Yeast Fermentation on Agro-Industrial Waste for Sustainable Biosynthesis of 2-Phenyl-Ethanol

Namrata Patil[1], Rajalaxmi S[2], Nargees Kousar[3], Dr.Narendra Kumar[4]

[1,2,3]M.Tech Biotechnology Student, R.V.College of Engineering, Bengaluru,India
[4]Assistant Professor, Department of Biotechnology,R.V.College of Engineering, Bengaluru,India

Abstract:
The aromatic alcohol 2-phenylethanol (2-PE) has a very high market demand because of its pleasant rosy scent. Consumers and safety regulations tend to favor natural methods of production over synthetic ones for this valuable compound because it is used in food, cosmetics, and pharmaceuticals. Natural 2-PE can be produced either through biotechnological or floral-based methods, such as the extraction of essential oils from roses, hyacinths, and jasmine. The inability to meet the high market demand and the high selling price are actually results of the rarity of natural 2-PE in flowers. Therefore, a biotechnological approach must be developed as a better option to the traditional industrial one that is more effective, affordable, and environmentally friendly. The most promising approach involves microbial fermentation, especially when yeasts are used. Using L-Phe as a precursor, many yeasts can synthesize 2-PE. The distinctive nutritional value of some agro-industrial waste and by-products makes them suitable media for microbial growth, including the production of 2-PE through yeast fermentation. This review provides an overview of the biotechnological production of 2-PE using a variety of yeasts to ferment synthetic media, byproducts, and waste from the agro-industrial sector.

Keywords: 2-Phenylethanol, microbial fermentation, yeast, agro-industrial waste, biochemical pathways, L-phenylalanine, biotechnological approach

1.INTRODUCTION

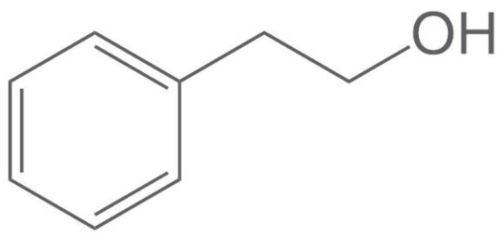

Fig-1: Structureof2-Phenyl-Ethanol

Using fragrances has become common over time. Nearly every human's daily life has been influenced by a vast array of flavors and scents. 2-Phenylethanol (2-PE), also known as 2- phenethyl alcohol, is a highly aromatic alcohol that is distinguished by having one of the most liked and desired scents, the rosy scent. Petrochemicals can be used to make 2- PE cheaply, but most food applications don't prefer this. The bio-derived compound is currently produced primarily by extracting the trace amounts found in rose petals, which is very expensive. Potentially, fermentation could offer a low-cost, naturally sourced substitute [1]. There are numerous uses for 2-PE in numerous product categories. It is frequently used as a preservative in pharmaceuticals, cosmetics, including perfumes, and a variety of herbal products. Additionally, 2-PE is used as an organoleptic enhancer of the finished product in the food and beverage industries[2]. For instance, this aroma is included in the mix of a variety of ice creams, candies, cookies, puddings, gelatins, cigarettes, and chewing gums[3]. The Flavor and Extract Manufacturers' Association (FEMA), the Joint Expert Committee on Food Additives (JECFA), the Council of Europe (COE), and numerous other international bodies have all given their approval to this flavor[4]. They give 2-PE an added value by considering it to be generally acknowledged as safe. Additionally, 2-PE has antifungal and antibacterial effects. It is possible to significantly boost the antifungal effects of fluconazole [FLU] and itraconazole [ITC] by mixing it with 2-PE. Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, as well as numerous fungal species like Candida albicans, Candida dubliniensis, Saccharomyces cerevisiae, Kluyveromycesmarxianus and many others growth can be inhibited due to 2-PE[5].

Because of these properties this substance is valued as an additive in products that are antiseptic, preservative, cleaning, and cosmetics.It is noteworthy that aromatherapy also makes use of 2-PE. The concentration of plasma adrenaline is shown to decrease by 30% and the sympathetic activity of humans by 40% upon exposure to rose oil's aroma [6].

Around 10,000 tons of 2-PE are produced globally each year, the majority of which is produced chemically, and its price ranges between 3 and 5 US dollars per kilogram[7]. The production of 2-phenylethanol by Saccharomyces cerevisiae in cassava wastewater from the starch industry was examined in a study. In order to increase the concentration of 2- phenylethanol and improve the efficiency of the conversion of glucose into 2-phenylethanol, the substrate was supplemented with glucose and L-phenylalanine.However, natural 2-PE, which is obtained from some flowers' essential oils and costs an estimated 1000 US dollars per kilogram, is much more expensive than chemically produced 2-PE[8].It is predicted that the actual cost of producing 2-PE through biotechnology would be around 220 US dollars per kilogram [9].

