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# Exploring the Microbial Production of Aromatic Fine Chemicals to Overcome the Barriers of Traditional Methods

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**ABSTRACT**

*Aromatic fine chemicals are compounds with great industrial value due to their particular properties such as antioxidant activity. However, the rising demand for these compounds is in risk due to many issues regarding their supply by the petrochemical industry. Although natural sources of aromatics such as plant extracts and lignin are not as attractive as oil because of the current barriers on the traditional methods of extraction and purification, the last advances in Metabolic Engineering and Bioprocess Optimization have enhanced the microbial conversion of biomass into aromatic compounds, overcoming many of these problems. This review compares the benefits and constraints of different technologies that have already been applied in the obtainment of aromatics from biomass and suggest a roadmap for profitable biorefineries through the co-production of aromatic fine chemicals and biofuels.*

**Keywords:** Aromatics, Biomass, Biorefinery, Fine chemicals, Metabolic engineering, Synthetic biology

## INTRODUCTION

Aromatic compounds are organic molecules containing benzene in their structures, which is characterized as a very stable cyclic functional group formed by six atoms of carbon. The principle behind this stability was elucidated in 1865 by August Kekulé who understood the alternation of double and single bonds in benzene [1]. This property is so important to the chemical industry that more than 40% of bulk chemicals, simple substances of low cost and high volume production, have an aromatic ring in their structure [2]. In addition, aromatic compounds with different levels of structural complexity are also present among the so-called fine chemicals, which are categorized as more valuable because of special properties such as antioxidant activity, allowing their application into important segments as the pharmaceutical and the agrochemical [3,4].

About 60% of the global production of aromatics comes basically from catalytic reforming or cracking units, which use naphtha fractions derived from oil to produce benzene, toluene and p-xylene (BTX) (Figure 1) [2,5-8]. Despite these compounds are the basic precursors of many aromatic molecules they are used mainly to obtain high-octane gas oil, creating a condition that submits the values of BTX to variations in gas oil global prices. Benzene is the most important building block used to produce aromatics, being alkylated with propylene to form cumene, a molecule that is then oxidized to generate acetone and phenol, hydroxylated benzene from which most of aromatic fine chemicals are made of. Alternatively, the partial oxidation of toluene to benzoate and its decarboxylation also creates phenol as product [9]. However, recent publications have demonstrated that the generation of BTX from petrochemical sources has limitations that put at risk the fine chemicals supply. The increasing replacement of naphtha by shale gas to obtain ethane, for example, has reduced the importance of catalytic reforming and cracking units, even though the demand for aromatics has risen significantly over the last years [10]. Alongside these problems, the replacement of oil to renewable sources is also an urgent concern in many countries due to the shortage of new reserves [11] and the environmental consequences of its consumption, such as global warming [12]. To tackle this, the consumption of biomass as feedstock has become a main target to the chemical industry although the scarcity of agricultural lands and the competition with the food and biofuel industries for commodities have imposed constraints on the development of this strategy [13]. In this context countries with a well-established leadership in agriculture as Brazil and the United States have invested millions to expand their biofuels industry by using agricultural waste as feedstock, resulting in second generation biofuels [14], a strategy that has been recently considered for aromatic fine chemicals [15]. However, the current thermochemical processes used to convert biomass residues into chemicals are highly expensive because of the recalcitrance of lignocellulosic material, requiring new technologies capable to turn them more feasible [16].

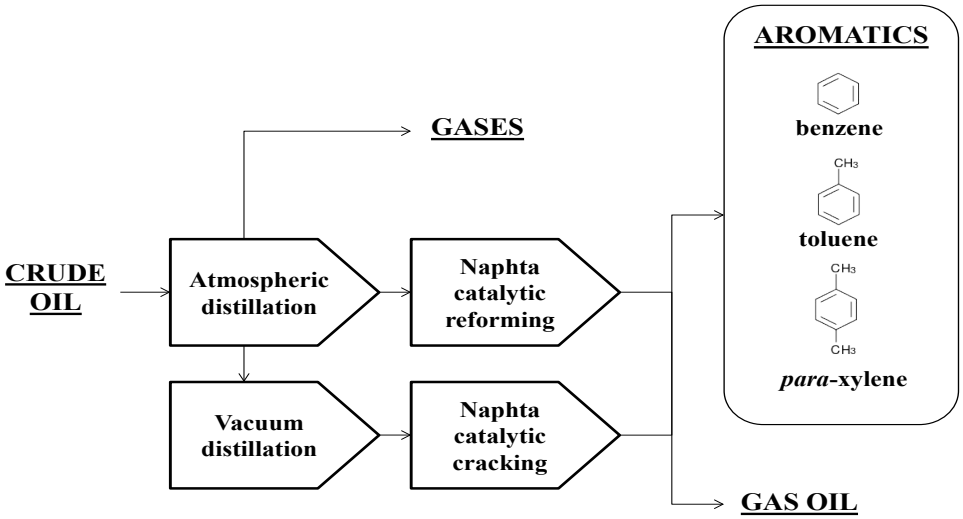


**Figure 1:** General scheme of aromatics production from petrochemical routes

One promising solution to this problem is the development of bioprocesses where microorganisms consume simple biomass sources to produce desired molecules from them, something that has showed a good potential to the industry of aromatics [17-27]. While these organisms are capable of consuming different nutrient sources, they can also produce phenolic metabolites that can be converted into different aromatic fine chemicals in environmental conditions after the application of simple genetic manipulation techniques [28]. In order to provide the current status on this subject and guidance for future researches this review aims to provide an insight on how the microbial production of aromatic fine chemicals can overcome the current problems faced by traditional methods. The benefits and limitations of these methods will be compared along with a view on the newest accomplishments biotechnology has achieved in the field. In the end, the perspectives on the incorporation of this approach in biorefineries will be discussed, considering key features that can make it a good strategy for a sustainable production chain.

