



Cite this: DOI: 10.1039/d5fb00661a

Received 7th October 2025  
Accepted 28th April 2026

DOI: 10.1039/d5fb00661a

rsc.li/susfoodtech

## Microbial enzymes and genetic design: driving innovation in food bioprocessing

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Among the multitude of functional foods available today, fermented products are uniquely positioned, with the global market for fermented foods anticipated to surpass \$400 billion by 2026, driven by growing demand for functional foods, probiotics, and environmentally friendly practices. The processes of food processing, preservation, and flavor enhancement all rely on microbial fermentation. The microorganisms that carry out fermentation, such as lactic acid bacteria, yeast, and molds, play a major role in enzymatic processes during fermentation. Our focus is on the key enzymes that govern carbohydrate, protein, and lipid metabolism during fermentation, as well as the previously mentioned CRISPR and synthetic biology tools to enhance strain efficiency, biosynthetic capacity, and the production of other enzymes. The synergistic understanding of advanced genetic technologies and biochemistry for enzymatic functions has enabled additional functional and non-functional applications in food, raised food sustainability concerns, and increased the demand for higher-quality foods.

### Sustainability spotlight

The integration of microbial enzymes and genetic engineering is revolutionizing sustainable food bioprocessing by enabling cleaner, more efficient, and resource-conserving production systems. Microbial enzymes enhance process efficiency through targeted biocatalysis, reducing energy consumption, minimizing chemical usage, and lowering industrial waste generation. Simultaneously, advances in genetic design and metabolic engineering facilitate the development of robust microbial strains capable of utilizing low-cost agricultural residues and food waste as renewable substrates. These innovations support circular bioeconomy principles by converting waste streams into high-value food ingredients, bioactive compounds, and functional biomolecules. Furthermore, sustainable enzyme-assisted bioprocesses contribute to reduced greenhouse gas emissions, improved water conservation, and enhanced food security while promoting environmentally responsible manufacturing practices. Collectively, microbial biotechnology and genetic innovation are driving the transition toward resilient, eco-friendly, and sustainable food technology systems for future global demands.

## 1. Introduction

The process of food fermentation has roots in ancient cultures that relied on natural microbial activity to preserve and flavor food, making it more nutritious. The growth of microbiology and biochemistry shifted fermentation from a traditional art to a meticulously monitored, scientifically refined bioprocess. This shift, with its core transformation, was driven by microorganisms—mainly bacteria, yeasts, and molds—that carry out fermentation through a multitude of enzymatic reactions (Fig. 1).<sup>1–3</sup>

These microorganisms are capable of producing various enzymes, such as those associated with carbohydrates, proteins, and even lipids, which serve as large constituents, breaking

them down into smaller bioactive fragments.<sup>5</sup> In dairy fermentation, for example, lactic acid bacteria produce enzymes such as lactase and proteases that convert lactose into lactic acid and proteins into peptides and amino acids. These changes also improve the texture, taste, and digestibility of the fermented dairy products. Similarly, during soy-based fermentations, molds such as *Aspergillus oryzae* produce proteolytic and amyolytic enzymes that hydrolyze soybeans' complex carbohydrates and proteins, thereby enhancing the umami flavor and nutritional value. These phenomena enhance not only the desirable attributes of the processed food, but also assist in extending its shelf life and safety through the production of organic acids and antimicrobial agents.<sup>6</sup>

The introduction of genetic engineering over the past few decades has drastically transformed food fermentation by enabling the alteration of microbial strains to improve fermentation outcomes. With the help of advanced technologies such as recombinant DNA technology, CRISPR-Cas systems, and synthetic biology approaches, scientists can now precisely amplify or silence specific genes in fermentation microbes.<sup>7</sup> This also enables a select positive change:

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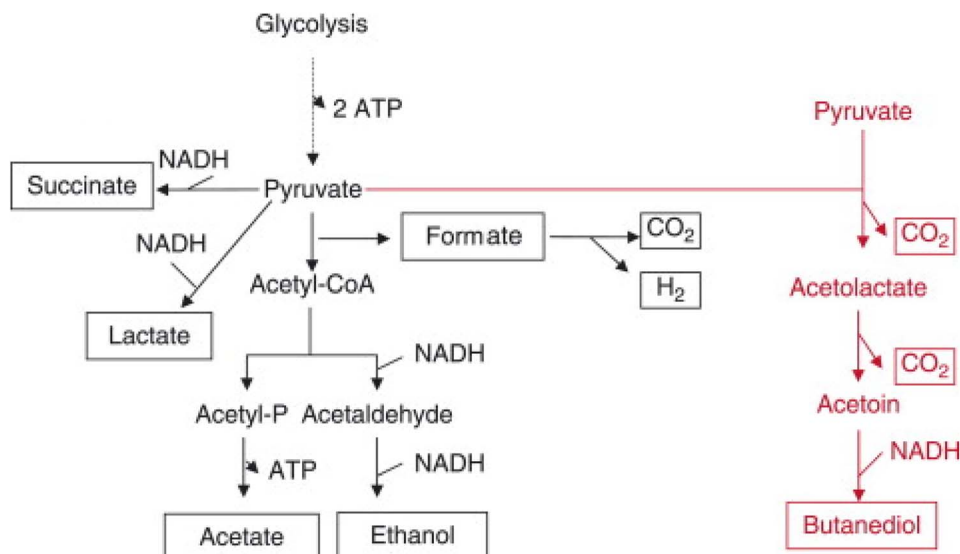


Fig. 1 Enzyme pathways in fermentation.<sup>4</sup>

overproduction of enzymes, increased stress resistance, and silencing of pathways that produce metabolically active, harmful byproducts. For example, some genetically modified strains of *Saccharomyces cerevisiae* now yield higher levels of aroma compounds in bread and alcoholic beverages, and some modified strains of *Lactobacillus plantarum* now produce higher levels of bioactive peptides in fermented vegetables (Fig. 2).<sup>8</sup>

These developments enhance the efficiency, uniformity, and safety of fermentation processes while expanding the development of new functional foods for specific consumer requirements, like low-lactose, gluten-free, and probiotic-rich foods.<sup>9</sup> Therefore, the merging fields of enzymology and genetic engineering related to food fermentation are one of the most emergent and important branches of food science, nutrition, and biotechnology.<sup>10</sup>

This review seeks to analyze contemporary approaches in food fermentation in regard to the individual contributions of major microbial species and their specialized enzymes. Furthermore, attention will be given to contemporary genetic

engineering strategies aimed at improving the effectiveness and utility of these microorganisms to aid in the creation of more economical, healthy, and high-quality fermented foods.