In the industry, 2-PE is chemically synthesized using one of three methods. The first reaction is the Friedel-Crafts reaction between ethylene oxide and benzene when aluminum chloride is present. Second, the hydrogenation of styrene oxide using a small amount of sodium hydroxide and a catalyst made of Raney nickel. Third is 2-phenylethyl hydroperoxide's oxidation of propylene. Toxic substances are used in these procedures like benzene, and styrene oxide, which are harmful to human health and the environment and can cause cancer. In addition, chemical synthesis requires high pressures, temperatures, and alkali or acid conditions. The formation of unintended by products is also a important aspect. The final product's quality is impacted by all of this, making purification challenging [10].

Natural 2-PE, on the other hand, can be obtained from the essential oils of many different flowers, including daffodils, hyacinth, lilies, and jasmine. Except for rose oil, which can contain up to 60% 2-PE, the 2-PE concentration in these flowers is too low. For instance, 2- PE, also referred to as a damask rose, is the main aroma compound produced by Rosa damascena[11] . Multiple separation steps are needed due to the low concentration of 2-PE in almost all types. The high market demand cannot be met due to the previously mentioned rarity of natural 2-PE in flowers, which requires time-consuming and expensive downstream processing.

The demand for natural methods of producing 2-PE increased as a result of its uses in cosmetic products, different foods, and pharmaceuticals, which sparked research into alternative biotechnological methods for producing natural 2-PE. The most promising technique involves microbial fermentation, particularly that which relies on yeasts to synthesize 2-PE[12]. Using the Ehrlich pathway or the shikimate pathway, respectively, yeasts can synthesize 2-PE using the substrates L-phenylalanine (L-Phe) or glucose[13]. In fact, if the substrates used in the process are of natural origin, biotechnological flavor and fragrance production is becoming more alluring because it results in a final product that is recognized as natural by the US Food and Drug Administration and by European law.The maximum concentrations of PEA that K. marxianus could produce in the three types of whey (SW, AW, and CV) were 0.96, 0.70, and 0.47 g L-1, respectively. It was discovered that the concentration of lactose in SW (where PEA was at its highest concentration) was 82 point 35 percent. Whey, a byproduct of the cheese industry, could replace the production of PEA by K. Marxianus. In addition to creating a "natural" product, the production cycle of microbial fermentation is quick and environmentally friendly, reducing the environmental pollution brought on by the chemical synthesis of 2-PE[14]. However, it should be noted that in order to be economically viable and compete with chemical synthesis methods, microbial fermentation techniques require highly productive yeasts and inexpensive feedstocks. Microorganisms can grow in environments that are provided by agro-industrial waste and byproducts with high nutritional value. There are many bioactive substances in agricultural residues. These leftovers can be used as a secondary source for the production of various goods, including biogas, biofuel, mushrooms, and tempeh as the primary ingredient in numerous studies and businesses. Utilizing agro-industrial wastes as raw materials can lower production costs and lower environmental pollution levels. Solid state fermentation (SSF) is a process that uses agricultural and industrial wastes to produce biofuels, enzymes, vitamins, antioxidants, animal feed, antibiotics, and other chemicals. Through SSF processes, a variety of microorganisms are used to produce these worthwhile goods[15].Microorganisms can use them as potential raw materials for the creation of products with added value through fermentation. Several fermentation techniques have been used to produce enzymes from agro- industrial wastes like sugar cane bagasse, corn cob, and rice bran. Recent research has shown that pretreatment techniques can multiply the yields of enzymes by several times. This process, known as "biorefinery," allows waste and by-products from one industry to be used as a raw material for another[16].This review will discuss the biotechnological production of 2-PE using various agro-industrial waste and by-products as feedstocks to ferment various yeasts. The production of 2-PE on synthetic media, the description of the yeasts capable of producing 2-PE, and the metabolic pathways involved in 2-PE biosynthesis will all be highlighted.The use of agro-industrial waste and by products as feedstocks for 2-PE production, as well as the methods employed to improve 2-PE productivity, will also be included.