## TRADITIONAL METHODS TO OBTAIN AROMATIC FINE CHEMICALS FROM BIOMASS

### Plant extract purification

The consumption of biomass as a source of aromatics has been done for a long time by mankind, mainly by obtaining phenolic compounds from plant extracts [28,29]. Such chemicals are part of the secondary metabolism of these organisms, which means that they are not directly connected to growth, although they offer competitive advantages, such as resistance against pathogens and predators [30]. Almost all aromatic compounds produced by organisms derive from the shikimic acid pathway, while a few come from the malonic acid pathway or from a combination of both routes, as flavonoids and their derivatives [31-33]. Also known as the biosynthetic pathway of aromatic amino acids (phenylalanine, tryptophan and tyrosine), the shikimic acid pathway begins with the condensation of a molecule of erythrose-4-phosphate (E4P), supplied by the pentose phosphate pathway, with phosphoenolpyruvate (PEP), derived from the Embden-Meyerhof-Parnas pathway, to form 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP). Then, new reactions lead to the formation of chorismate (CHO), which can be converted into other intermediates to form aromatic amino acids (Figure 2). In the end of the pathway, phenylpropanoids can be formed from phenylalanine and tyrosine, being grouped into simple phenylpropanoids, coumarins, lignin precursors or flavonoids, which is the largest group of plant phenolic compounds, covering anthocyanins (natural dyes), isoflavones (antimicrobials) and condensed tannins (antioxidants) (Figure 2) [33,34].

Currently, the main process to obtain aromatic compounds from biomass is the extraction and purification of these compounds from medicinal and aromatic plants (MAPs) [28,34,35]. In this case, unlike the production of commodities as sugar and vegetable oils, which can be produced in large volumes by species of high productivity, MAPs are often seasonal wild strains grown in small areas. Furthermore, due to the fact that such compounds are normally present in small concentrations along with several by-products with similar characteristics in complex polymeric structures, sophisticated purification techniques must be employed, increasing the costs of the process [36]. To tackle this, the use of fungi and bacteria in both submerged (SmF) and solid-state fermentation (SSF) has become a good strategy

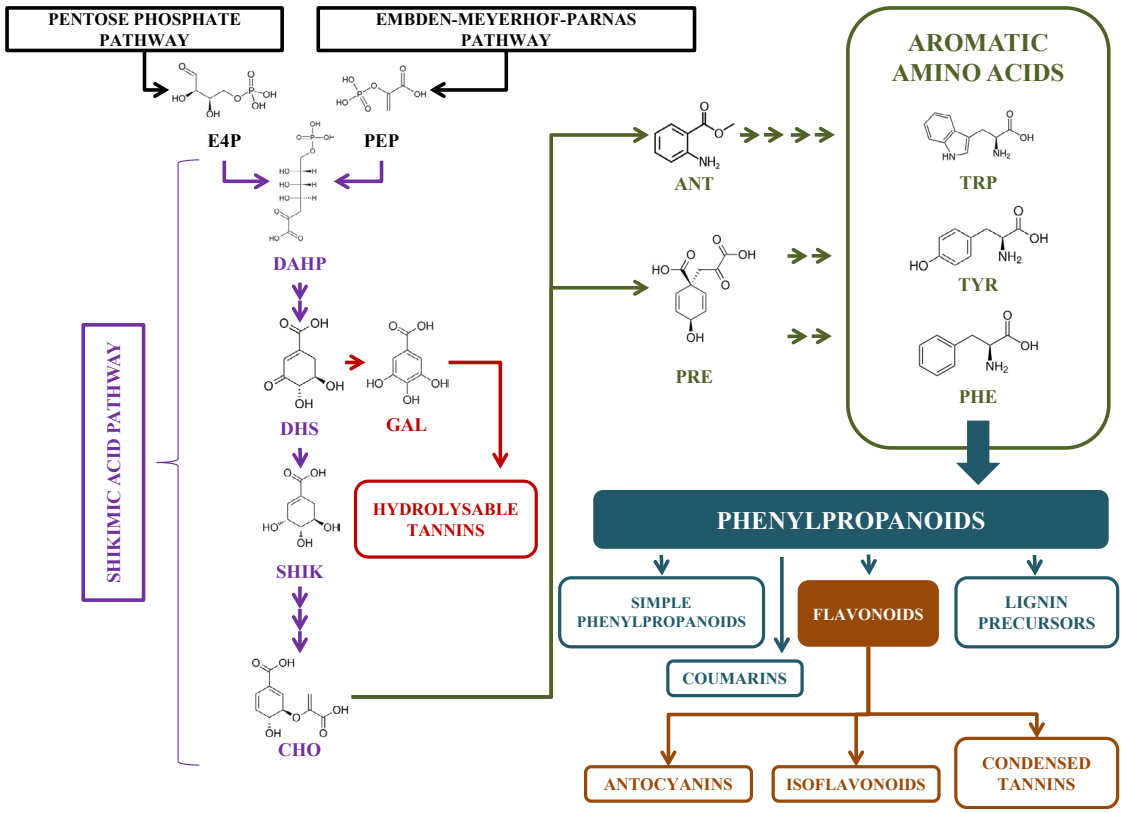


**Figure 2:** General scheme of the shikimic acid pathway and its phenolic derivatives

E4P: Erythrose-4-Phosphate; PEP: Phosphoenolpyruvate; DAHP: 3-Deoxy-D-Arabino-Heptulosonate 7-Phosphate; DHS: 3-Dehydroshikimate; GAL: Gallic Acid; SHIK: Shikimic Acid; CHO: Chorismate; ANT: Anthranilate; PRE: Prephenate; TRP: Tryptophan; TYR: Tyrosine; PHE: Phenylalanine

known as bioconversion, exploring their metabolism and enzymes to turn complex phenolic polymers into simpler molecules, enriching the broth with the desired molecules [37]. As an example, to produce the antibiotic trimethoprim, gallic acid must be isolated from hydrolysable tannins of some trees by tannase producing fungi [38]. Even though more effective than the physicochemical hydrolysis, this process still requires a large number of steps, making it less attractive to replace petrochemical routes.