## 2. Microorganisms in food fermentation

Microorganisms serve as the machinery, transforming raw materials into preserved, flavorful, and nutritionally rich foods. Primarily, these microorganisms include bacteria, yeasts, and molds, which are either selected or present in the fermentation environment. Each group contributes uniquely to the sensory, nutritional, and safety aspects of the fermented food.<sup>11</sup> The metabolic activities of these microorganisms are primarily influenced by the enzymes they produce, which break down macromolecules into simpler molecules. A breakdown of the three groups of

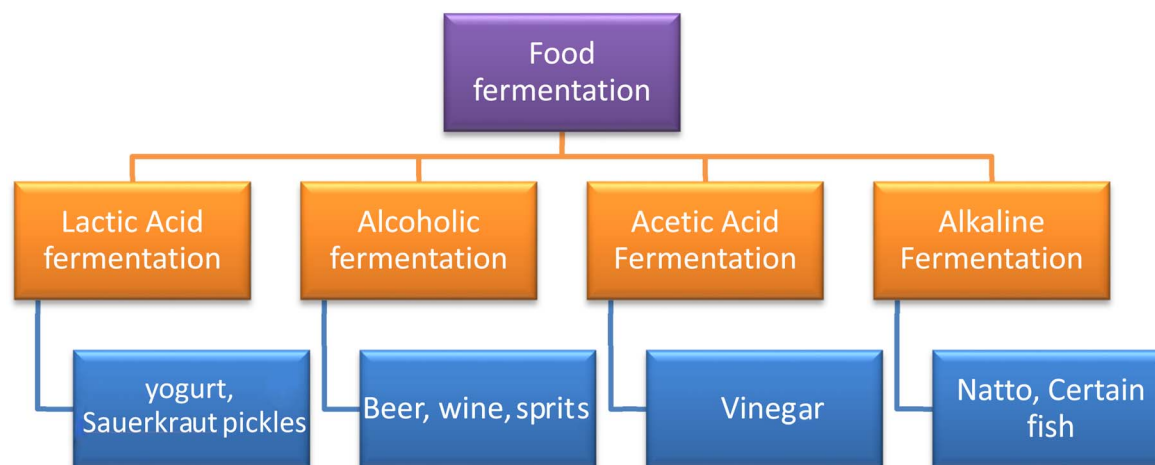


Fig. 2 Food fermentation.



microorganisms that are most commonly used in food fermentation is given below:

### 2.1 Lactic acid bacteria (LAB)

Lactic Acid Bacteria (LAB) are among the most critical microorganisms used in food fermentation. This includes the genera of *Lactobacillus* (now known to be split into several genera, including *Lacticaseibacillus*), *Leuconostoc*, *Lactococcus*, and *Streptococcus*. LABs are used in the fermentation of dairy products (yogurt, cheese), vegetables (kimchi and sauerkraut), and even meats (fermented sausages). One distinguishing feature of LAB is that they primarily ferment carbohydrates to lactic acid. Two fermentative metabolic processes can be carried out by LAB, depending on the species and strain. These are homofermentative processes, which mainly produce lactic acid, and heterofermentative processes, which produce lactic acid along with other byproducts such as ethanol, acetic acid, and carbon dioxide. The formation of lactic acid decreases food pH, inhibiting pathogens and spoilage microorganisms, thereby improving food safety. Moreover, their action plays a major role in the food's texture through acid-induced protein coagulation, as seen in yogurt and cheese, and in flavor and aroma through volatile compounds. Other LAB strains are considered probiotics and can provide health benefits when consumed in sufficient amounts.<sup>12</sup>

### 2.2 Yeasts

Yeasts are vital in the fermentation of alcohol and in the leavening of other foods. They are unicellular fungi, and one of the best-known species is *Saccharomyces cerevisiae*, also known as baker's yeast. It is an important raw material for bread and alcoholic beverages like beer and wine. *S. cerevisiae* breaks down sucrose to produce carbon dioxide and ethanol. During the fermentation of bread, the carbon dioxide released helps the dough to rise, while the ethanol, along with aroma compounds, gives flavor and alcohol to the fermented product.<sup>13</sup>

Other than *S. cerevisiae*, non-*Saccharomyces* yeasts such as *Pichia*, *Candida*, and *Kluyveromyces* are also presumed for the more advanced level of taste and aroma of the fermented foods. These yeasts can synthesize secondary metabolites such as higher esters, alcohols, aldehydes, and acids, which undoubtedly affect the product's taste and aroma. For example, *Kluyveromyces marxianus*, which can ferment lactose, is used in dairy fermentations, and *Pichia anomala* can produce antimicrobial compounds that aid in food preservation.<sup>14</sup> The variety of metabolic activities and the versatility of these microorganisms are useful for both large- and small-scale fermentation systems, processes that are made easier by the flexibility of the yeasts.

### 2.3 Molds

Filamentous fungi play a significant role in fermentation processes for producing East Asian food products such as sake, miso, tempeh, and soy sauce. Cross-fermentative practices include the use of molds such as *Rhizopus oligosporus* and *Aspergillus oryzae*, which are widely used in food fermentation.

*Aspergillus oryzae*, also known as koji mold, has captured the attention of many due to its powerful enzymatic weapons: amylases (sugar benders), which split starch; proteases (muscle benders), which decompose protein in raw materials into peptides; and lipases (fat destroyers). They convert raw materials such as seeds and grains into their core constituents, enhancing their quantifiable value and contributing to the marvelous taste of numerous fermented Asian cuisines. *Rhizopus oligosporus* is also used in tempeh and oxidized soya bean cakes. In addition to serving as a major source of proteins and carbohydrates in soybean, it plays a significant role in promoting the aggregation and structural integration of soybean components, leading to the formation of a compact and stable cake matrix.<sup>15</sup>

Moreover, molds have been shown to help detoxify certain raw materials, break down antinutritional factors and increase the overall nutritional value of fermented foods. Using molds in the fermentation process requires precise environmental control to avoid the development of pathogenic molds, guaranteeing the safety and quality of the resulting products.<sup>16</sup>

## 3. Enzymology of fermentation microorganisms

The initial steps in food processing to produce appetizing, palatable food products with extended shelf life include adding nutritional and quality value tailored to specific target markets. All this can be achieved thanks to the catalytic activities of the fermentation microorganisms. The process of fermentation is accompanied by uncontrolled enzymatic reactions of confounded macromolecules, carbohydrates, proteins, and lipids breaking down into simpler building components: sugars, amino acids, and fatty acids (Fig. 3).<sup>17</sup>

These enzymatic reactions enhance the digestibility and bioavailability of nutrients as well as creating diverse flavor, aroma, and textural compounds characteristic of the sensory profile of fermented foods.<sup>19</sup> A summary of the major classes of enzymes involved in microbial fermentation is provided below:

### 3.1 Carbohydrate-degrading enzymes

Every food fermentation process involves carbohydrate-degrading enzymes that further hydrolyze complex polysaccharides into simpler sugars that can be metabolized by fermentative microorganisms. Moreover, these carbohydrate-degrading enzymes may modulate the perceived palatability of the food substrate through changes in viscosity and sweetness.

