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Review

Effect of Microparticles on Fungal Fermentation for Fermentation-Based Product Productions

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Abstract: Ranging from simple food ingredients to complex pharmaceuticals, value-added products via microbial fermentation have many advantages over their chemically synthesized alternatives. Some of such advantages are environment-friendly production pathways, more specificity in the case of enzymes as compared to the chemical catalysts and reduction of harmful chemicals, such as heavy metals or strong acids and bases. Fungal fermentation systems include yeast and filamentous fungal cells based on cell morphology and culture conditions. However, filamentous fungal fermentation has gained attention in the past few decades because of the diversity of microbial products and robust production of some of the most value-added commodities. This type of fungal fermentation is usually carried out by solid-state fermentation. However, solid-state fermentation poses problems during the scale-up for industrial production. Therefore, submerged fermentation for value-added products is usually preferred for scaling-up purposes. The main problem with submerged fungal fermentation is the formation of complex mycelial clumps or pellets. The formation of such pellets increases the viscosity of the media and hinders the efficient transfer of oxygen and nutrient resources in the liquid phase. The cells at the center of the clump or pellet start to die because of a shortage of resources and, thus, productivity decreases substantially. To overcome this problem, various morphological engineering techniques are being researched. One approach is the use of microparticles. Microparticles are inert particles with various size ranges that are used in fermentation. These microparticles are shown to have positive effects, such as high enzyme productivity or smaller pellets with fungal fermentation. Therefore, this review provides a background about the types of microparticles and summarizes some of the recent studies with special emphasis on the fungal morphology changes and microparticle types along with the applications of microparticles in filamentous fungal fermentations.

Keywords: filamentous fungal fermentation; microparticles; microparticle-enhanced cultivation; MPEC; fermentation-based products

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1. Introduction

Fermentation has always been a crucial part of human civilizations even before the extensive knowledge of microbiology and industrial biotechnology. Ancient humans used to make bread, beer, and many other fermentation-based products without the knowledge of underlying microbiological principles [1]. Records of bread and alcoholic beverage making can be traced back to the Egyptians (5000 BC) and Babylonians (6000 BC) [2]. At the end of the nineteenth and start of the twentieth century, the enhanced knowledge of microbiology helped in the development of more sophisticated fermentation-based products. The microorganisms varied from simple prokaryotic bacteria to mycelial-forming eukaryotic fungi. Each microorganism is known for specific fermented products and commodities that make it an industrial producer of that product. Among these microorganisms, fungi are known for many products, including bread, alcoholic beverages, pharmaceuticals, vitamins, food

additives, biofuels, enzymes, organic acids, fatty acids, and sterols [3]. The use of fungi as fermentation producers has increased significantly over the last decades [4]. Currently, only the plant biomass-hydrolyzing fungal enzyme industry is worth $4.5 billion market and is expected to increase to $9.74 billion by 2026 [1].

There are many different types of fungi including, yeasts, mushrooms, and filamentous fungi or molds. Each differs in terms of its cell structure, growth patterns, and generation time [2]. All fungi are, however, eukaryotic cells, which makes them different from bacteria [3]. Filamentous fungi are gaining attention due to the robust production of bioenergy-related enzymes, such as cellulases and hemicellulases [5]. There has been greater research interest in developing mechanisms for the industrial fermentation of filamentous fungi to produce a diverse range of products with general categories of food-related items, alcoholic beverages, juice, pulp, bioenergy, and detergent industries. There are many kinds of filamentous fungal genera that have gained attention in their production of a specific or diverse range of products. Some examples include *Aspergillus*, *Trichoderma*, *Penicillium*, and *Acremonium* species, among many others.