**Organic residues valorization**

Another important approach used to explore the production of chemicals from biomass is the valorization of organic residues, which uses physicochemical or biological techniques to recover valuable molecules from agricultural waste. The main criterion to select this biomass source is its qualification as a residue, covering from discarded fruits and vegetables or animal waste and meat until lignocellulosic material, such as wood or bagasse [39]. While physicochemical methods target the separation of fine chemicals from these residues, biological fermentation strategies aim to detoxify the feedstock in order to remove by-products and use them as nutrient sources to the microbial production of different compounds. The valorization of passion fruit (*Passiflora edulis*) seeds is an example of the application of physicochemical approaches to obtain aromatic fine chemicals [40]. In order to solve the degradation and loss of bioactivity of phenolic compounds caused by maceration and organic solvents sophisticated techniques started to be considered, as the ultrasound-assisted and supercritical fluid extractions. Although these techniques result in extracts with superior concentration of phenolics with high rates of antioxidant activities, the problems related to their purity are still present. To cope with that, filamentous fungi have been extensively used in SSF to allow the valorization of specific molecules from organic residues. As an example, the fermentation of cauliflower (*Brassica oleracea* L. var. *botrytis*) outer leaves by the fungus *Aspergillus sojae* has enabled a higher recovery of less glycolylated forms of kaepmferol when compared to extracts before fermentation [41]. It happens due to the production and release of many enzymes by this microorganism, making it not just capable to detoxify the feedstock, but also to promote bioconversions. However, in spite of the benefits of fungal SSF, sometimes these organisms can release undesired molecules during the process as a result of their complex secondary metabolism, making product recovery still difficult [37].

**Lignin depolymerization**

The primary source of aromatics in biomass is lignin, a polymer that sustains the cell walls of most plants. Unlike cellulose and hemicellulose, which are sugar polymers also present in plant cell walls, lignin has a polyphenolic structure that confers resistance to chemical and biological degradation, due to its high hydrophobicity [42-44]. Its biosynthesis occurs basically by an irregular and highly branched polymerization of three phenolic compounds: trans-p-coumaryl alcohol (phenyl), coniferyl alcohol (guaiacyl) and synapyl alcohol (syringyl), collectively known as monolignols [42]. The high availability of lignin in nature is of extreme interest to a bioeconomy, being estimated that over 30% of existing organic carbon is stored in its structure, which gives it the status of the second most abundant biopolymer in the planet [43,45]. In addition to this benefit, lignin is not consumed by the food industry, unlike other types of biomass such as starch, sucrose and vegetable oils, often being treated as an agro industrial residue [15]. With a production that reaches 60 kton/year, the lignin obtained as a by-product from the production of cellulose has the potential to provide both bulk and fine chemicals such as vanillin, one of the key molecules used by the industry of fragrances and also highly targeted for biopolymers production [46-50]. Recently, the company Virent has developed a process that integrates the aqueous phase reforming of lignocellulosic materials with catalysts to create a biobased gasoline blend with a high content of aromatics [51]. With that blend, it is possible to recover BTX, allowing the establishment of a high-potential refinery in which aromatic fine chemicals can be produced by traditional chemical methods.

Various techniques have been developed to isolate aromatic compounds from lignin. However, before this step, the carbohydrates cellulose and hemicellulose must be removed from the lignocellulosic material to reduce impurity by techniques such as the Kraft process, in which wood is subjected to high temperatures in the presence of sodium sulphide in alkaline conditions, resulting in a black liquor rich in soluble lignin [52]. In addition, heating the material with sulphide, sodium hydroxide, or organic solvents (the organosolv process) can also be employed, along with the hydrolysis of carbohydrates by enzymes or steam explosion [44,53,54]. After such removal, the lignin can be depolymerized in simple aromatics by thermal, chemical or biological procedures (Figure 3). Despite all these advances, the difficulties related to the extraction and purification of lignin fibers result in high costs when compared to the phenolic compounds derived from petrochemical routes or MAPs [55]. Furthermore, given the high recalcitrance of lignin, the costs of its depolymerization are also high, mainly because of the use of chemical catalysts and thermal consumption [43,44,54,56]. However, microbial lignin valorization has been considered a potential strategy due to the capability of many organisms not just to produce enzymes that can depolymerize its complex structure but also to grow in the presence of inhibitory compounds released during this process and to consume lignin monomers as carbon sources to produce many compounds in a consolidated bioprocess (CBP) [57].

## MICROBIAL CONVERSION OF BIOMASS INTO AROMATICS: A NEW APPROACH WITH GREAT PERSPECTIVES

**Potential sources of biomass as non-expensive feedstock**

To guarantee the success of a microbial production of biochemicals choosing the right feedstock is of extreme importance, as it will guide decisions on the microorganism selection, such as the necessary metabolic changes to be implemented. Because simple carbon sources, such as vegetable oil, starch or sucrose, are easily obtained from agricultural commodities, the use of these feedstocks in biotechnological processes has been the main strategy adopted by bioprocess engineers [58]. However, given that these raw materials are used both for food and biofuel production, the price of sugar undergoes a series of influences, featuring high volatility [59]. As a result, biomass derived from agro-industrial waste, such as lignocellulose, gained great relevance in the last decade, making it a great bet for a bioeconomy [60].

Cellulose is the major component of lignocellulosic materials being a highly recalcitrant glucose polymer that normally needs to be depolymerized prior to the consumption of its monomer in a bioprocess. However, it comprehends similar treatments to the isolation of lignin (Figure 3) which not just requires highs amounts of energy and chemicals but also releases inhibitory compounds in the process. Although the cost of lignocellulose is very low compared to the cost of sucrose (about 30-45US$/dry ton and 350-371US$/ton, respectively) [58], the process of obtaining simple sugars from this biopolymer is still very expensive, mainly due to the use of hydrolytic enzymes for its depolymerization [61]. The lack of a highly efficient enzymatic consortium and intellectual property issues tend to keep the cost of these biocatalysts extremely high, reaching around US$5.38/kg [62].

