Microbial Fermentation Technology

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Fermentation is one of the earliest biotechnological methods of food preservation and processing to be extensively applied in the world: foods (fermented food, food additives, functional materials and live probiotics); intestines (aids digestion and promotes absorption, synthetic bioactive substances, inhibits harmful bacteria, diabetes, cardiopathy and allergy); and industry (energy, soil transformation and sewage treatment). The current trends in fermented-based vegetable foods are growing. Fermentation has been used for ages as a safe technique for food preservation, and it uses minimal resources. Fermentation is related to a wide range of catabolic biochemical procedures in both eukaryotes and prokaryotes. Yeasts are eukaryotes; they can use oxygen while also having the ability to live without oxygen. The lactate fermentation process consists of glycolysis and some alternative steps.

microbial fermentation protein

1. Fermentation Technologies

1.1. Solid State Fermentation (SSF)

Solid state fermentation (SSF) is a fermentation technique performed by different industries like the pharmaceuticals, textile, food, etc., to produce metabolite microorganisms using solid support in place of the liquid medium [1][2]. Compared with submerged fermentation (SmF), SSF has different benefits like direct use of agricultural and industrial residues as carbon sources and leading in affordable cost; however, systematic analysis of genome-wide gene expression in filamentous fungi under various cultivation conditions, namely SSF and SmF, is scarce [3][4][5]. The microbiological components of SSF can happen as single pure cultures, mixed identifiable cultures or totally integrated indigenous microorganisms; some SSF technologies, e.g., tempeh and oncom production, need the selective growth of organisms such as molds that need low moisture levels to carry out fermentation with the assistance of extracellular enzymes secreted by fermenting microorganisms [6][7]. However, bacteria and yeasts, which need higher moisture content for effective fermentation, can also be used for SSF, but with a lower yield [8]. The most important advantages of solid state fermentation are: (1) it produces a minimum amount of waste and liquid effluent, thus it is not very damaging to the environment; (2) solid substrate fermentation employs simple natural solids as the media; (3) low technology and low energy expenditure require less capital investment; (4) no need for sterilization, less microbial contamination and easy downstream processing; (5) the utilization of agro-industrial residues as substrates in SSF processes provides an alternative avenue and value-addition to these otherwise under- or non-utilized residues; (6) the yield of the products is reasonably high; (7) bioreactor design, aeration process and effluent treatment are quite simple; and (8) many domestic, industrial and agricultural wastes can be fruitfully used in SSF. The limitations of solid state fermentation are: (1) microorganisms that tolerate only low moisture can be used; (2) precise monitoring of SSF (e.g., O_2 and CO_2 levels, moisture content) is not possible; (3) organisms grow slowly, and consequently, there is a limitation in product formation; and (4) heat production creates problems, and it is very difficult to regulate the growth environment [9][10][11][12]. The variety of enzymes produced in SSF are: Naringinase (Orange and grapefruit rind), Polygalacturonase (Apple bagasse and wheat bran), α-Amylase (Rice husk, banana husk, millet, water melon husk, lentil bran, wheat bran and maize oil cake), Manganese peroxidase (Pineapple leaf), Lipase (Sunflower seed and sugarcane bagasse), Protease (Wheat bran and soybean meal), Cellulase and hemicellulase (Corn straw, rice husk, grass powder, sugarcane barbojo and sugarcane bagasse), Ellagitannase (Sugarcane bagasse, corn cobs, coconut husk and candelilla stalks), Phytase (Wheat bran) and Laccase (Poplar sawdust) [13]. Lipids produced in SSF are: γ-Linolenic acid (*Mortierella isabellina*), Gamma linolenic acid (*Mucor rouxii*), Oleic acid and Palmitic acid (*Mortierella isabellina*), Lipids (*A. oryzae*), Oleic acid and Palmitic acid and Linoleic acid (*Mortierella isabellina*), Lipids (*Mortierella isabellina*) and Lipids (*Aspergillus tubingensis* TSIP9) [13].