There are mainly two microbial fermentation system types: solid-state and submerged fermentations. In solid-state fermentation, the microorganisms have limited moisture content and are cultivated on a solid substrate. In submerged fermentation, on the other hand, the microbial media is in a liquid state with freely available nutrients to all microbial cells. Solid-state fermentation is usually used for mold, while submerged is used for bacteria and yeast cultures. Regardless of the fermentation type, every fermentation product and microorganism requires specific culture conditions with upstream and downstream processing techniques. For example, in the case of yeast cells, which are freely suspended in the media, the use of spargers or propellers is efficient in the distribution of nutrients and oxygen in the bioreactor. However, the same type of instrument can disrupt the mycelium in the filamentous fungal bioreactor, which consists of heavy mycelial clumps or pellets and can result in the premature death of the fungal cells and their inability to produce the required product [2]. Therefore, airlift or bubble column reactors are used for such types of fermentation [2]. The main challenge in submerged fungal fermentation is the formation of mycelial clumps or pellets, which disrupts the even distribution of oxygen and nutrients.

The mycelial growth of filamentous fungi under submerged conditions can occur in many forms. The basic vegetative form is like a tubular filament and is known as a hypha. The hypha is formed through the germination of a single reproductive spore. The growth and spreading of a hypha into branches results in the formation of a complex structure known as mycelium. When grown in a free liquid phase or submerged conditions, these mycelial structures can take many different forms based on genetics, inoculum properties, and culture conditions [6]. The mycelium can either disperse into loosely branched structures or can aggregate into dense clumps or pellets [4]. The same fungal species can form two different forms of mycelial structures based on the culture conditions and product. For example, in the case of *Aspergillus niger*, pectic enzyme production culture conditions produce filamentous growth, while pellets are produced under the fermentation conditions for citric acid [4]. Another example is *Penicillium chrysogenum*, for which the pelleted form is preferred in the production of penicillin while it shows other morphological forms as well. After the initial stationary and early growth phases, the production of mycelial clumps and pellets becomes problematic as the viscosity of the medium increases with the growing mycelial mass [4]. Therefore, many strategies have been developed to control the physiology of fungal cells under submerged fermentation conditions.

One strategy that has recently gained attention to control fungal physiology under submerged conditions is the use of microparticles. Microparticles are inert particles of various sizes that can affect the morphology of the microbial cells in fermentation without chemically interacting with the media or the microbial cells. Many types of microparticles have been studied recently for their effect on fungal morphology and the production of various fermentation-based products [7–9]. There are many hypotheses as to why the addi-

tion of a low concentration of microparticles changes fungal morphology and productivity under different submerged conditions. One hypothesis is that microparticles affect the size of the mycelial clumps or pellets, thus facilitating the oxygen transfer to the fungal cells [10]. Another one is that it affects the interaction between fungal hyphae. The main advantage of using microparticles is their ease of application. The addition of microparticles after preparing the microbial media is straightforward. After the fermentation stages, the microparticles can be filtered out from the broth along with the fungal biomass. They can also be separated from the biomass using centrifugation and washing, although this technique has not been reported in the literature so far. The separated microparticles can be recycled for the next fermentation batch. Currently, the most prominent type of research on microparticles in fungal fermentation is their effect on yield, fungal physiology and overall changes in the fermentation run. However, more research is needed to see if such particles can be washed out of the broth for the next fermentation cycle. While the research on the effect of microparticles on fungal productivity and morphology is going on, it is imperative to review the types and concentrations of microparticles in the media that are giving positive results. Therefore, this review article provides a background about the types of microparticles and their effect on fungal fermentation, as well as summarizes the studies conducted in the last few years regarding to the microparticle-enhanced cultivation (MPEC) strategies.

2. Advantages and Disadvantages of Fungal Fermentation

Just like any other microbial fermentation, fungal fermentation requires specific requirements, including inoculum size, aeration, agitation, and nutrient composition. Fungal fermentations can be of many kinds based on the type of fungi. The yeast fermentation can be easily controlled as compared to the filamentous fungal fermentation because of their differences in the cell structure and formation. The yeast fermentation is mostly done in submerged fermentation. Filamentous fungal fermentation, which is a promising technique for various enzymes and other value-added products, is predominantly solid-state fermentation. However, solid-state fermentation, on the other hand, has many disadvantages, ranging from the difficulty in obtaining the even distribution of nutrients to the problems in scale-up [11]. Therefore, submerged fungal fermentation has been researched extensively for filamentous fungal species such as *Aspergillus niger*, *Trichoderma reesei*, and many others.