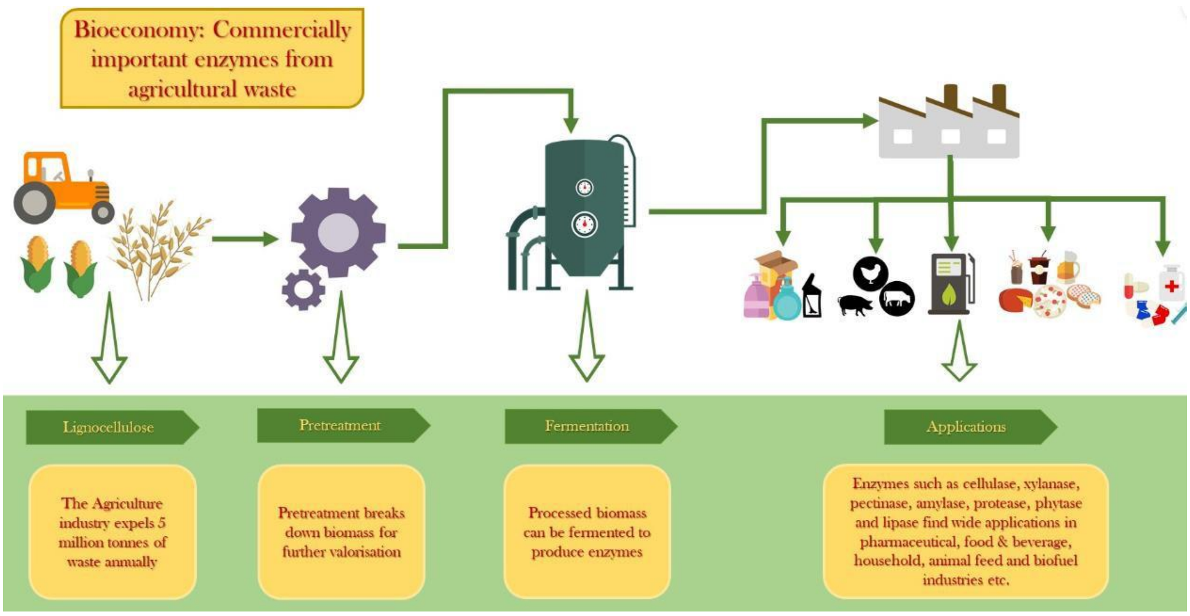

Fig-2: Bioconversion ofAgro-IndustrialWastestoIndustriallyImportantEnzymes

Yeasts that produce 2-PE:

Yeasts are microorganisms with significant synthesis capacity. They are able to transform carbohydrates and nitrogen sources into various complex molecules, particularly different flavor compounds. In fact, many yeast species are capable of producing 2-PE and since this production is strain-specific, it is possible to observe a significant difference in 2-PE production level between various strains of a single species. It is noteworthy that a significant increase in 2-PE yield has recently been achieved by creating numerous metabolically engineered yeast strains[17].

One well-known and promising microorganism for its capacity to produce 2-PE is Saccharomyces cerevisiae, a significant eukaryotic model organism. Despite being a desirable microbe for 2-PE production, Saccharomyces cerevisiae has a low natural yield, and over- expressing one or two key genes in S. cerevisiae did not significantly enhance 2-PE[18].

Numerous studies have demonstrated that other 'non-conventional' yeasts, such as S. cerevisiae, are also capable of producing 2-PE, and some of them exhibit a higher capacity for aroma metabolite production than S. cerevisiae. The production of 2-PE by Pichia pastoris has been previously observed, though to varying degrees and the others are Candida utilis, Phellinus ignarius, Ischnodermabenzoinum, Geotrichumpenicillatum, Aspergillus niger, Metschnikowiachrysoperlae, Clavisporalusitaniae, Kluyveromycesmarxianus, Kluyveromyces lactis, Pichia fermentans, Pichia anomala, Pichia membranaefaciens[19].

Applications of 2-Phenyl Ethanol

- The food, fragrance, and cosmetics industries all use 2-Phenylethanol (2-PE), a crucial flavor and fragrance compound with a rose-like aroma.
- As a result, it is frequently used in flavors and perfumes, especially when rose odor is desired.
- Cigarettes incorporate it as an additive.
- Due to its stability under basic conditions, it is also utilized as a preservative in soaps.

- Since it has antimicrobial qualities, it is interesting.

Biochemical Pathways for 2-PE Production:

The normal metabolic process allows yeast cells to produce 2-PE. In yeast cells, 2-PE can be produced in two distinct ways: either de novo through the shikimate pathway or through the bioconversion of L-Phe through the Ehrlich pathway.Using constitutive promoters, important genes such as ARO8 and ARO10 of the Ehrlich pathway for 2-PE synthesis and important transcription factor ARO80 in Saccharomyces cerevisiae were re-regulated. At the same time, the impact of the nitrogen source in synthetic complete (SC) medium with L-phenylalanine (L-Phe) on Aro8/Aro9 and Aro10 was examined. The outcomes demonstrated that ammonium sulfate in SC + Phe medium significantly inhibited the aromatic aminotransferase activities of ARO8 over-expressing strains [20].