Another option coming from a different agricultural waste is the raw glycerol resulting from the transesterification of vegetable oils during biodiesel production. Although glycerol is considered a molecule of high market value for

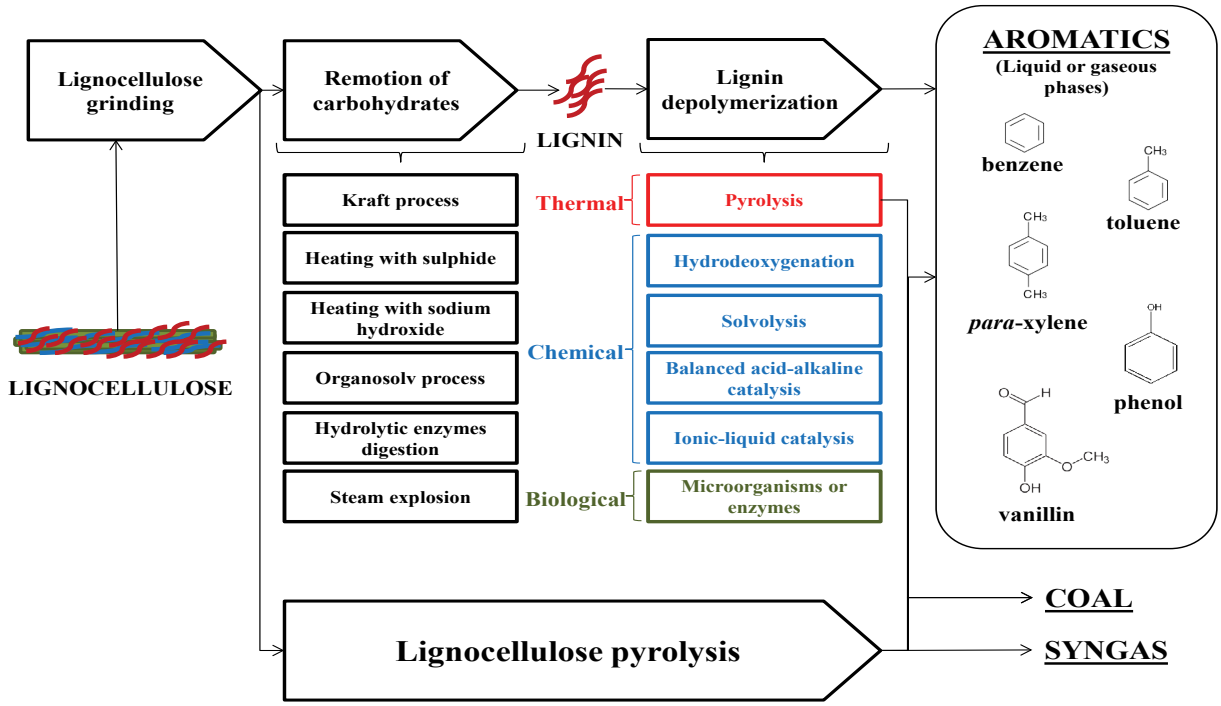


**Figure 3:** General scheme of aromatic compounds extraction from lignocellulose

its direct application in cosmetics and toiletries, the raw glycerol has some contaminants such as methanol, salts and traces of metals, which requires purification processes that lift its price [63]. However, many reports indicate the existence of microbial strains capable of consuming raw glycerol as a carbon source, such as a strain of *Escherichia coli* that produces amino acids for the food industry, a strain of *Clostridium butyricum* that produces 1,3-propanediol and one of *Pseudomonas putida* that produces p-hydroxybenzoic acid, an aromatic fine chemical [64-66]. Given the rising production of biodiesel in the world, the availability of raw glycerol will increase in the years to come [67]. Furthermore, this feedstock has also lower prices, compared to sucrose (231-319US$/ton) [58], making it an excellent candidate for the biotechnological production of aromatic compounds.

**Bacterial strains as promising industrial hosts**

The main advantages of using unicellular microorganisms in industrial processes involve the rapid growth in liquid medium and rapid mobilization of carbon sources. The yeast *Saccharomyces cerevisiae* and bacteria such as *E. coli* and *Clostridium acetobutyricum* are main examples of species that are widely used in the production of simple bioproducts such as ethanol [68-70]. Besides these criteria, the choice of a microorganism shall also include its ability to grow in non-expensive carbon sources, an appropriate metabolism that produces intermediates of interest and available tools for genetic manipulations [68,71]. Regarding the production of aromatics, the chosen microorganism must be also capable of growing in culture media with high concentrations of these compounds, which tend to be toxic for the cells, and have a rich metabolism that generates intermediates with aromatic rings. A species that meets all of these criteria is *P. putida*, an environmental aerobic saprophytic gram-negative bacterium that is able to grow in environments with high titers of aromatic compounds. This is possible due to a singular metabolism capable of producing cofactors with high reducing power in its primary pathways along with a capability to consume aromatic compounds as carbon sources [57,72-74]. From the point of view of sustainable feedstocks, *P. putida* is capable of consuming very efficiently raw glycerol coming from biodiesel production. At the same time, it is resistant to inhibitory lignocellulosic compounds, which makes it a promising species for the consumption of second generation sugars [57]. Although it is not able to consume pentoses from hemicellulose, a polymer that is also present in lignocellulosic materials, scientists have synthetically inserted genetic circuits into *P. putida* strains that demonstrated high efficiency in the production of p-hydroxybenzoic acid from the combined consumption of the pentose xylose with glycerol [27,75]. It is important to emphasize that alongside these advantages, the simpler genome structure of bacteria make them more appealing than eukaryotic cells, as yeasts, for strain development through genetic manipulation.

**Bioprocess design through metabolic engineering and synthetic biology**

*Construction of synthetic routes from a systemic analysis and the key role of the shikimate pathway*

Recent advances in the so-called Omic Sciences, which are responsible for sequencing genomes, transcriptomes, proteomes, metabolomes and fluxomes of a large number of organisms, have allowed scientists to work with biological systems with some degree of predictability. Now, it is possible to interfere with such systems using simple concepts brought from Engineering to convert any feedstock into high-added value substances through biochemical pathways [76]. The way in which the biocatalytic potential of microbial cells can be explored in a bioprocess involves steps ranging from the choice of the product of interest to the construction of bioreactors due through the selection and optimization of the cell lines used as biofactories. Initially, all data collected from the "omics" of an organism are integrated to form a network of information about the metabolic fluxes involved in the production of a compound, allowing simulations in silico that anticipate important decisions regarding genetic modifications. For example, in silico analysis can rely on metabolic maps constructed from the host genome to define the best option of carbon sources. In this field, the elementary mode analysis has been used with great success, guiding the choice of feedstocks before any experimental procedure [77]. It consists in grouping all the chemical reactions present in a cell to define the simplest non-reductant routes responsible for converting a substrate into a desired compound. With this technique, scientists could evaluate, for example, that among glucose, ethanol and glycerol, the highest maximum yield that could be reached during the microbial production of p-aminobenzoic acid was obtained by the combination of glycerol and ethanol (0.92) [78]. Together, these advances contributed to a paradigm shift in which the deletion, replacement or expression of single genes by Genetic Engineering became the management of a group of genes connected by regulated metabolic networks, starting the field of Metabolic Engineering [79].