Organic acids produced in SSF are Citric acid (*Aspergillus niger* DS 1, *Aspergillus niger* CECT-2090, *Aspergillus niger* PTCC-5010), Lactic acid (*Lactobacillus delbrueckii*, Lactobacillus casei, Lactobacillus amylophilus GV6), Gluconic acid (Aspergillus niger ARNU-4, *Aspergillus niger*) and Ellagic acid (*Aspergillus niger*, *Aspergillus niger* GH1) [13]. Cashew and guava byproducts were successfully subjected to solid state fermentation for protein enrichment through single-cell protein and then included in cereal bars for human nutrition, and the addition of protein-enriched byproducts is a substitute to add nutritional and economic value to cereal bars [14]. The addition of 0.1% and especially of 0.5% solid state fermentation product (Synergen^{TM}) could markedly improve growth performance and feed efficiency of lupin diets [15]. Fermentation of de-oiled rice bran (DORB) resulted in decreased in vitro protein digestibility; fermentation of DORB with *Rhizopus oryzae* increases the n-6 fatty acid profile; and fermentation leads to reduction in phytate and trypsin inhibitor activity of DORB [16]. Inoculation of suitable cellulolytic microbes to enrich protein content and improve in vitro digestibility of herbage with solid state fermentation for chicken feed is the prospective method for animal husbandry, agriculture and substantial management [17].

The protein constituent of fermented pangola grass increased from 5.97–6.28% to 7.09–16.96%, and the in vitro digestion increased from 4.11–4.38% to 6.08–19.89% with the inoculation of cellulolytic microbes by solid state fermentation; this procedure may enrich protein content, increase in vitro digestibility and boost the quality for animal feeding [18]. Fermentation by *Bacillus subtilis* increased the nutritional quality of soybean meal (SBM), and fermentation principally decreases trypsin inhibitor and beta-conglycinin in SBM [19]. It has been reported that the solid state fermentation of aquatic macrophytes in the production of crude protein extraction is encouraging, which makes aquatic macrophytes a potential source and thus is suitable to the long-term ecological restoration of eutrophic lakes [20]. The electronic nose (e-nose) technique was designed to monitor the SSF process of protein feed and the application of linear and non-linear algorithms in calibrating the discrimination model using e-nose data [21]. *Pleurotus ostreatus*-based solid state fermentation of mechanically managed canola meal increased its protein constituent, and fungal fermentation degraded glucosinolates and phytate up to 98.8% and 75.8%, respectively [22]. Solid state fermentation increased protein and amino acid constituents of soybean meal (SBM), and *B. subtilis* brought about a greater impact to increase protein and AA than *A. oryzae* [23].

Solid state fermentation with *Rhizophus obligosporus*, according to nitrogen compounds balance, helped to increase the nutritional value of the grains and the digestibility of its protein in lupin [24]. Solid state fermentation revealed better enzyme activity than submerged fermentation for both raw and processed canola meal [25]. Solid state fermentation of pineapple peels with *Trichoderma viride* ATCC 36,316 resulted in protein production, and protein enriched peels from an on-farm fermenter had higher protein content than the conical flask experiment's product, 16 and 14.89%, respectively [26]. Solid state fermentation enriches fruit and vegetable discards in protein and amino acid profile, highly improving their suitability as animal feed, and *Rhizopus* fermentation of fruit and vegetable leachate leads to a 31% protein biomass, being a valuable alternative protein [27]. SSF involved the consumption of mainly amylopectin instead of amylose and non-resistant starch instead of resistant starch irrespective of the Australian sorghum variety, and all fermented samples were found to have increased protein content [28]. A novel solid state fermentation with *Bacillus subtilis* was applied to produce fermented chickpeas, and chickpea proteins were degraded to low molecular weight peptides during fermentation [29]. Fermentation-assisted hydrolysis increased the protein quality of soybean mean, and fermentation-assisted hydrolysis decreased the potential antigenicity of soybean meal [30]. Solid state fermentation was conductive to boosting drumstick (*Moringa oleifera* Lam.) leaf nutritional value, and protein content was also increased [31]. It has been reported that solid state fermentation leads to an effective approach to increasing the quality of proteins sources, such as rapeseed cake, as well as increasing the enzyme activity of endoglucanase, acid protease, xylanase and phytase [32]. It was found that SSF decreased the organic matter and reduced the sugar content of the fermented product, while crude protein and fiber fractions were improved; SFF led to a stabilized feed ingredient enriched in protein but at the expense of digestibility reduction [33].