The Ehrlich pathway is regarded as the biotechnological strategy that is most effective and a more appealing choice from an industrial standpoint. The development of this procedure has received the majority of attention in biotechnology . Using L-phenylalanine as their only source of nitrogen, yeast cells create 2-PE through this mechanism. Three steps are needed, the first of which involves turning L-Phe into phenylpyruvate. The amino acid transaminase isoenzymes Aro8p and Aro9p catalyze this transamination reaction in the model yeast S.cerevisiae to create phenylacetaldehyde, phenylpyruvate is secondarily decarboxylated. The three pyruvate decarboxylase isoenzymes Pdc1p, Pdc5p, and Pdc6p, as well as the phenylpyruvate decarboxylase enzyme Aro10p, catalyze the second step of the Ehrlich pathway. The third step is the reduction of phenylacetaldehyde to 2-PE by alcohol dehydrogenases (Adh1p, Adh2p, Adh3p, Adh4p, and Adh5p) and formaldehyde dehydrogenase Sfa1p. Since high concentrations of l-Phe are required to switch cell metabolism to the Ehrlich pathway, the natural process is significantly enhanced when l-Phe is present in the media.

However, yeasts have the ability to make 2-PE from their metabolism's intermediate molecules (e.g. erythrose-4-phosphate (E4P), and phosphoenolpyruvate (PEP). This is demonstrated by the shikimate pathway's de novo synthesis of 2-PE, which occurs. Simple sugars are converted to 2-PE along this pathway[21]. 3-deoxy-D-arabinoheptulosonate-7-phosphate (DAHP) is produced through catalysis from PEP and E4P, which are products of the pentose phosphate pathway and glycolysis, respectively. Following that, a series of intermediaries such as shikimate, chorismate, prephenate, , phenylpyruvate are produced.Next, the Ehrlich pathway receives phenylpyruvate, a byproduct of the shikimate pathway. Decarboxylation of phenylpyruvate to phenyacetaldehyde and dehydrogenation of phenylpyruvate to 2-PE follow the process[22].

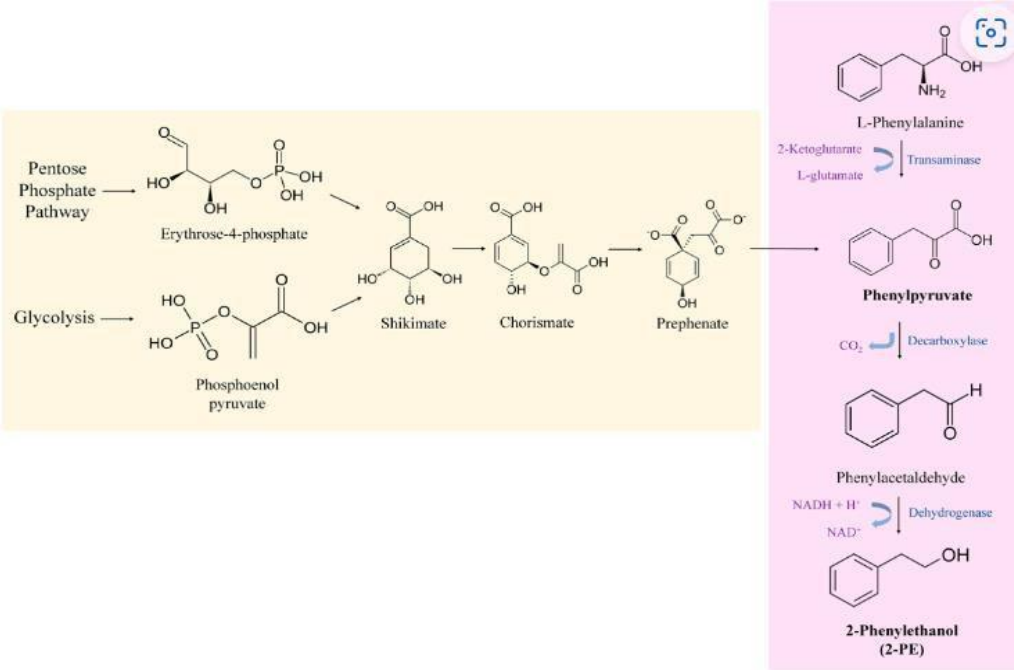

Fig -3 Shikimate and Ehrlich metabolic pathways are both involved in the production of 2-PE in yeasts from L-Phe.