- Amylases are a class of enzymes that break down starch, a polysaccharide that is made of glucose monomers, into smaller fermentable sugars like maltose and glucose. Many microbes can utilize these sugars as substrates. Amylases produced by *Aspergillus* and *Bacillus* species are widely used in the fermentation of cereals and starchy products. Amylases from *Aspergillus oryzae* also hydrolyze starch in rice or soybeans during the fermentation of sake, miso, and soy sauce, thereby aiding yeasts and bacteria in subsequent fermentation steps.<sup>20</sup>



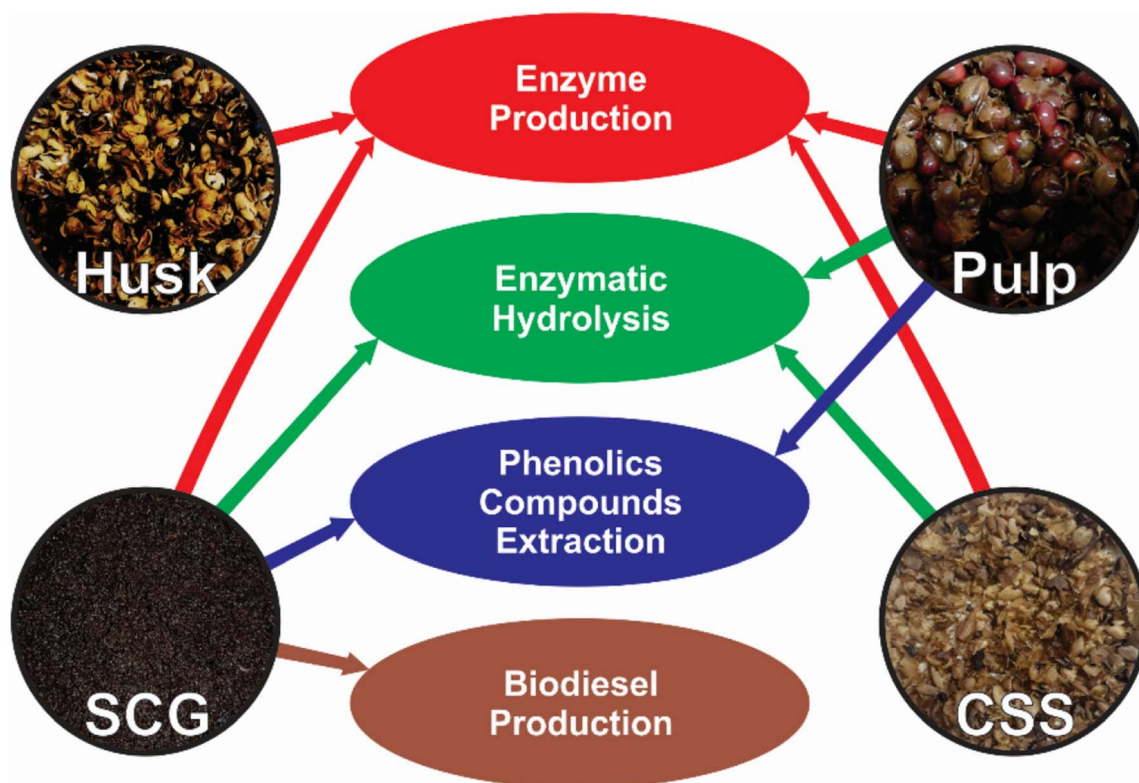


Fig. 3 Enzymatic application in food fermentation:<sup>18</sup> coffee silverskin (CSS) and spent coffee grounds (SCG).

- Cellulases are enzymes responsible for the hydrolysis of cellulose, a structural polysaccharide found in the cell walls of plants, into glucose and cellobiose. These are especially relevant during the fermentation of plant materials such as vegetables or legumes. Through the cleavage of fibrous elements, cellulases augment the release of nutrients and bioactive compounds and soften the texture of the vegetables while improving the fermentative microbial accessibility.<sup>21</sup>

- Sucrose and lactose are disaccharides that can be hydrolyzed by invertases and  $\beta$ -galactosidases into glucose and fructose, and glucose and galactose, respectively. These enzymes hold great importance in the fermentation of dairy and fruits. For example, during the fermentation of milk,  $\beta$ -galactosidase from *Lactobacillus* or *Kluyveromyces* species hydrolyzes lactose, producing lactic acid in yogurt and kefir. This is useful not only for lactose malabsorbers but also for the sour taste and creamy mouthfeel of fermented dairy products.<sup>22</sup>

### 3.2 Proteolytic enzymes

Proteolytic enzymes include specific proteases and peptidases that cleave proteins into smaller peptides and free amino acids. These reactions are essential to the multi-stage fermentation processes that determine the flavor, texture, and nutritional value of constituent parts in many fermented foods. Some LAB, for example, *Lactobacillus helveticus*, mold-like *Aspergillus oryzae*, and some species of *Rhizopus*, produce a broad range of proteolytic enzymes during fermentation. For example, in cheese production, starter cultures are incorporated as the first

step. Then, after the addition of adjunct microorganisms, proteases act to degrade casein, the main protein in milk. This proteolytic activity results in the generation of various salts that may be further broken down into seething volatile compounds such as sulfurous aldehydes and acids.<sup>23</sup>

Moreover, in fermented soybean derivatives such as miso, soy sauce, and tempeh, molds such as *Aspergillus oryzae* and *Rhizopus oligosporus* cleave miso soy proteins, boosting umami taste and producing highly active proteases that further cleave them, enhancing umami flavor and making the dish easier to digest. Aside from these, they also change the texture of the food by reducing anti-nutritional components, which include protease inhibitors found in props legumes.<sup>24</sup>

### 3.3 Lipases and esterases

Lipases and esterases are enzymes of biological importance in the flavor development of certain fermented foods, particularly those involving molds and surface-ripened cheeses, because they act on lipids.

- Lipases aid in the hydrolysis of triglycerides into freely available fatty acids and glycerol. The former can undergo further microbial metabolism to produce volatile compounds such as esters, lactones, and ketones, all of which contribute to the diverse scent of fermented products. For instance, *Penicillium roqueforti*, which is incorporated in certain blue cheeses such as *Gorgonzola* and *Roquefort*, contains lipase, which acts on triglycerides to generate free fatty acids, corroborating the



pungent taste that is peculiarly found in seasoned blue cheeses like cheddar.<sup>25</sup>

- Esterases, as the name suggests, contribute to the generation of esters, budding compounds that impart the scent of fruits and flowers. As *Geotrichum candidum* grows, it hydrolyzes surface milk fat while adding floral notes. It also facilitates the activity of the aforementioned lipases, contributing to lipid hydrolysis and promoting the development of a softer texture in ripened soft cheeses with desirable sensory characteristics.<sup>26</sup>

In combination, these enzymes are needed for fermentations involving fat as the action of esterases and lipases improves the breakdown of lipids into critical aroma-building blocks, contributing to the heterogeneity of taste and the overall sensory appeal.