Nowadays the chemical synthesis of DNA sequences are available at very low prices, along with a large number of techniques using restriction enzymes or non-enzymatic assemble of DNA [80-82]. This enabled the development of a work front named Synthetic Biology, which aims to standardize the construction of genetic circuits that can change the behavior of any organism or build new biological processes under certain regulation [83-85]. Furthermore, fine genome editing techniques, such as homologous recombination through suicide vectors or the Clustered Regularly Interspaced Short Palindromic Repeats edition system (CRISPR-Cas9), have also allowed the expression of genetic circuits without plasmids, since genetic modifications can be transferred directly into the genome, making them stable [86,87]. Over the last years the main focus of this approach has been the biotechnological production of bulk chemicals [88] prioritizing these substances according to market demands in terms of volume, and their potential to act as chemical platforms for higher value-added compounds through well-known chemical routes [48,58,89]. However, due to the constant volatility of sugar prices and the high costs related to lignocellulose processing, researchers have concluded that the great potential of Metabolic Engineering to leverage a sustainable bioeconomy should consider the production of fine chemicals exclusively through biotechnological routes [90]. Thus, the potential of promoting biocatalytic reactions to produce high value molecules has enlightened engineers to the feasibility of the process, given that the costs of the feedstock are offset by the low cost of bioconversions and the high value of the final products. As a result, the biotechnological production of fine aromatic chemicals has gained great prominence because of its importance to the industry not in terms of volume demand but in terms of financial return [91,92].

To obtain aromatic fine chemicals, the shikimic acid pathway has been exploited as a metabolic platform, since this is not only present in plants but in almost all microorganisms, including fungi and bacteria [93]. Considering all the tools available, it is possible to build cell factories not only to obtain the compounds that integrate this biochemical pathway but also other compounds that can be generated by synthetic ramifications (Figure 4) [17-26,94]. Amongst these bioproducts gallate, protocatechuate, p-hydroxybenzoate and p-coumarate are highlighted along with catechol as antioxidants and drug precursors, while vanillin, 2-phenylethanol and phenylacetaldehyde can be used as flavors and fragrances. Moreover, salicylic acid is applied as an agrochemical [94-96], the aromatic amino acids phenylalanine, tyrosine and tryptophan are consumed by the food industry and phenolic acids such as the phenyl-lactic acid can form biopolymers with superior properties to those synthesized from lactate [97,98]. Some studies have also shown that engineered *Escherichia coli* and *S. cerevisiae* can also produce complex phenylpropanoids as resveratrol and curcumin from p-coumarate [25,99,100] and researchers have recently developed a metabolic platform based on the shikimate pathway that generates artificial aromatic amines in *E. coli* targeting the market of biopolymers [101].