Factors affecting 2-PE production

The amount of 2-PE that yeasts can produce is dependent not only on the species but also on the media's composition and the fermentation environment.The Ehrlich pathway predominates over de novo synthesis, which typically predominates at low amino acid concentrations, if amino acids, particularly L-Phe, are the sole source of nitrogen in the medium[23]. However, L-Phe will be metabolized via the cinnamate pathway in the presence of more readily available nitrogen sources, restricting the formation of 2-PE. As a result, providing L-Phe as the only nitrogen source allows for the production of high concentrations of 2-PE. The cost of using L-Phe as a substrate for the production of 2-PE must be taken into consideration[9]. Therefore, metabolic engineering is being used to increase the aromatic amino acids pathway (AAA) for supplying L-Phe for the bioconversion and also to overexpress important Ehrlich pathway enzymes.

The availability of nutrients, nitrogen sources, and sugars in the culture media, on the other hand,have an impact on the fermentation conditions, which in turn affect the metabolic activity of the microorganisms. Furthermore, changes in temperature, pH, oxygen availability, and air flow rate have a significant impact on the production of 2-PE by affecting microbial growth, microbial community structures, or key enzyme activities.

2-PE Production via Fermentation on Agro-Industrial Waste and By-Products

There are a lot of residues produced annually by the agri-food sector. In actuality, 1.3 billion tons, or one third, of the food produced for human consumption globally each year is wasted . The amount of food waste today is alarming, so strategies for reusing it have been constantly developed and improved. Actually, the majority of agro-industrial waste and by- products are high in nutrients like complex carbohydrates, proteins, lipids, and numerous nutraceuticals [24]. The potential to value these residues is therefore very high. The presence of these nutrients in these raw materials demonstrates suitable conditions for the development of various microorganisms, particularly yeasts. Microorganisms have the ability to use these raw materials in fermentation to create high-value product. The use of agricultural and food by-products as a more affordable substrate for the production of 2-PE has a number of benefits, including their wide availability and nutritional richness, the reduced cost of producing this aroma molecule, the ability to create a "natural" product, and the environmentally friendly process [12].

Submerged fermentation (SmF) is the most popular bioprocess for converting L-Phe into 2- PE. SmF is regarded as an expensive and energy-intensive production process because it uses sterilized synthetic media as substrates and complex reaction systems that require large amounts of essential resources to achieve high titers . Solid-state fermentation (SSF) is a substitute technology that has recently attracted attention. SSF has been suggested as a sustainable and cost-effective way to produce 2-PE from renewable sources in addition to the high-value added molecules that fungi typically produces [25] . SSF frequently exhibit high production yields and rates with relatively minimal energy and water needs. The amount of waste and hazardous materials produced is minimal, and energy efficiency is high . The SSF is influenced by a variety of factors, including moisture content, water activity, the media's pH and temperature, the sources of carbon and nitrogen, and the substrate's particle size. Finally, SSF has demonstrated its effectiveness as a cost-effective and environmentally friendly tool for the valorization of various organic solid waste in order to produce valuable aroma compounds. Substrates, enzymes, and yeasts are typically loaded into the reactor in full at the start of batch processes using SSF systems.

The following are a few examples: whey, grape must, corn stover, sugar beet molasses, sugarcane bagasse, tobacco, cassava water, red apple pomace, etc., are readily available and inexpensive agro-industrial byproducts that have proven to be top-notch natural substrates for the productionof 2-PE from L-Phe by various yeasts.