1.2. Submerged Fermentation (SmF)

SmF is a procedure in which the growth of microorganisms happens in a liquid broth medium, which is escalated with mandatory nutrient to have a better cultivation of microorganisms, and this consists of accurately growing the selected microorganisms in closed reactors with medium fermentation and a high concentration of oxygen [34][35]. Bacteria are usually utilized as a source in this procedure as it needs high moisture content [36]. Submerged fermentation, using *Trichoderma viride* ATCC 36,316 on cassava peel, particularly on unpretreated cassava peel for 3 to 4 days, improved crude protein content of cassava peel 8-fold and true protein constituent 22-fold [37]. Although submerged fermentation (SmF) is responsible for the majority of current enzyme industries, it has been reported that solid state fermentation (SSF) can produce higher enzyme yields in laboratory scale. The non-enzyme proteins in SSF were active in fungal mycelia growth and condition, while those in SmF were more associated to stress tolerance and glycometabolism [38]. The solid state fermentation step improved the protein content in waste bread by 161%, and the fermented product has potency to be applied as nutrient rich feed [39]. Production in solid state fermentation was two times higher than submerged liquid fermentation, and this significant difference in yields of hydrophobins underlines the appropriateness of solid substrate fermentation procedure along with the addition of oil cakes to boost the yields [40]. Sustainable production of mycoproteins and surface-active proteins can be progressed by growing a marine fungal strain for shedding light on the potentiality of an integrated methodology that promotes the circular economy [41]. A novel magnetic field technology aid for submerged fermentation was performed; the morphology of mycelium was altered significantly after magnetic field treatment;

the scale-up magnetic field fermentation notably enhanced mycelium biomass; and the magnetic field increased fermentation by stimulating the expression of genes [42]. Cellulase activities of micoorganisms changed according to various conditions, and solid state fermentation indicated better enzyme activity than submerged fermentation [42]. An isolate of *Aspergillus niger* was assessed for citric acid production and enriched protein mycelium using molasses and whey for the fermentation medium, and utilizing industrial wastes of cheese whey fortified with beet molasses increased the consistent, economical, large-scale yield of citric acid by protein enriched *A. niger* [43]. Among different microorganisms, *Fusarium venenatum* is the most prevalent species to be successfully utilized in food industry, and it has been applied to produce mycoprotein as food being under the trade name Quorn, and mycoprotein indicates satiation characteristics which can be a solution for obesity by enabling people to obtain a healthier diet with low fat and high fiber content [44]. It has been reported that *Vitreoscilla hemoglobin* has profitable advantages on improving total protein secretion and cellulase activity of *Trichoderma reesei* in submerged fermentation [45]. Benefits and disadvantages of Solid State Fermentation and Submerged Fermentation are presented in **Table 1**.

Table 1. Comparison of Solid State Fermentation and Submerged Fermentation.

Types	Advantages	Disadvantages
Solid State Fermentation	Substrates need less pretreatment in comparison with liquid media	Low moisture level can restrict the growth of microorganisms
	The medium is easily available, simple, and inexpensive	A problem in removing metabolic heat in large scale
	Forced aeration is usually easier	Problems and difficulties in monitoring the process parameters
	Contaminations are restricted since the moisture content is low	
	Simple fermentation equipment	
	Minimized and simplified downstream process and waste disposal	
	High volumetric productivity	
Submerged Fermentation	Simplicity of measuring process parameters	Utilization of expensive equipment and costly media
	Even distribution of microorganisms and nutrients	Expensive and complex downstream procedure and difficulty in the waste disposal
	Capability to control and monitor growth conditions	High power consumption
	Accessibility of high-water content for	

Types	Advantages	Disadvantages
	the growth of microbes	

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Fermentation engineering, which is one of the most important components of modern biotechnology, has been extensively applied in areas including food, pharmaceutical and chemical industries, energy and environmental protection [46]. Various methods, such as microscopy, product and substrate evaluation, toxicity tests or biomass monitoring assist in generating a complete picture of the strains' characteristics and demands and enable control over precise fermentation procedures. Yeasts are eukaryotic single-cell microorganisms that act during the pulque fermentation procedure, providing appropriate aromatic constituents, proteolytic and lipolytic activities; producing carbon dioxide and ethanol; and helping bacterial growth by producing vitamins, amino acids and other metabolites [46]. Yeast fermentation procedures are alcoholic fermentations, beer fermentation, wine fermentation, cider fermentation; non-alcoholic fermentation of yeasts are coffee fermentation, bread fermentation and chocolate fermentation. Yeasts are eukaryotic, unicellular microfungi that are extensively distributed in the natural environment [47][48]. They are included in a group of organisms termed fungi, which also consists of molds and mushrooms [49][50], and they can have both negative and positive impacts on fermented products consumed by animals and humans [51][52][53].