There are many advantages of filamentous fungal fermentation as compared to yeast or bacterial fermentation. The most prominent advantage is the diverse range of proteins, enzymes, and many secondary metabolites that are produced by filamentous fungal species and are not produced by other microbial species [1,12]. Another advantage is the higher enzyme titers as compared with other microbial enzymes [13]. In addition, bacterial cells require a more sophisticated nutrient composition as compared with fungal cells. The bacteria and yeast prefer simple carbon sources such as glucose or other mono and disaccharides. On the other hand, there are many fungal species that can thrive on complex and diverse carbon sources such as cellulose, hemicellulose, and pectin [14,15]. There are many studies that have been conducted to show the effectiveness of complex and unconventional carbon sources that can be used to produce value-added products by using fungal fermentation. Table 1 shows some of such studies in the literature.

Table 1. Examples of complex carbon sources for the production of value-added products from fungal fermentation systems.

Carbon Source	Fungal Strain(S)	Value-Added Products	References
Spent hydrolysates	*Trichoderma reesei* Rut C-30	Enzymes	[15]
Mixture of dry leaves	*Aspergillus niger*	Cellulase	[16]

Table 1. *Cont.*

Carbon Source	Fungal Strain(S)	Value-Added Products	References
Aguamiel	Aspergillus niger GH1, *Aspergillus niger* PSH and *Aspergillus oryzae* DIA-MF	Fructooligosaccharides (FOS)	[17]
Whole maize flour	*Aspergillus niger*	Citric acid	[18]
Corn cob and sawdust	*Aspergillus niger*	Xylanase	[19]
Corn cobs	*Aspergillus flavus* AW1	Xylanase	[20]
Sorghum xylans	*Aspergillus fumigatus* RSP-8	Xylanase	[21]
Algerian date varieties	*Aspergillus niger* ATCC 16888	Citric acid	[22]
Rice husk, cottonseed cake, and red gram husk	*Aspergillus niger* MTCC 872	Lipases	[23]
Distillers dried grains with solubles (DDGS)	*Aspergillus niger* (NRRL 330) and (NRRL 567)	Cellulases and hemicellulases	[24]
Sunflower stalks	*Aspergillus* sp.	Xylanase	[25]
Sugarcane bagasse	*Mucor circinelloides*	Single cell oil	[26]
Babassu cake	*Penicillium simplicissimum*	Lipase	[27]
Agro-industrial bioproducts	*Moniliella* SB9 and *Penicillium* sp. EGC5	Polygalcturonase and pectin lyase	[28]
Sweet potato extract	*Gongronella butleri* USDB 0201	Chitosan	[29]
Wheat and soybean bran	*Aspergillus niger* and *Aspergillus flavus*	Lipase	[30]
Textile waste	*Trichoderma reesei* ATCC 24449	Cellulase	[31]
Waste streams from ethanol and bread	*Neurospora intermedia*	Ethanol, biomass and a feed product	[32]

The main disadvantage of filamentous fungal fermentation, as compared with yeast and bacterial fermentation, is the undesirable production of complex mycelial structures, such as fungal pellets that make the even distribution of oxygen and nutrients difficult in submerged fermentation. Therefore, solid-state fermentation is mostly preferred over submerged fermentation for filamentous fungi. However, the build-up of temperature, oxygen, pH, and moisture gradient makes the scaling-up of solid-state fermentation very challenging [11]. Therefore, there is a pressing need for the development of submerged fermentation techniques that can help in the cultivation of filamentous fungal strains under submerged fermentation. The productivity and yield are also lower under submerged conditions as compared to solid-state fermentation. The main reason is the substrate inhibition at higher sugar levels in liquid suspended-cell fermentation [33]. There have been many approaches proposed in the recent literature to overcome such problems. The most prominent strategy is the optimization of aeration and agitation to control the size of the mycelial clumps. However, a recent development is the use of microparticles to control the fungal morphology and to release the product more efficiently into the liquid phase [34].