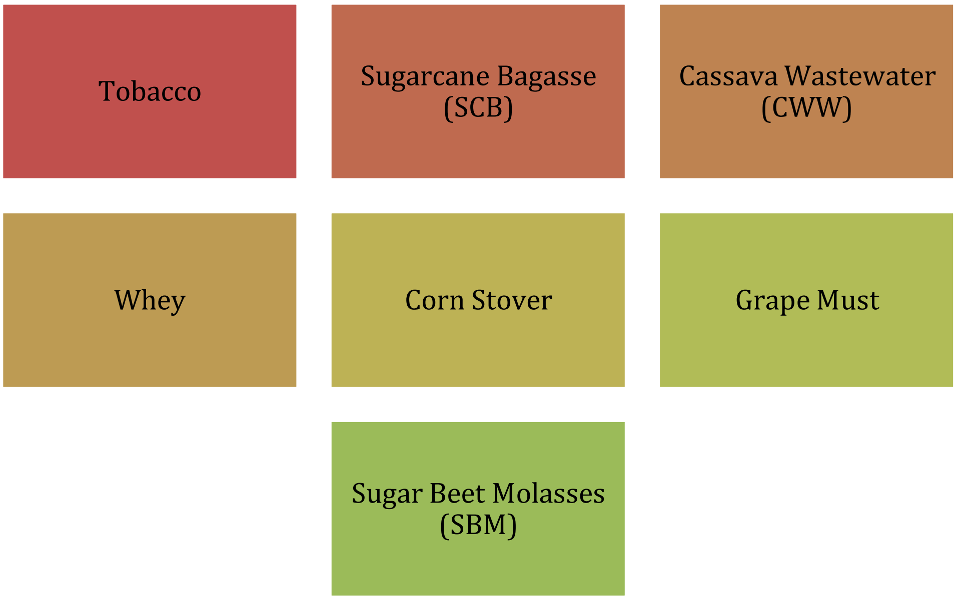

Fig -4 : DifferentAgro-Industrial Waste

Conventional Strategies to Increase the 2-PE Production

High 2-PE concentrations prevent yeast cell growth. This inhibition is undesirable in a process that aims for high yield. To avoid the inhibition restraint, there are several strategies. The optimization of fermentation conditions, strain mutagenesis, and culture medium composition are common techniques for increasing yield.

First, during the fermentation process, the medium composition has a significant impact on the titer and yield of metabolites. The fermentation process is impacted by the nitrogen sources, including L-Phe, carbon sources, vitamins, and minerals added to the media. The amount of 2-PE produced may also be influenced by the culture temperature, initial medium pH, shaking rate, and fermentation time .

Another method of getting around the product inhibition is by genetically altering the microorganism to make it more tolerant to the product. For instance, S. cerevisiae strain was able to produce 2.61 g/L of 2-PE by overexpressing ARO8 and ARO9, which is a 36.8% higher yield than the wild type strain. Increasing 2-PE yield may also be accomplished through the application of synthetic biology, a developing field that aims to apply engineering principles to biological systems to make them more predictable and controllable. To create non-native metabolites artificially, specific genetic toolboxes can be expanded [26]. For example, a variety of tactics have been created to support engineered Y. lipolytica to produce aromatic compounds, including 2-PE and to make product extraction easier.Y. lipolytica can grow on a variety of inexpensive substrates[27].

Third way to get around product inhibition is to remove 2-PE from the fermentation medium right after its biosynthesis is allowing the yeasts to keep making this aromatic compound. Many in situ product recovery (ISPR) techniques can help with this. ISPR have a number of benefits, such as the reduction of yeast inhibition brought on by product toxicity, the stabilization of the product, and the facilitation of downstream processing. The methods can be categorized into two-phase extraction, in situ product adsorption, solvent immobilization, and organophilic pervaporation depending on the various separation tools used in ISPR . Two-phase extraction is a straightforward, low-cost, and straightforward to use at an industrial scale ISPR technique. The biotransformation takes place in an aqueous phase, and 2-PE extraction occurs continuously in an organic phase. The organic phase's solvent must be highly selective towards 2-PE and perform efficient 2-PE recovery without impairing either the aqueous phase's biotransformation or the extraction's quality of the 2-PE [28]