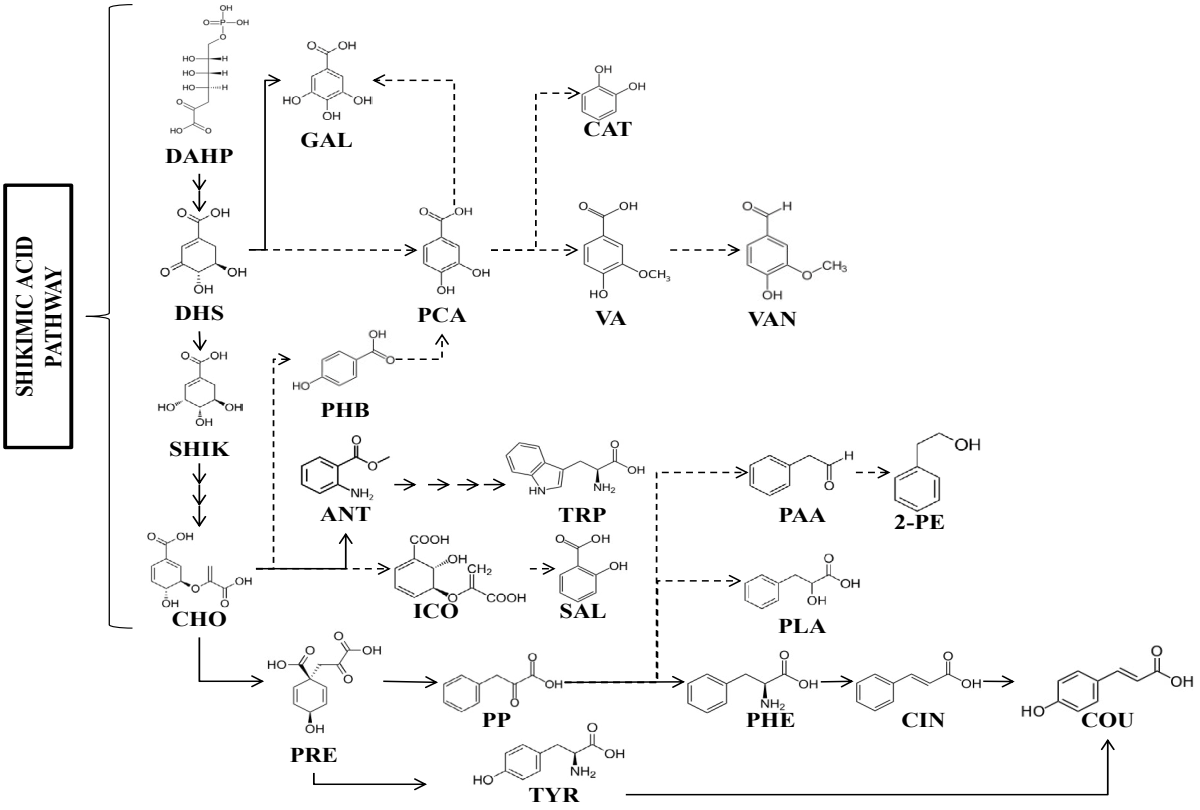


**Figure 4:** Examples of compounds that can be obtained from the shikimic acid pathway

Solid arrows: Natural routes in bacteria; Dashed arrows: Synthetic routes. DAHP: 3-Deoxy-D-Arabino-Heptulosonate7 Phosphate; DHS: 3-Dehydroshikimate; GAL: Gallic Acid; SHIK: Shikimic Acid; CHO: Chorismate; ANT: Anthranilate; PRE: Prephenate; TRP: Tryptophan; TYR: Tyrosine; PHE: Phenylalanine; PHB: *p*-Hydroxybenzoate; PCA: Protocatechuic Acid; CA: Catechol; VA: Vanillic Acid; VAN: Vanillin; ICO: Isochorismate; SAL: Salicylic Acid; PLA: Phenyllactic Acid; PP: Phenylpyruvate; PAA: Phenylacetaldehyde; 2-PE: 2-Phenylethanol; CIN: *trans*-Cinnamic Acid; COU: *p*-Coumaric Acid

Besides the biotechnological production of aromatic compounds, the insertion of synthetic routes at the shikimic acid pathway has long been exploited for the production of cis,cis-muconic acid, a non-aromatic derivative of catechol that can be converted by chemical routes into adipic acid in order to synthesize the polymer nylon-6,6 and also into terephthalic acid to produce polyethylene terephthalate (PET) (Figure 5) [102-104]. As a consequence, the arguments for using the shikimic acid pathway as a metabolic platform are even stronger not just because of the production of aromatic fine chemicals, but also for the production of non-aromatic compounds normally derived from BTX. Many companies have already patented the microbial conversion of biomass into aromatics and their derivatives (Table 1), however, only few of them explore it as a marketing strategy. Companies as Amyris, Evolva and Genomatica are examples of them [105-123].

*Shikimate pathway regulation through fine genetic manipulation*

Even though no aromatic ring is formed in the shikimate pathway, the structural modifications its intermediates suffer are extremely important to allow the formation of phenolic compounds. Because of that, the regulation of this pathway has been extensively studied and modified to enhance the microbial production of aromatics and other molecules such as cis,cis-muconate [26,102,124]. To begin with, the synthesis of DAHP in the beginning of the pathway can be done by three DAHP synthase isoenzymes (AroG, AroF and AroH), which are inhibited by allosteric and transcriptional regulations of the amino acids L-phenylalanine, L-tyrosine and L-tryptophan, respectively. To overcome this, mutant versions of these enzymes that are resistant to feedback inhibition have already been obtained in *E. coli* [125,126]. Furthermore, the gene aroE, which encodes the enzyme dehydroshikimate dehydratase, which is allosterically inhibited by shikimate, have been consistently replaced by its orthology diB in *E. coli*, which encodes for a dehydroshikimate dehydratase that suffers no inhibition [102,127]. Another important strategy used to enhance the carbon flux through this pathway is the analysis of the organism's metabolic network, so pathways that may be draining important intermediates or co-factors can be edited. This analysis can help researchers to design knockout or modular pathway engineering strategies that can provide new ways to cycle co-factors, to generate intermediates