## 4. Enzyme engineering and directed evolution in food fermentation

The development of enzyme engineering technology has greatly enhanced microbial fermentation processes in food production. Unlike conventional fermentation processes, which rely on existing enzymes, modern biotechnological tools can now create specific enzymes optimized for different, often challenging fermentation conditions. Enzyme engineering is primarily achieved through rational design and directed evolution. In rational design, the chef alters specific amino acids in an enzyme's active site to improve catalytic efficiency, substrate selectivity, and thermal or pH stability, using the enzyme's 3D structure data.<sup>27</sup> Comparing this approach with directed evolution, which mimics natural selection in a lab

setting, reveals a stark difference. It entails developing a vast collection of enzyme variants subjected to random mutagenesis, and thereafter subjecting them to high-throughput screening to select the best-performing ones. This technique has achieved great success in developing heat-resistant proteases for cheese production and acid-resistant  $\beta$ -galactosidase for yogurt fermentation, enabling robust fermentation processes without compromising product quality.<sup>28</sup>

In addition to the approaches focused on single enzymes, other researchers are pursuing the creation of 'fusion enzymes' which combine multiple catalytic domains into one polypeptide chain. These multi-step-designed XX enzymes perform sequential biochemical transformations in a single step, improving efficiency and reducing production time. Another approach of considerable importance is immobilization of enzymes, which consists of binding the enzyme to solid supports to increase their stability or reusability, as well as ease of separation from the fermentation matrix. This method is particularly beneficial in continuous fermentation systems, as well as in industries where the cost-effectiveness and durability of the enzyme are paramount.<sup>29</sup>

Another case of enzyme engineering in food fermentation is the modification of  $\alpha$ -amylase from *Bacillus licheniformis*. The action of this enzyme has been altered to enable its use at higher temperatures in rice-based fermentations in Asia, such as sake and other traditional beverages. This type of modification enables saccharification at high temperatures, thereby increasing contamination risks, shortening processing times, and reducing energy consumption. These advances are essential to modern fermentation systems, which are designed to be sustainable and economical.<sup>30</sup>

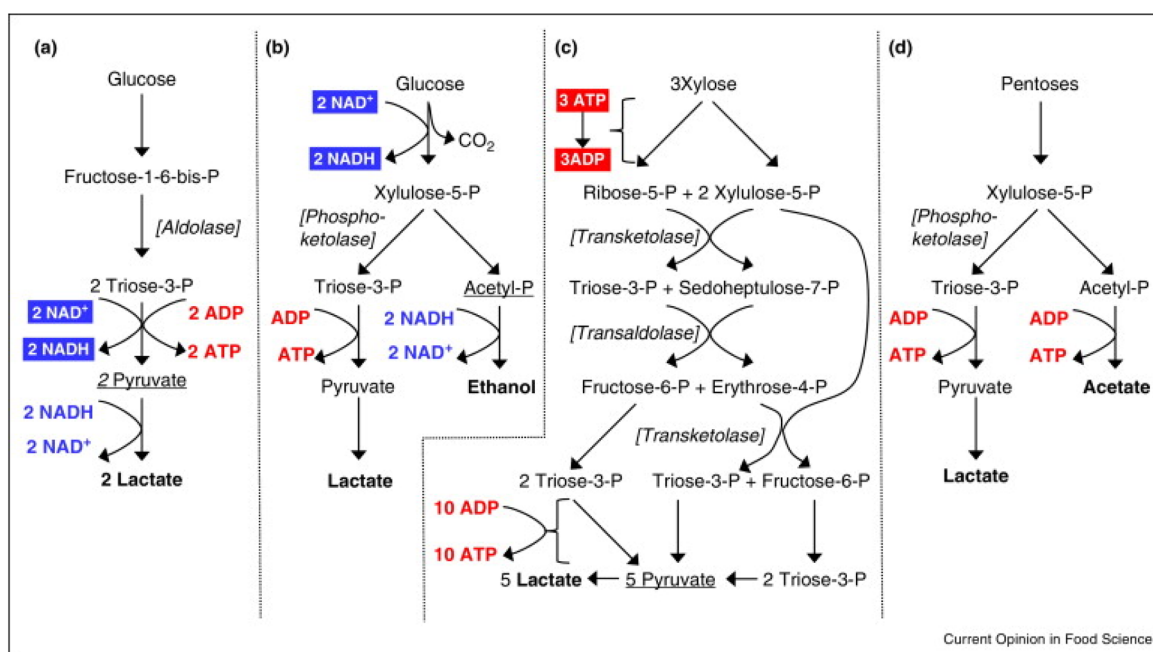


Fig. 4 Enzymatic reaction pathways.<sup>32</sup> (a) Glucose metabolism via glycolysis to pyruvate and lactate. (b) Glucose fermentation via pentose phosphate pathway yielding ethanol and lactate. (c) Pentose phosphate pathway and sugar interconversions leading to lactate production. (d) Pentose metabolism via PPP producing pyruvate, lactate, and acetate.



These developments in enzyme engineering are likely to occur amid stagnating growth in the food industry, which is shifting toward precision fermentation and a greater focus on sustainability. Engineered enzymes provide greater control over biochemical pathways, enhance the nutritional and sensory attributes of fermented foods, and enable the valorization of non-conventional feedstocks such as agricultural waste. With integrated computation modeling, structural biology, and high-throughput screening, we expect enzyme engineering to offer more efficient, resilient, and flexible systems that will meet the shifting demands of the health and environment-conscious consumer for tailored fermentation processes (Fig. 4).<sup>31</sup>

## 5. Genetic engineering of fermentation microorganisms

Recent advances in genetic engineering have greatly increased the potential to optimize microbial strains used in food fermentation processes. Unlike traditional fermentation processes, which depend on naturally occurring or selectively cultivated microorganisms, the enhancement of microbial performance through rational modification is a more precise approach.<sup>33</sup> By manipulating microbial genomes, researchers can enhance enzymatic and metabolic activity and tailor microbes to defined industrial, nutritional, or sensory objectives. Further below are the specific goals, methods, and practical illustrations of genetic engineering pertaining to fermentation microorganisms.<sup>34</sup>

### 5.1 Goals of genetic modification

The primary aims of genetically modifying fermentation microbes focus on improving functionality, operational efficiency, and the safety of the modification.<sup>35</sup> These are engineered to augment the fermentation process and improve the attributes of the final food product.