3. Fungal Cell Growth Characteristics (Morphology) and Effects on the Product Formation

Fungal microorganisms, widely used in biotechnological processes, exhibit different complex morphological forms, such as mycelium, clumps, and pellets [34]. These forms could vary accordingly, due to features of submerged cultures, and process performance in a bioreactor is directly affected by morphological varieties [35]. Morphology affects the yield of upstream and downstream processes. The fermentation conditions, such as the rheology of broth, mass, and oxygen transfer rate, are varied depending on the morphology. Accordingly, productivity is affected by the alteration of these conditions. Furthermore,

biomass formation affects purification steps. Compact biomass formation can be easily removed from fermentation broth by comparison hyphal formation [36].

The production of various fungal products, which are target products or second metabolites, could differ based on the morphological characteristics of fungal microorganisms. For example, the production yield of the other metabolites is generally higher in pellet forms, or mycelia forms are effective fungal microorganisms for enzyme production [36]. Although there are some approaches like these in the literature, choosing a convenient morphological form, such as mycelial or pellet, is one of the most important challenges for production in optimum yield. The viscosity of broth increases as mycelium forms and the flow behavior of broth turns into pseudoplastic [37]. Similarly, mass transfer rates, particularly gas-liquid oxygen transfer, are negatively affected. Moreover, higher oxygen input and agitation rate are required for production at the desired levels [4]. On the other hand, the pelleted growth form does not have as strong an impact as mycelium on viscosity. However, there are a few disadvantages that originate from the nature of pellet form morphology. During the fermentation process, biomass density increases and larger non-homogeneous pellet structures, which have less porosity, begin to form. Under these conditions, lower nutrient uptake occurs with the effect of an intense hyphal network, and lack of substrate has an impact on metabolism and leads to autolysis in the central part of pellet, eventually. The constitution of smaller pellet forms are generally desirable for fungal fermentations to avoid nutrient limitation and autolysis [35,38]. Therefore, many of the research studies in the literature indicated that the product yield can be improved by controlling the morphology of fungal microorganisms with different strategies.

4. Conventional Methods for Controlling Fungal Cell Growth in Liquid Fermentation

Various conventional techniques were developed and used in fungal submerged fermentation to overcome these limitations, which are derived from the morphological properties of fungi. These techniques, also known as morphology engineering, focus generally on modifying the environmental conditions of fermentations to improve productivity. There is plenty of research in the literature about various factors which affect morphology in different fermentations type. These factors, known as environmental inputs, are temperature, pH, dissolved oxygen concentration, agitation, impeller type, reactor type, and media composition. In this review, some of the research on the effects of these inputs were summarized. In addition, some factors related with preferred fungi for production were also investigated, such as cultivation type, inoculum concentration, age of mycelium, and viability of spore [4,36,39].

The effects of inoculum on the morphology and yield were investigated in many research studies in the literature. For example, Liu et al. [40] demonstrated that the effects of inoculum on *Rhizopus oryzae* (NRRL 395) (ATCC 9363), *R. oryzae* (ATCC 20344), and *R. oryzae* (ATCC10260). They reported that the probability of forming pellets increased at high inoculum spore concentrations (up to 3 $\times$ 109 spores/L). In another study, when the initial spore concentrations of *Aspergillus terreus* (ATCC 20542) were below 2 $\times$ 109 spores/L, the biosynthesis of the second metabolite, such as geodin, was observed [41]. The correlation between pH and the aggregation of *Aspergillus niger* conidia were also investigated. It was demonstrated that the number of pellets was higher at pH 4.0 than at pH 7.0. In addition, productivity also decreased with increasing of at extreme pH values [42].