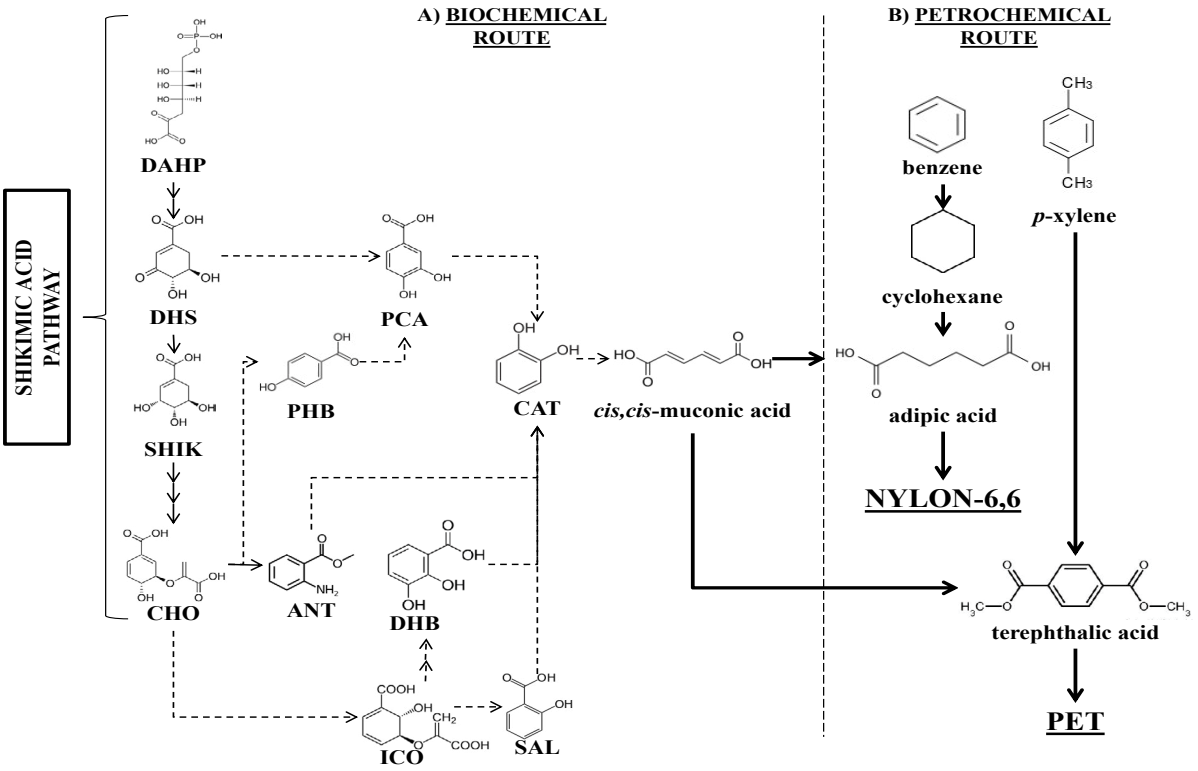


**Figure 5:** Biotechnological routes to produce *cis*,*cis*-muconic acid in bacteria. (A) Examples of synthetic biochemical pathways (dashed arrows) capable to generate *cis*,*cis*-muconic acid from the shikimic acid pathway; (B) Petrochemical routes for adipic acid and terephthalic acid DAHP: 3-Deoxy-D-Arabino-Heptulosonate 7 Phosphate; DHS: 3-Dehydroshikimate; SHIK: Shikimic Acid; CHO: Chorismate; ANT: Anthranilate; PHB: *p*-Hydroxybenzoate; PCA: Protocatechuic Acid; ICO: Isochorismate; SAL: Salicylic Acid; DHB: 2,3-Dihydroxybenzoate; CAT: Catechol

**Table 1:** Examples of companies with patents on the microbial production of aromatic compounds from biomass

| Aromatic compound | Main substrate | Main microorganism | Company | Reference |
|---|---|---|---|---|
| Vanillin | Sucrose | *S. cerevisiae* | Evolva | [108,109] |
| | Sucrose | *S. cerevisiae* | IFF | [108] |
| | Feluric acid | *Aspergillus niger* | Kraft General Foods | [110] |
| | Feluric acid | *Streptomyces setonii* | Givaudan | [111] |
| Resveratrol | Glucose | *S. cerevisiae* | Evolva | [112] |
| | Glucose | *Yarrowia lipolytica* | Du Pont | [113] |
| Aromatic amino acids | Glycerol | *E. coli* | Ajinomoto | [65] |
| | Glucose | *E. coli* | Du Pont | [114] |
| *para*-Hydroxybenzoic acid | Glucose | *E. coli* | General Eletric | [115] |
| Terephtalic acid (produced from *cis*,*cis*-muconic acid) | Glucose | *E. coli* | Amyris | [103] |
| | Glucose | *E. coli* | Genomatica | [116] |
| *p*-Coumaric acid | Glucose | *E. coli* | Du Pont | [117,114] |
| 2-Phenyllactic acid | Phenylalanine | *Pseudomonas gladioli* | IFF | [118] |
| 3-Phenyllactic acid | Glucose | *Brevibacterium lactofermentum* | Ajinomoto | [119] |
| Phenylacetaldehyde | Milk | *Lactococcus lactis* | Nestec | [120] |
| *trans*-Cinnamic acid | Glucose | *E. coli* | Du Pont | [114] |
| 3,4-Dimethoxycinnamic Acid | Feluric acid | *E. coli* | Symrise | [121] |
| Gallic acid | Tannic acid | *A.niger* | Zunyi Beiyuan Chemicals | [122] |
| Protocatechuic acid | Glucose | *B. lactofermentum* | Ajinomoto | [123] |

or even to couple the bioproduct synthesis with primary metabolic routes [71,127,128]. Lastly, given the fact that the main role of the shikimate pathway is to provide aromatic amino acids to the cell, swerving the synthesis of

these molecules to produce aromatic fine chemicals may create a condition of auxotrophy to the organism. To solve this, scientists have regulated the expression of genetic circuits only in the late growth phase by using, for example, promoters of the genes phoA or pstS from *E. coli* [129,130], or even by creating tunable switches that modulate the production in strains that can grow with no addition of aromatic amino acids in the broth [131].

**Bioprocess optimization tools for better scale-up conditions**

When a microbial strain is developed not just factors as its metabolic network, growth and tolerance to high concentrations of the final product must be considered, but also, parameters related the industrial production of the target molecule in a bioreactor. In this level, it is important to measure the accumulation of the bioproduct in the broth (titer), the rate of this accumulation (productivity) and the efficiency to convert the carbon source into the bioproduct (yield) [132]. Although these factors are intrinsically influenced by the cell physiology, several conditions may modulate them, such as medium composition, pH, aeration, agitation, temperature and carbon source concentration along with the regime of cultivation (batch, fed-batch or continuous). To help bioprocess engineers to plan experiments for bioprocesses optimization several statistic and informational tools have been developed, supporting a design-of-experiments methodology (DoE) [133]. Recently, scientists have also applied this method to correlate the expression of several genes with violacein production in *E. coli*, creating an opportunity for a multiplexed combinatorial analysis that combines metabolic variables with many parameters of a bioprocess [134].

**Product recovery: No more extraction techniques**

One of the main benefits of the bacterial production of aromatic fine chemicals is the facilitation of product recovery due to the biological decontamination of the broth by many strains [135] and the reduced release of secondary metabolites when compared to filamentous fungi [136]. It can make downstream processes less expensive and allow the adoption of waste streams as substrate. Recent studies show that the sum of the costs related to the hydrolysis, detoxification and downstream treatment during lignocellulose bioconversion correspond to 45% of the total costs, revealing the importance of these steps to a successful bioprocess [137]. Regarding the recovery of aromatic fine chemicals, while the traditional routes employ complex extraction methods, such as maceration, supercritical fluid extraction, the high hydrostatic pressure (HHP) and microwaved-assisted extraction [37], the recovery of these compounds from the culture medium after a SmF process does not require any of them once most microorganisms release the molecules in the broth [138]. Furthermore, traditional methods such as lignin valorization normally result in a mixture of phenolic compounds with similar physicochemical properties, which require fine separation tools such as nonionic macroreticular polymeric resins or molecular imprinted polymers [138]. Although the same situation may happen in bioprocesses, a reduced number of these contaminants will be presented if strains that naturally degrade aromatic compounds, such as *P. putida*, were used. At last, another advantage of this and other bacteria is the fact that once they are tolerant to organic solvents a two-phase system can be applied during the cultivation, combining the culture media with organic solvents to rapidly remove aromatic compounds while they are being released by the cells [73].