Yeast is applied as a starter culture in bread and cheeses, as well as in beer, wine and other alcoholic fermentation products, but they can also propose spoilage in foods, such as yogurt, salads, fruit juice and mayonnaise [54][55][56][57]. In addition to being extensively applied in the production of beverages, foods and pharmaceuticals, yeasts play significant functions as model eukaryotic cells in improving our knowledge in the biomedical and biological sciences [58][59][60][61]. Processing methodology of fermented vegetables had a significant impact on eukaryotic microbial communities in comparison with the raw material and packing, and under the same process techniques, raw materials had a noticeable effect on eukaryotic microbial communities compared with packaging [62]. Omics Database of Fermentative Microbes (ODFM) is a data management system that combines comprehensive omics knowledge for fermentative microorganisms [63]. Yeast fermentation altered the volatiles of the larvae without boosting mortality, and it can also significantly improve intensity of fruity flavor volatiles [64].

Hydrocolloids supplementation led to the immobilization of yeast cells via flocculation, providing a protective impact on the physiological characterization of large yeast during high gravity brewing [65]. Low-temperature fermentation is regarded to enrich the aroma of wine; it can increase ethyl acetate, ethanol and ethyl butanoate synthesis, and it can also decrease phenylethanol, acetic acid and phenylethyl acetate synthesis [66]. Supplementation of protein hydrolysate is an important technique for boosting the salt tolerance of soy sauce aroma-producing yeast [66][67][68]. The application of baker's yeast in fermentation or rice bran for extraction of protein concentrate can be more effectively managed to increase the extraction yield in comparison to natural fermented and untreated rice bran [69].

3. Prokaryotic Microorganism Species and Fermentation Technology

Prokaryotes are typically simple, single-celled organisms; they have ribosomes to make proteins, a membrane and a cell wall to contain the contents of the cell, and their DNA is packed up in the middle of the cell [70][71][72][73][74]. Certain prokaryotes, consisting of some species of Archaea and bacteria, use anaerobic respiration, which can be discovered in soil and in the digestive tracts of ruminants, like cows and sheep [75][76][77][78]. Many prokaryotes can switch between aerobic respiration and fermentation, depending on the availability of oxygen [79][80][81]. The group of Archaea called methanogens decreases carbon dioxide to methane to oxidize NADH, and some sulfate-reducing bacteria and Archaea are anaerobic, decline sulfate to hydrogen sulfide to regenerate NAD^+ from NADH [82][83][84]. Archaea consists of an individual domain of organisms with discrete biochemical and genetic distinctions from bacteria, and methane-forming methanogens comprise the prevalent group of archaea in the human gut microbiota [85]. In anaerobic systems without inhibition by NH_3-N, organic acids created from acidogenesis are fermented to acetate and H_2, and the ordinary distribution of the electron flow to methane is 67% acetate and 33% H_2 [86].

Dissimilarities in the constitution and activity of the rumen microorganisms may have a role in variation in host feed adaptability through their impact on feed digestion, fermentation and CH_4 production [87]. Halophilic archaea consisted of 74.5% of the microbial communities in fermented fish, and archaea may have a function in both fermentation and health benefits of fermented fish [88]. Up to now, archaea have been categorized into 5 phyla, namely *Korarchaeota*, *Crenarchaeota*, *Nanoarchaeota*, *Euryarchaeota* and *Thaumarchaeota* [89][90][91][92]. IntensiCarb^{TM} (IC) is an innovative technology that permits coinciding thickening and anaerobic fermentation in a single treatment step; IC can increase both volatile fatty acid (VFA) and hydrolysis yields compared to control fermenter, and IC produced condensate at higher quality without solids and low nutrient constituents [93][94][95][96][97][98][99][100][101][102].

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