Apart from these approaches, inputs, such as agitation and aeration, were studied to define their impact on morphology and productivity. Chen et al. [43] reported that pellets were not formed when the agitation speed was 200 rpm, but L-malate production and *A. oryzae* pellets number were increased by increasing of agitation speed to 600 rpm. They also reported that, although the increasing aeration rate improved pellet number and product concentration initially, they decreased subsequently. It was determined that lower agitation speed and milder shear stress were improved pellet formation and enzyme production due to reducing of broth viscosity in fed-batch fermentation [44]. The effect of volumetric power input, which was controlled by different agitation speed and aeration

rate, were investigated to improve of pellet morphology of *Aspergillus niger* (AB1.13) and glucoamylase productivity. Results indicated that pellet concentration and production of glucoamylase were increased with increasing of volumetric power input by aeration rate [45].

Most of the productivity problems that originated from the morphological properties of fungi can be overcome by using conventional methods at small scales. However, conventional methods have limited impact because of the high energy requirement and non-feasible large-scale applicability [36,39]. Therefore, they have been less efficient and preferable in comparison with new morphological engineering techniques as microparticles or genetical modifications.

5. Microparticles for Controlling Fungal Cell Growth in Liquid Fermentation

Filamentous fungi can grow in different forms in submerged cultures. These forms are dispersed hyphae, compact pellets and loose clumps [46]. These growth forms directly affect the primary or secondary metabolite production tendencies and process control [35]. Therefore, there are many conventional strategies to control filamentous fungi growth in liquid media. However, none of them is efficient enough by itself to control filamentous fungi growth and enhance productivity, as discussed in previous section. In recent years, microparticle addition to the fermentation media has been evaluated as a unique strategy for controlling filamentous fungi growth.

The main advantage of this method is the researcher friendly ease of application, because there is no need for any genetic manipulation of microorganisms. Any type of filamentous fungi can be used to carry out the alteration of cell morphology and productivity on submerged fermentation with microparticle addition (Figure 1). Microparticles do not interfere with the product and can easily be removed by any type of filtration or centrifugation technique [10,47]. The other benefits of microparticle usage for filamentous fungi fermentations include enhancing mass transfer and productivity, reproducible/repeatable results, and controlling overgrowth of fungi. However, the concentration of the microparticle is a key parameter because addition of low or high microparticle has an adverse effect on its performance.

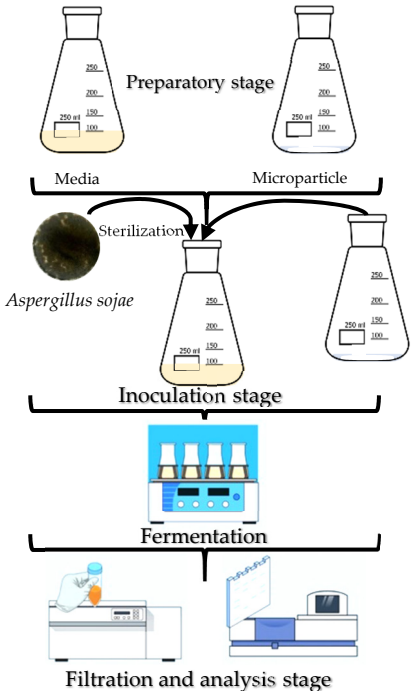

Figure 1. Microparticle usage strategy for shake flask fermentations.

5.1. Microparticles

Researchers use different microparticles to enhance the productivity of organic acids, enzymes, pigments, and other value-added products. Some of them are summarized in Table 2.

Table 2. Microparticles and their applications for biological processes.