## BIOREFINERIES AND THE BIOTECHNOLOGICAL PRODUCTION OF AROMATIC FINE CHEMICALS

A biorefinery is an industrial complex where biomass is converted into chemicals and energy (heat, electricity and fuel). As the production of biofuels generates residues such as lignocellulose, an industrial model in which these materials become inputs for the production of bulk and fine chemicals has become widely suggested in order to leverage viable biorefineries [13,139,140]. As an example, a techno-economic analysis of a biorefinery where succinic acid is biotechnologically produced from the crude glycerol of a biodiesel facility showed that this co-production increases the profits by 60% [139]. In this new context, the production of aromatic fine chemicals in biorefineries linked to the production of biofuels could be a great opportunity to be explored, given the market trends for these compounds, combined with the consolidation of tools that now favor the construction of proper engineered strains. Considering all the advantages and limitations discussed for the available processes and feedstocks it is possible to imagine a roadmap of short and long-term biorefinery models targeting the co-production of aromatic fine chemicals and biofuels (Figure 6). In the short-term, high-value aromatics could be produced from raw glycerol along with biodiesel, leading to new investments in lignocellulosic waste processing. It would further enable the consumption of biomass residues from the bioethanol industry as well, integrating more industrial chains to the system in the long-term (Figure 6). This roadmap makes clear the central role of the microbial conversion in the whole process, as it may use different types of feedstocks in a versatile way.

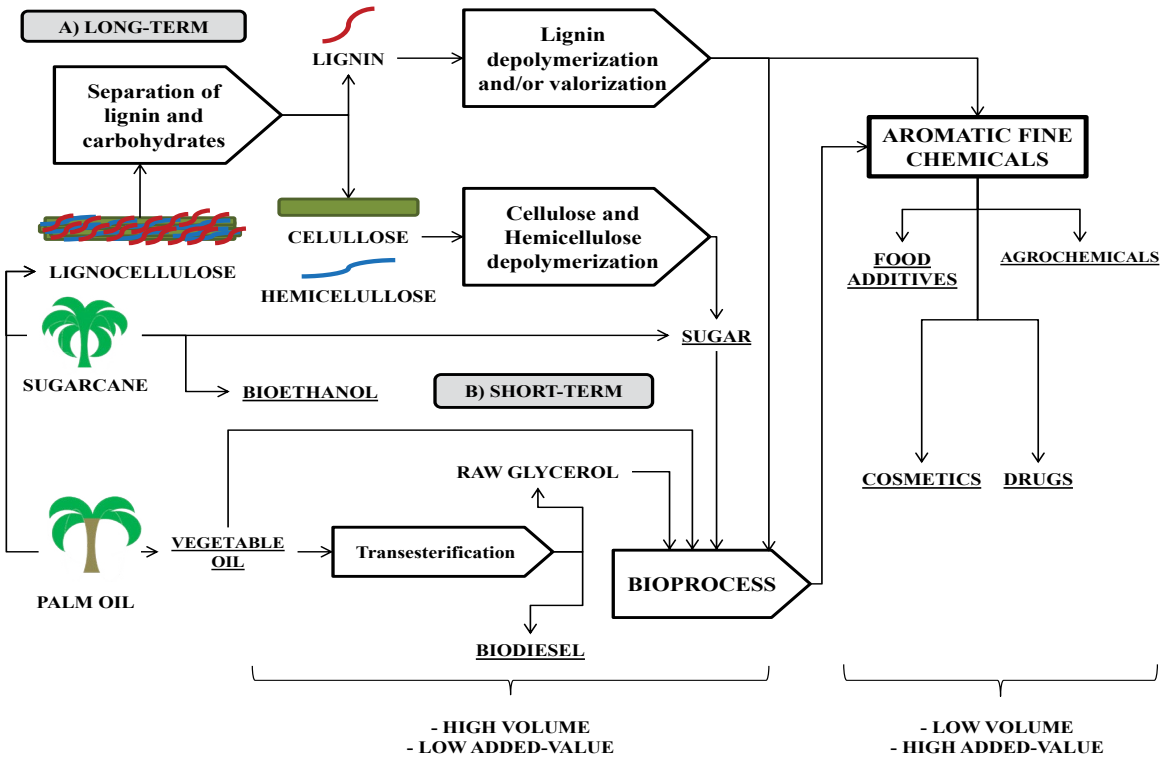


**Figure 6:** A general model for a biorefinery system with aromatic fine chemicals. (A) A short-term strategy. (B) A long-term strategy

## CONCLUSION

Considering the advantages of microbial conversions of biomass into aromatics it is possible to conclude that the biotechnological production of aromatic fine chemicals is capable to overcome the current barriers of traditional methods (Table 2). Microorganisms are not just capable of consuming different feedstocks, overcoming the barrier of biomass availability but are also able to enrich the target molecules in the broth through the bioconversion of polymeric structures and the degradation of contaminants. At the same time, many tools are available, helping the development of better strains and optimized processes, which makes this approach even more attractive. In initial stages, in silico analysis of metabolic networks can allow the decision making about what biochemical routes must be engineered with synthetic biology techniques. As a result, enhanced microbial strains can be subjected to bioreactor experiments designed by statistic tools in order to optimize the conditions of cultivation in industrial scale. At last, all of these advantages make the microbial production of aromatic fine chemicals a good strategy to leverage feasible biorefineries, establishing a possible roadmap where the consumption of simple agro industrial residues can provide short-term incomes that may be used in long-term plans to afford investments in lignocellulose processing.

**Table 2:** Advantages of the microbial conversion of biomass into aromatic fine chemicals

| Traditional methods | Main barriers of traditional methods | Advantages of microbial conversion |
|---|---|---|
| Plant extract purification | Seasonality of medicinal and aromatic plants and low amount of biomass | Feedstock versatility |
| Plant extract purification and organic residues valorization | Need of extraction techniques | Recovery directly from the culture medium |
| Plant extract purification, organic residues valorization and lignin depolymerization | Pre-treatment (thermochemical and/or enzymatic) | Production and release of hydrolytic enzymes during the biological conversion (Consolidated bioprocess) |
| Plant extract purification, organic residues valorization and lignin depolymerization | High concentration of contaminants | Enrichment by bioconversion and biodecontamination |

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