, though impressive in mass even currently, are envisaged as non-sustainable due to them being non-renewable, their impending reduction in terms of mass and accessibility, and the pollution derived from their massive utilization. In this framework, biorefineries are conceptually considered as refineries whose resource is biomass, a renewable and accessible raw material whose variety reduces energy and resource dependence. Chemicals from biomass, as succinic or fumaric acid, are thus, platform chemicals that have the potential to complement and even replace petrochemicals as monomers in the polymer industry. However, although succinic acid is now known to have an adequate price (1.17 $/kg) in comparison to its petrochemical counterpart (2.86 $/kg) for the Myriant process [91], fumaric acid is only produced at the present time from benzene or from butanes. Using as reference the lonely LCA analysis performed for succinic acid and, in particular, for Myriant's process, it can be envisaged that bioprocesses to fumaric acid will be, in the worst scenario, as sustainable as the process of the reference is, with a worse use of raw materials (if compared to the petrochemical counterparts), but a much better use of energy and a high reduction in the global warming potential (GWP). By the end of the last century, costs for bio-based fumaric acid, even in the best case, almost doubled costs of fumaric acid obtained from benzene [92]. It is probable than the optimization of the rotary biofilm contactor coupled to adsorption, which quadruplicates productivity of the batch operation in stirred tanks, reaching equal or higher fumaric acid titer, should result in a notable reduction in operational costs. Further improvement of this process could result in operating adsorption under a simulated moving bed mode. Though $CaCO_3$ is known to enhance fumaric acid productivity, hybrid operation and the use of Na_2CO_3 should result in a process that resembles that of succinic acid, with low-cost fumaric acid as the end product.

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