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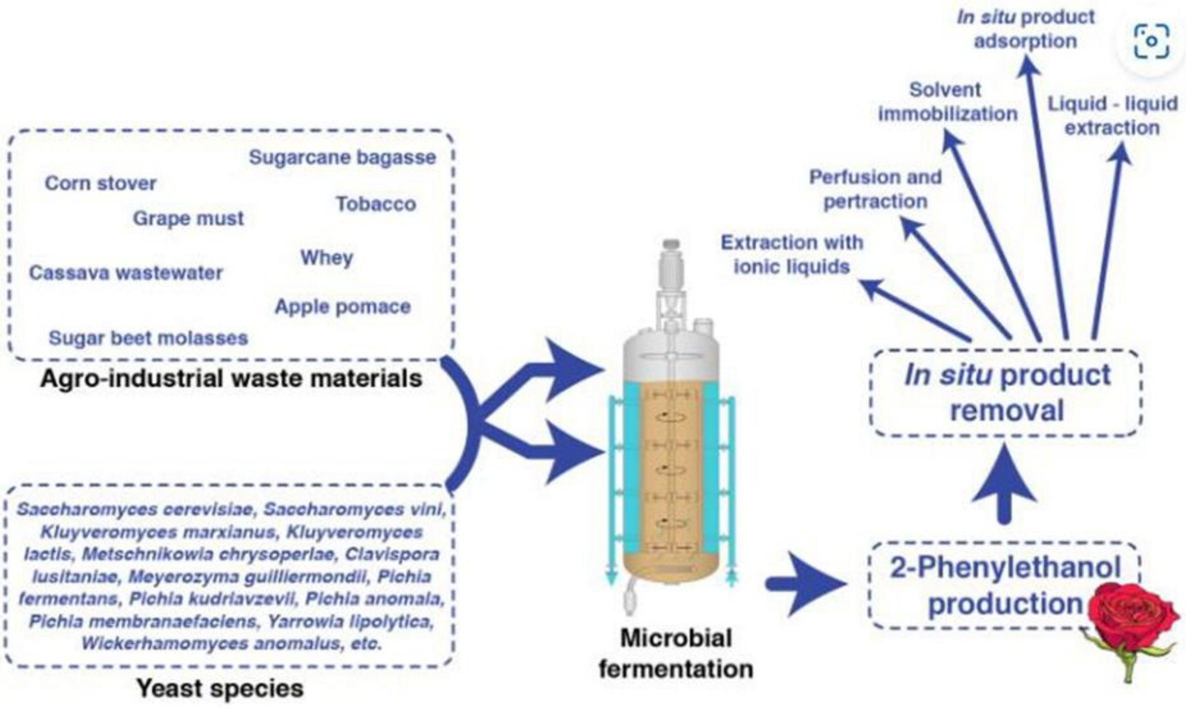

Fig -5: 2-PE production by microbes. Different yeast species ferment a variety of agro industrial wastes and byproducts to produce 2-PE.

Sustainability and Societal Concerns:

By modifying Bacillus licheniformis and using agro-industrial wastes as feedstocks, a sustainable platform for 2-PE biosynthesis was created. In the beginning, B licheniformis endogenous pathway for catabolism of sucrose was introduced CscB from Escherichia coli W and SucP from Bifdobacteriumadolescentis was replaced.

The effects of using crude glycerol and molasses as feedstocks on the production of 2-PE were then looked into. The production of 2-PE was increased from 2.21 g/L to 5.74 g/L by optimizing the molasses pretreatment techniques and medium components. The raw materials used were 50 g/L pretreated molasses, 15 g/L crude glycerol, and 7 g/L corn steep liquor powder. Finally, using fed-batch fermentation in a 5-L fermenter, we identified the PE24 strain and produced the highest amount of 2-PE, with a titer of 6 point 43 g/L. This study offered a practical method for 2-PE sustainable biosynthesis [29].