- Productive modification of genes in microbes with fermentative capabilities, particularly with the aim of enhancing enzyme production, tends to be universal. This goal can be achieved by introducing or overexpressing the genes encoding critical proteolytic and amylolytic enzymes. From a chef's viewpoint, enhanced flavor development and faster fermentation are major outcomes of the effective breakdown of food's constituents. For example, engineered strains of *Aspergillus oryzae* that overproduce proteases can significantly increase protein hydrolysis during soy fermentations, leading to strong umami.<sup>36</sup>

- The production of some health-promoting compounds like gamma-aminobutyric acid,<sup>37</sup> B-group vitamins such as folate and antioxidants can be carried out more readily through genetic engineering. By altering the metabolic pathways of certain microorganisms, it is possible to boost their production of health-promoting compounds and position them for development into functional foods fermented to enhance nutritional value.

- Tolerance to low pH (acidic) temperatures, high ethanol concentration, or high salt concentration must be heightened for optimal spoilage-free fermentative performance. Genetic modifications are one way to aid fermentation strains' stability in extreme environments, resulting in consistent production, which minimizes batch failures.<sup>38</sup>

- Reducing spoilage or pathogenic characteristics is important for sustaining quality and safety. Some wild-type strains exhibit traits such as toxin synthesis, metabolic antibiotic resistance, and the production of non-essential metabolites. Engineering, in many cases, can modify such strains by systematically disabling specific genes and render the microorganisms efficient for food application.<sup>39</sup>

### 5.2 Genetic tools and techniques

A variety of sophisticated molecular biology technologies now exist that allow the editing and reprogramming of the genomes

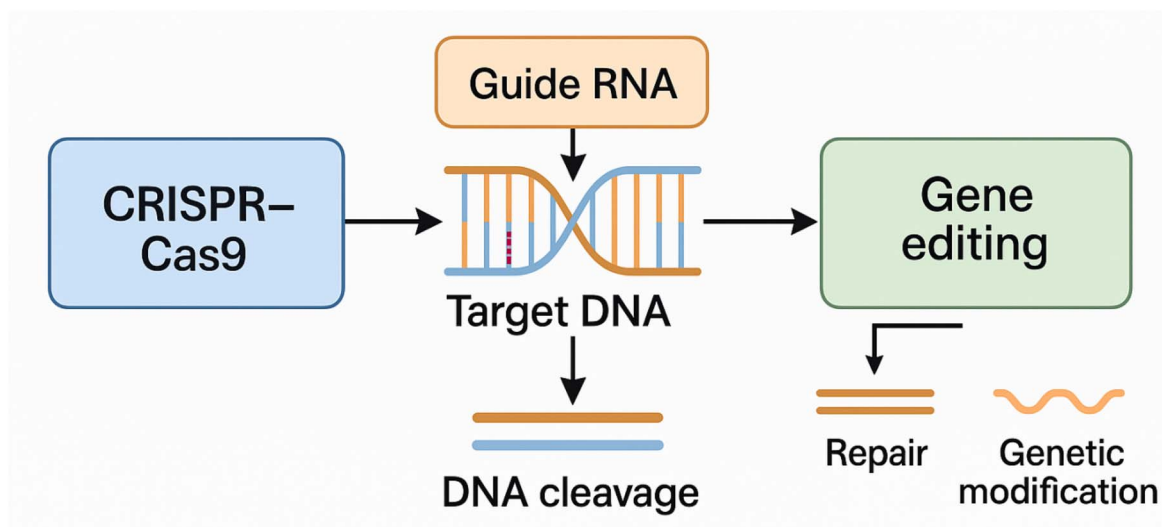


Fig. 5 CRISPR/Cas9 guide mechanisms in common food microbes. Created with <https://BioRender.com>.



of the microorganisms used for fermentation. These technologies have profoundly changed the ways in which researchers refine and regulate microbial activities.

- CRISPR/Cas9 technology enables specific, precise, and effective alterations to be made to DNA sequences within a living organism as highlighted in Fig. 5. This technique employs a guide RNA to locate the desired region within the genome, and the Cas9 enzyme subsequently cleaves it. This permits the precise, regulated control of gene insertion, deletion, and modification.<sup>40</sup>

CRISPR has been used on lactic acid bacteria (LAB), yeasts such as *Saccharomyces cerevisiae*, and even molds like *Aspergillus* species to improve LAB's ability to tolerate acid, express enzymes, and produce metabolites.<sup>40</sup>

- Recombining DNA from different sources requires implanting exogenous DNA fragments into a microorganism's genome, which allows new genes to be expressed or existing ones to be overwritten. This has been done by inserting biosynthetic genes for vitamins into hosts or by restructuring fermentation metabolic pathways to create specific metabolites or enhanced products.

- Synthetic biology goes both ways, enabling the development and assembly of entire gene circuits or metabolic pathways from scratch. This allows microorganisms to be endowed with more complex capabilities, such as controlling enzyme production so that they are produced in different amounts at different stages of fermentation, or having multiple enzymes act together for a combined effect. New possibilities are arising with the application of synthetic biology in developing microbial consortia with a designed profile to be used in fermentation.<sup>41</sup>

The practical applications of genetic engineering in food fermentation are evident in its success stories and benefits. One of these successful cases involves *Lactobacillus plantarum*, which was modified to increase folate production. Folate is one of the B vitamins important to the health of people, especially to the growth of cells and the synthesis of DNA. After modifying the metabolic pathway of folate biosynthesis, a strain of *L. plantarum* was developed that could produce much greater amounts of folate in yogurt and fermented vegetables. This is an improvement for both the cultivation and the nutrition of these foods, which should help many populations suffering from folate deficiency.<sup>42</sup>

The modification of *Saccharomyces cerevisiae* for xylose fermentation is another example. The wild-type *S. cerevisiae* has low efficiency in fermenting xylose, a sugar found in lignocellulosic biomass. Through metabolic engineering, advanced fermentation processes have been developed that enable the conversion of agricultural byproducts into ethanol and other constituents for use in food fermentation. These processes make fuller use of the available resources to improve the yield and sustainability of bioethanol production.<sup>43</sup>

Moreover, protease-overproducing genetically modified strains of *Aspergillus oryzae* have been developed for soy fermentation. In commercially used soy protease miso and soy sauce, proteolytic enzymes are involved in proteolysis and flavor enhancement. Genetic engineering has resulted in the

development of *A. oryzae* strains with improved protease expression, which increases the fermentation rate and augments the levels of flavor compounds, glutamate, and aspartate. With this benefit, these engineered molds help increase the consistency of low protein products, shorten the production times, and thus improve the efficacy and reliability of the fermentation process.<sup>44</sup>

### 5.3 Safety considerations in genetic engineering

The incorporation of genetic engineering into fermentation processes brings many safety issues that require thorough evaluation. Before any food production using genetically modified (GM) microorganisms, intensive risk assessments are conducted to assess potential risks to humans, ecosystems, and biological diversity, including the possibility of new allergens, toxins, and gene transfer to unrelated organisms. Post-market surveillance is crucial after the approval of GM organisms. In addition to basic monitoring, long-term studies to evaluate potential negative outcomes for consumers, assessing the usefulness of the GM microorganisms to determine appropriate intent, and checking for harmful byproducts must be conducted. Strenuous health evaluations are paramount for GM microorganisms in food, especially for imprinted products with long shelf lives. Safety considerations also require taking traceability, labeling, and their inter-regional regulations into account by marking the modified foods for elective patient-based choices. The stipulated modification is self-contained, with strict command protocols to ensure that the unintentional release of GMOs into the environment does not occur. Control of these GM organisms is essential through rigorous measures to prevent uncontrolled proliferation.<sup>37</sup>

As seen with the GM microorganisms used in fermentation, adequate safety surveillance and practices involving clear communication at every stage of the product lifecycle are necessary to ensure product safety. The development of food fermentation has been enhanced by the fields of microbial enzymology and food genetic engineering. Table 1 summarizes research conducted to date in this field, showcasing the microorganisms used, the genetic or enzymatic targets, and their applications. This synthesis helps clarify what is currently available and determine what can be pursued in other research in later stages.<sup>45</sup>

## 6. Applications of fermentation in sustainable food systems

Fermentation processes contribute to technological and biomaterial innovations in waste management, resource management, and environmental protection. In relation to these sustainable approaches, fermentation assists in sustainability through waste valorization. By-products from the agricultural and food industries, such as fruit and vegetable scraps, grains, and food remnants, enable fermentation processes (Fig. 6). For instance, functional products such as vinegar or even fermented animal feed can be produced from food waste and low-value perishables. In addition, agricultural by-products



Table 1 Summary of previous research on microbial enzymology and genetic engineering in food fermentation

| Reference | Focus of study  | Microorganism <sup>46</sup> involved              | Application area                            |
|-----------|---|---|---|
| 47        | Folate overproduction in <i>L. plantarum</i> via genetic modification | <i>Lactobacillus plantarum</i>                    | Functional fermented dairy/vegetables       |
| 48        | Non- <i>Saccharomyces</i> yeast contributions to food aroma           | <i>Pichia</i> , <i>Kluyveromyces</i>              | Alcoholic beverages and fruit fermentations |
| 49        | Proteolytic activity in LAB for flavor development                    | <i>Lactobacillus helveticus</i>                   | Cheese, yogurt                              |
| 50        | Role of amylases in cereal fermentation                               | <i>Aspergillus oryzae</i> , <i>Bacillus</i>       | Miso, soy sauce, sake                       |
| 51        | Xylose fermentation by engineered <i>S. cerevisiae</i>                | <i>Saccharomyces cerevisiae</i>                   | Bioethanol, waste valorization              |
| 52        | Protease overproduction in <i>A. oryzae</i> for soy fermentation      | <i>Aspergillus oryzae</i>                         | Soy-based food fermentation                 |
| 53        | Probiotic potential of LAB in fermented foods                         | Lactic acid bacteria                              | Fermented foods (general)                   |
| 54        | Role of lipases/esterases in cheese flavor development                | <i>Penicillium roqueforti</i> , <i>Geotrichum</i> | Surface-ripened cheeses                     |
| 55        | CRISPR editing of LAB for enhanced acid resistance                    | Lactic acid bacteria                              | Probiotic-rich dairy                        |
| 56        | Recombinant DNA use for enzyme expression in molds                    | <i>Aspergillus</i> , <i>Lactobacillus</i>         | Industrial enzyme production                |
| 57        | Bioactive compound production in fermented foods                      | <i>Lactobacillus</i> , <i>Bifidobacterium</i>     | Smart foods with health benefits            |
| 58        | Precision fermentation for flavor and safety control                  | Selected microbial consortia                      | Tailored food fermentation                  |
| 59        | Multi-omics in fermentation optimization                              | Mixed microbial communities                       | Fermentation system design                  |
| 60        | Regulatory and safety aspects of GMOs in food                         | Genetically engineered microbes                   | Policy, compliance, labeling                |
| 61        | Comprehensive review of microbial enzymology in fermentation          | Lactic acid bacteria, yeasts, molds               | Fermented food industry outlook             |

## Microbial fermentation: A pathway to improved nutrient bioavailability

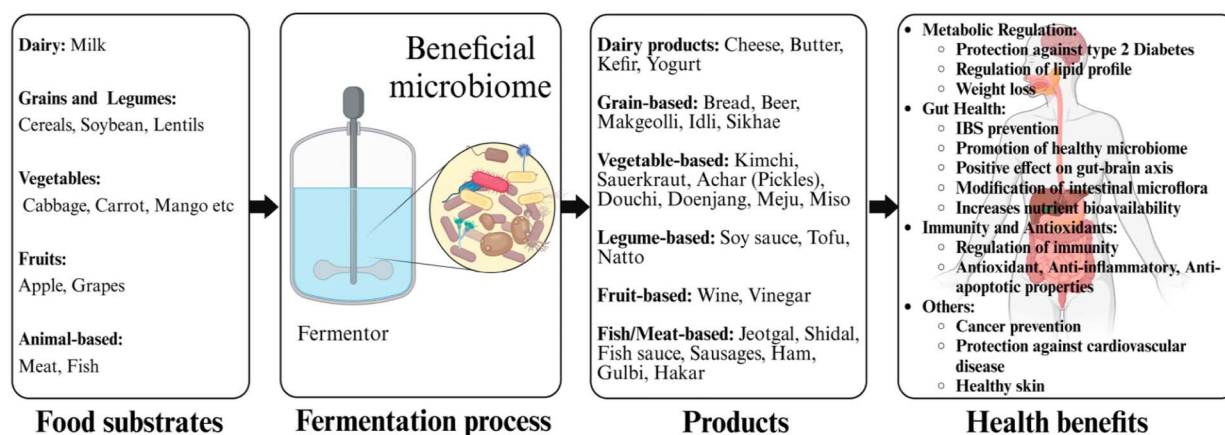


Fig. 6 Microbial fermentation in food: a comprehensive overview of the fermentation process, substrates, products, and associated health benefits. Created with <https://BioRender.com>.<sup>46</sup>

such as corn husks and cereal straw are now commonly utilized in biofuel or bio-based chemical production through fermentation, which supports the circular economy movement. Alterations to raw materials are important for changes in sustainability.<sup>62</sup> The use of plant or insect materials in fermentation reduces dependence on resource-intensive animal farming. Take, for example, insects as a protein source with low emissions because they require less land, water, and feed than livestock. Fermentation also embraces plant-based ingredients like legumes and peas, aiming towards sustainable dairy or vegan products. And last but not least, one of the main goals nowadays is to reduce the carbon footprint through fermentation. Precision fermentation is a method of producing proteins,

fats, and other ingredients using synthesized microorganisms. Compared to traditional animal farming, it has a more positive environmental impact. It could help decrease GHG emissions, land area, and water usage, contributing to an improved food production system.<sup>63</sup>