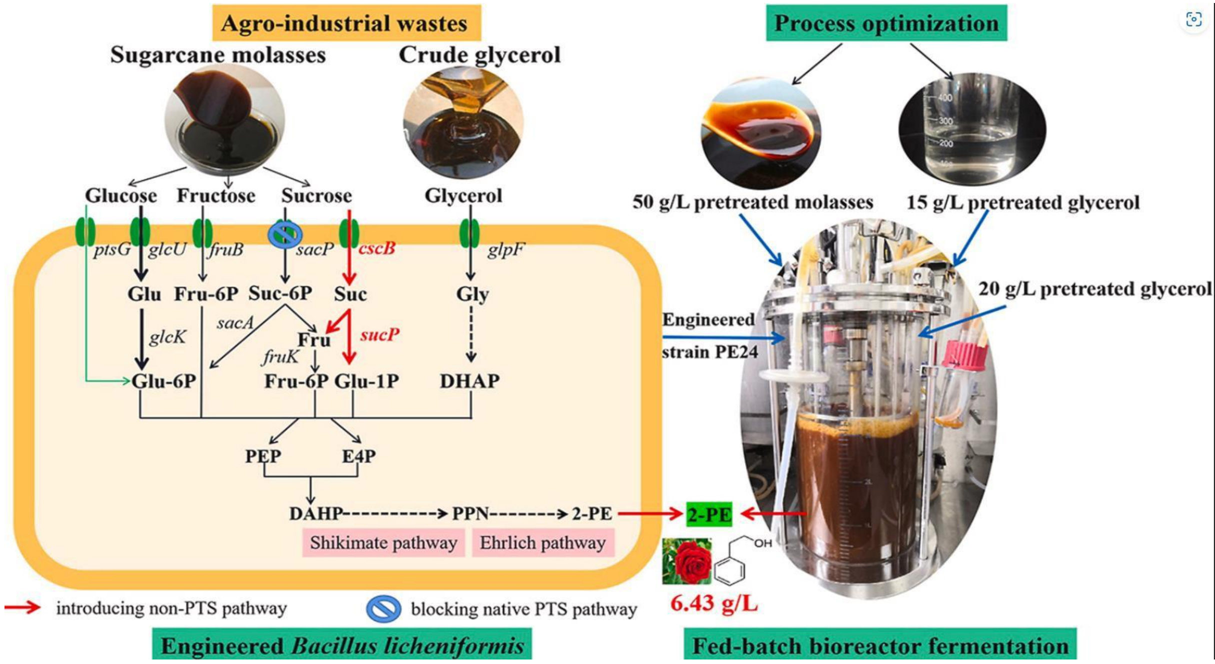

Fig-6 :Sustainableproductionof 2-Phenyl Ethanol

2-phenylphenol harmful effects include :

- neurotoxicity, acute toxicity, and toxicity to reproduction and development.
- increases the likelihood of corneal damage, lesions, and irritated respiratory tracts.
- causes weakness, depression of the central nervous system, and inhibition of deoxyribonuclease.
- causes dark urine, sweating, fainting, and symptoms of diarrhoea, nausea, and vomiting.
- damaging to the kidneys, skin, and eyes.
- negatively impacts both the central nervous system and reproductive health.

Therefore, it is necessary to create a biotechnological approach that is more effective, affordable, and environmentally friendly than the traditional industrial one.

Wastes or residues from the agro-industrial sector are abundant in nutrients and bioactive substances. Since these wastes have a variety of different compositions, including proteins, sugars, and minerals, they ought to be viewed as "raw material" rather than "wastes" for other industrial processes. Through fermentation processes, the microorganisms may be able to use the waste as starting materials for new growth.

The agro-industrial wastes can be used in SSF processes to produce a variety of significant beneficial compounds as a solid support.
Using agricultural and agro-based industry wastes as raw materials can help to lower production costs and contribute to waste recycling, which helps to create a more environmentally friendly environment.

Cytotoxicity of 2-PE:

The inhibitory effect of this alcohol on the yeast cells, which prevents the accumulation of higher 2-PE contents, restricts the bioproduction of 2-PE via yeasts. In fact, it has been discovered that 2-PE can prevent the growth of numerous yeast species at concentrations of 2 to 4 g/L . 2-PE is strain-specific and not equally toxic to all species of yeast.

At 2.6 g/L of 2-PE, K. marxianus CBS 600 was completely inhibited, whereas S. cerevisiae GIV 2009 required 4 g/L of 2-PE to have the same effect. The growth rate of an Australian ethanol-tolerant S. cerevisiae strain used to make cider was reduced by 75% in the presence of 2.5 g/L of 2-PE, it is important to note[30] .

Yeast viability and growth are lowered once the reaction system reaches a specific concentration of 2-PE. The inhibition phenomenon involves a wide variety of mechanisms. Alcohols firstly make the cell membrane more permeable, which encourages ion leakage and reduces the transport of amino acids and glucose. Second, a high 2-PE concentration reduces the cell's ability to breathe. This deficiency is partially attributed to the induction of "petite" mutations, which cause yeast cells to produce dysfunctional mitochondria, and partially results from a direct inhibition of respiration. Furthermore, yeasts are susceptible to both exogenous and endogenous alcohols, including ethanol, which is produced by cells. Because it is a by-product of the yeast fermentation, ethanol interacts with 2-PE in a way that increases its toxicity[31].

Conclusion

Chemically produced 2-PE costs $3.05 USD/kg, naturally produced 2-PE (from flowers) costs $1,000 USD/kg and biotechnologically produced 2-PE costs about $220 USD/kg.

The biotechnological route must outperform conventional methods, which requires highly productive yeasts and inexpensive feedstocks.

In actuality, different yeast species, including S, are able to synthesize 2-PE on synthetic and agro-industrial byproducts using L-Phe as a substrate because the production of 2-PE is strain-specific. cerviviae, K. Karl Marx, P. P. Kudriavzevii. yeasts as well as Y. lipolytica.

Agricultural and food waste materials and by-products can also be used as substrates for the production of 2-PE because of their availability, affordability, and high nutrient content. In order to obtain these aroma compounds in a more sustainable and environmentally friendly manner, the "from waste to product" principle is applied.

Tobacco, cassava water, grape must, corn stover, sugar beet molasses, sugarcane bagasse, and red apple pomace were just a few of the agro-industrial by-products that were successfully used as substrates for the biosynthesis of 2-PE.

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