### 6.1 Industrial scale vs. small-scale fermentation

The difference between industrial-scale fermentation and small-scale or artisanal fermentation is remarkable and comes with its own unique challenges and opportunities. At an industrial level, fermentation processes are optimized for efficiency, scalability, and consistency. Large-scale fermentation is considered efficient because it is usually automated. This



automation includes the use of bioreactors that regulate temperature, pH, and nutrient levels for optimal microbial growth. The industrial setting focuses on maximizing production volume while minimizing costs.<sup>64</sup> This is the most common way to meet consumer expectations, which leads to product standardization. Such a high-volume production model is ideal for mass-market foods; however, it greatly limits the product's uniqueness. On the other hand, small-scale or artisanal fermentation places great emphasis on the quality and uniqueness of the end product, including creativity and sensory characteristics such as smell and taste. Artisanal methods, however, are flexible and better suited to catering to niche markets and flooding them with unique flavors, textures, and other sensory attributes. Small-scale production has proven to be more labor-intensive and to require a manual skill set, thereby increasing production costs.<sup>65</sup> However, artisanal fermentation still offers opportunities for innovation and experimentation with new flavors or traditional techniques, appealing to consumers seeking authenticity. Not to forget that such techniques are also more efficient in terms of sustainability, as the ingredients used are often locally sourced. The decision to use industrial or small-scale fermentation depends largely on how one balances efficiency, cost, and product individuality.<sup>66</sup>

## 6.2 Consumer trends and market impact

Recent years have seen favorable changes in the global market for fermented foods, driven by shifts in consumer preferences and rising demand for functional foods. A greater emphasis on wellness, particularly gut health, is driving growth in market demand. The fermented foods sector is expected to surpass \$400 billion by 2026, owing to probiotics-rich foods, nutraceuticals like kombucha, and tailored nutrition products gaining market traction.<sup>67</sup> There is heightened awareness among people of the benefits of consuming probiotics, including improved digestive health, boosted immunity, and overall health support. This shift in awareness has increased demand for yogurt, kimchi, sauerkraut, and kefir, which are now essentials in most pantries. The expansion of personalized nutrition also contributes to this shift, as there is a growing demand for tailor-made health solutions like low-lactose, gluten-free, or probiotic foods.<sup>68</sup> Also, sustainability is emerging as a key motivator for consumers, with many opting for fermented products with plant-based ingredients or that support ethical and eco-friendly business practices. The increase in demand for functional fermented foods is significant, as shoppers seek products with advanced bioactive properties that help boost immunity, improve digestion, and enhance cognitive function. This pressure is forcing manufacturers to come up with innovations to fulfill these changes in consumer preference.<sup>69</sup>

## 6.3 Emerging trends and applications

Food fermentation has become a highly controlled, innovative, health-focused biotechnology as our understanding of it and related technologies has evolved. Newer developments in the

domain are enhancing the focus of fermented foods far beyond preservation and flavoring. Through systems biology, multi-omics technologies, and genetic engineering, researchers and industries can precisely tailor fermentation systems and functional food products to consumers' requirements.<sup>70</sup> Below is a discussion of the trends I believe will have the most significant impact and their applications:

### 6.4 Functional fermented foods

One of the most important trends shaping the future of nutraceuticals is the creation of functional fermented foods that aim to address nutritional gaps and deliver specific health benefits. From engineering more sophisticated fermentation microorganisms, it has become feasible to conceive strains that synthesize vitamins (B-group vitamins – folate, B12), antioxidants, bioactive peptides, and amino acids during fermentation. Such bioactive compounds can enhance gut health (immunity), cardiovascular wellness, and cognitive function.<sup>71</sup>

For instance, strains of *Lactobacillus* and *Bifidobacterium* have been modified to produce more GABA (gamma-aminobutyric acid), a neurotransmitter known to reduce anxiety and lower blood pressure. Likewise, the increased production of folate by *Lactobacillus plantarum* or antioxidant-rich peptides in fermented dairy products contributes to a new class of “smart” foods designed for use as dietary supplements within normal eating routines. This phenomenon is rapidly expanding the market for fermented products among consumers who are more conscious about their health and who use food for preventive and therapeutic nutrition.<sup>72</sup>

### 6.5 Precision fermentation

Precision fermentation is an emerging technology that adopts a more systematic approach in the fermentation industry. Unlike traditional fermentations that rely on undefined microbial populations, precision fermentation uses well-defined microbial strains or consortia. Defined consortia are groups of selected microorganisms that share specific genetic and metabolic characteristics. These microbes are either selected or engineered to perform specific tasks, such as enzyme production, spoilage prevention,<sup>21</sup> or flavor enhancement. Compared with traditional fermentation, precision fermentation is far more flexible and enables detailed modifications to achieve desired outcomes. By modifying microbial composition and the expression of relevant enzymes, precision fermentation provides control over the safety and sensory attributes of fermented products. For example, plant-based products that undergo fermentation face challenges with bitterness and off-flavors that affect their overall taste and market appeal. Additionally, the use of consortia enables organisms to interact synergistically, with one organism producing the compounds needed by another, leading to more complex profiles and flavors. Also, through microbial biosynthesis of proteins, fats, and flavor molecules, precision fermentation integrates with sustainable food technologies to develop meat, dairy, and egg analogs without animal byproducts. This is part of global



movements aimed at formulating sustainable, eco-friendly, and ethically sound food systems.<sup>5,25</sup>

### 6.6 Multi-omics approaches

The combination of metagenomics, transcriptomics, proteomics, and metabolomics is providing scientists with a new perspective on optimizing food fermentation. These methods provide a functional, systems-level understanding of a microbe's activity and function, thus enabling a reasonable understanding of enzyme actions, gene expression, and metabolite formation.<sup>73</sup>

- Metagenomic analysis enables scientists to investigate the entire genetic potential of unculturable microbial communities residing within fermented foods. This research broadens the scope of discovering novel metagenomic enzymes and genes responsible for flavor, texture, and health-related metabolites.

- Transcriptomics aids in understanding RNA expression and provides information on genes that are transcribed during fermentation under specific conditions. This information assists in optimizing fermentation processes and identifying the controlling processes for enzyme activation.

- Metabolomic analysis of fermentation concentrates on the end products like organic acids, esters, alcohols, and peptides. Linking specific microbial activities with the resultant end products in terms of flavor and nutrition improves understanding of these nutritive outputs.

Together, these tools analyze the complete dynamics of the fermentation ecosystem. This understanding provides in-depth insights into the rational design and enhancement of microbes, fermentation strategies, and even the automation of intensive tasks. Additionally, these tools can be utilized in developing advanced predictive models for self-regulated fermentation behavior, expediting novel product development and optimizing industrial processes.<sup>30,68,73</sup>

### 6.7 Safety and regulatory considerations

Microorganisms have been used in the fermentation of foods for decades, and due to the roles of *Lactobacillus*, *Saccharomyces*, and *Aspergillus* species in the fermentation process, they are considered GRAS (Generally Recognized As Safe) by the FDA. Like other forms of biotechnology, the use of genetic modification results in more stringent safety evaluations in risk analysis frameworks, regulatory scrutiny, and the assessment of GMOs in food systems.<sup>41,48</sup> Risk evaluation is highly diverse with the use of genetic modification *via* recombinant DNA technologies, CRISPR/Cas9, and synthetic biology. Certain parameters, such as allergenic and toxic potential, and the stability of the introduced genes, along with environmental evaluation, are set by regulatory agencies. This is crucial when GM microorganisms are incorporated into foods intended for direct consumption, rather than simply aiding food processing.<sup>2,39</sup> The degree of acceptance and oversight still tends to differ by region due to how policies are formulated and structured:

- In the United States, the policy of the USDA is known as the product-based approach. This means that a genetically modified food or microorganism will get approval if it contains no

harmful characteristics and is, by and large, similar to its non-GMO version. CRISPR-edited organisms that do not introduce foreign DNA may sometimes bypass GMO classification depending on the context.<sup>74</sup>

- In contrast, the European Union takes a more stringent, process-based approach to policy. Genes that have been edited or generated using recombinant technologies are treated as GMOs and must comply with exhaustive pre-market approval, labeling, and even traceability requirements, regardless of whether foreign DNA is present. This is indicative of both public concern and regulatory conservatism within the EU.<sup>60</sup>

These legal frameworks pose significant barriers to the commercialization of genetically engineered fermentation microbes globally. Not only do scientists and companies have to deal with the technical side of the issue, but they must also address compliance, documentation, and even consumer education. In the process of gaining approval and public trust, Liu and others cite the necessity of strict safety data.

## 7. Case studies in genetic engineering and advanced fermentation techniques

Numerous real-life case studies underscore the benefits of biotechnology and modern fermentation processes. These include the genetic engineering of *Lactobacillus plantarum* to overproduce folate, an essential B-vitamin. This development has enabled the production of fortified yogurt and fermented vegetables to treat endemic nutritional insufficiency in certain populations. Another example is the engineering of *Saccharomyces cerevisiae* strains capable of fermenting xylose, a sugar obtainable from agricultural byproducts. This has enabled the use of lignocellulosic biomass from corn stover and wheat straw for bioethanol production, thereby promoting sustainable fuel production. In soy products from Asia, *Aspergillus oryzae* has been genetically modified to enhance soy protease production, thereby improving soy fermentation and enhancing the flavor and textural properties of soy sauce and miso.<sup>10,26,55</sup> This makes processes more efficient, with shorter time frames, while maintaining greater consistency across products and enhancing umami taste. These case studies illustrate how genetic engineering, combined with modern fermentation technologies, can create more efficient, high-quality, nutritionally dense foods and meet the rising global need for sustainable, functional, and health-beneficial foods. Although microbial biotechnology and fermentation science have advanced significantly, fundamental hurdles still hinder the full use of engineered microorganisms in food production. These challenges are multifaceted, covering technical, socio-cultural, and regulatory domains, and require a comprehensive approach for effective solutions. The use of genetically modified organisms (GMOs) in food is currently constrained by stubborn regulatory barriers, which are among the most important hurdles. Innovation pipelines can be severely stunted by international inconsistencies, slow approval processes, and costly compliance. These hurdles disproportionately affect smaller businesses that lack the resources to navigate complex systems.



Moreover, many promising fermentation technologies remain commercialized and unfunded despite their potential value, useful when financed within a public-private partnership model.<sup>42,54</sup>

Consumer attitudes can facilitate or impede the adoption of genetically engineered fermented foods. For example, in Europe and in some parts of Asia, consumers express skepticism around the safety, unnaturalness, and environmental impacts of GMOs. Unfortunately, this skepticism is accompanied by a disinformation campaign and low levels of awareness. Trust can, however, be built by developing effective communication and outreach programs that implement transparent labeling, convey accurate information, and inform the public about other principles of fundamental science. Acceptance of engineered fermentation products can, therefore, be built on such trust. Another challenge focuses on maintaining traditional fermented-food practices while employing modern genetic tools. Flavorful, artisanal, traditionally fermented products are valuable for more than just their taste; they also hold cultural significance and often embody skilled craftsmanship. Employing genetically modified strains may worsen consumers' perceptions of sensory attributes and, in turn, of authenticity. Balancing innovation and cultural preservation can be achieved through considerate strain design for modern tools integration into traditional work.<sup>8,56,60,71</sup>

Looking ahead, several promising directions are emerging. As sustainability becomes ever more important, so does sustainable fermentation with special priority on low-input systems, waste valorization, and low energy use. Engineered microbes could play a significant role in achieving circular food economies by converting underutilized agricultural residues into food products or bioactive compounds that promote health. Also, optimization of bioprocesses will be important. Such control, background scaling up of processes, multi-omics data, automation, and consistent monitoring allow for greater control during fermentation. From an economic perspective, these technological improvements will enhance the cost efficiency of fermentation, making their processes easier. The application of artificial intelligence to the design of microbial strains is one of the most exciting developments. With the help of machine learning and AI, it is possible to analyze genomics, transcriptomics, and metabolomics and suggest the best fermentation conditions. This model will further speed up the advancement of innovations by minimizing empirical testing and redefining the construction of next-generation fermentation systems.<sup>28,37,46,70–72</sup>

## 8. Conclusion

The combined disciplines of microbial enzymology and genetic engineering have elevated food fermentation from an artisanal practice into a highly sophisticated biotechnological process. Through the actions of naturally occurring and synthetic enzymes, fermentation catalyzes the transformation of food products by enhancing their flavor, texture, and preservation, and, most importantly, elevating their nutritional value and functional potential. Existing design tools, such as CRISPR and

synthetic biology technologies, coupled with multi-omics, enable the tailoring of microorganisms and their control systems to precise industrial, consumer, and ecological requirements. The ability to customize enzymes and microorganisms enables the production of tailor-made bioactive-enriched fermented foods with improved digestibility to meet specific dietary needs. Furthermore, fermentation processes sustain food supply by mitigating waste through valorization, reducing carbon footprints, employing unconventional raw materials, and utilizing materials that would otherwise go to waste, reinforcing the importance of fermentation in addressing global food supply and environmental issues. Despite progress, many issues remain, including public scrutiny of the use of genetically modified organisms, legislative barriers, and the preservation of traditional attributes. In the future, active implementation of advanced artificial intelligence, real-time bioprocess monitoring, and enhanced safety application frameworks will be necessary to scale trusted innovations. Fundamentally, harnessing microbial enzymology together with genetic engineering provides extraordinary opportunities for developing the next generation of functional, safe, and sustainable fermented foods, satisfying both the present food requirements and the anticipated challenges of future food systems.

## Conflicts of interest

The author declares there are no conflicts of interest.

## Data availability

The authors confirm that the data will be made available on request.

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