Microparticle	Microorganism	Product	Reference
Talc	*C. fumago*	Chloroperoxidase	[10]
	A. niger	Fructofuranosidase	[47]
		Enzyme Production	[48]
		Inulinolytic activity	[37]
	M. isabellina	Lipid production	[49]
	A. ficuum	Phytase	[9,50]
	R. oryzae	Lactic acid	[51]
	A. sojae	β-mannanase	[7,52–54]
		Hydrolytic enzymes	[55]
		Polygalacturonase	[56]
	C. globosum	β-D-glucuronidase	[57]
	A. nidulans	Echinocandin B	[58]
	S. gilvosporeus	Natamycin	[59]
	M. purpureus	Yellow pigments	[60]
	P. dubia	Morphology regulatory	[61]
		Polysaccharides	[62]
Al-oxide	*C. fumago*	Chloroperoxidase	[10]
	A. niger	Enzyme Production	[48]
	A. ficuum	Phytase	[9,50]
	R. oryzae	Lactic acid	[51]
	A. sojae	β-mannanase	[7,52–54]
		Hydrolytic enzymes	[55]
		Polygalacturonase	[56]
	M. purpureus	Yellow pigments	[60]
	P. dubia	Morphology regulatory	[61]
		Activated sludge anaerobic digestion	[63]
	Trichoderma viride	Cellulase	[64]
	C. globosum	β-D-glucuronidase	[57]
	A. terreus co-culture	Lovastatin	[65]
		Penicillin G	[65]
		Oxytetracycline	[65]
		Laccase	[65]
SiO_2	*C. unicolor*	Laccase	[66]
	P. sapidus	Laccase	[66]
	M. purpureus	Yellow pigments	[60]
		Activated sludge anaerobic digestion	[63]
TiO_2	*M. purpureus*	Yellow pigments	[60]
		Activated sludge anaerobic digestion	[39]
	Rhodopseudomonas sp.	Hydrogen production	[67]
		Sludge anaerobic fermentation	[68,69]
ZnO	HAU-M1	Hydrogen production	[70]
		Activated sludge anaerobic digestion	[63]
	Rhodopseudomonas sp.	Hydrogen production	[67,71]
		Sludge anaerobic fermentation	[68,69,72]
Titanium silicone oxide	*A. niger*	Enzyme Production	[73]
OSA-SNa	*M. purpureus*	Monascus red pigments	[74]

There are many different microparticles, such as talc, aluminum oxide, kaolin, nanoclay, florisil, aluminum silicate, titanium silicate, aluminum titanate, zirconium silicate, silicium oxide, etc. [75]. All of them have different cost, particle size (vary from 5 μm to 250 μm), chemical composition and surface properties (hydrophobicity or hydrophilicity). The selected microparticle is a key parameter on the fungal cell morphology and productivity.

Some of the microparticles could also increase the ion content of media when incubated overnight. These ions are Mg (0.097–18.738 mg/L), Si (29.9–136.5 mg/L), Ca (0.807–45.105 mg/L), Na (124.31–359.5 mg/L), fluoride (0.014–5.126 mg/L), chloride (0.11–44.87 mg/L), nitrate (0.181–7.399 mg/L), phosphate (0.729–1.16 mg/L) and sulphate (0.216–803.56 mg/L), respectively. Although these ions' effects were not as prominent as the presence of the microparticles, their content should be considered to evaluate the results [75]. It is recommended to analyze particle size, leached particle, ion content and scanning electron micrographs to carry out the effect of microparticles on cell morphology and productivity.

The most studied microparticles are talc powder and aluminum oxide. Microscopic analysis of both materials showed that microparticle diameters change from 1 to 20 μm for aluminum oxide and 1 to 42 μm for talc powder respectively [10]. They are generally used in the same study to see the difference between the productivity values. Both improve cell viability, cell growth controllability, and repeatability. It is not possible to generalize microparticle type and concentration for all products because each microparticle has different chemical and physical properties, such as particle size, elements (Mg, Si, O, H, Al, Ti, Fe etc.); molecular weight, density, etc. Furthermore, a microparticle can play different roles in different filamentous fungi fermentation because of fermentation type, cell growth characteristics, pH, aeration or agitation rates, inoculation ratio, carbon or nitrogen concentration, etc. A higher agitation rate provides lower cell diameter sizes or cell disruption. Lower pH values can limit not only cell growth but also productivity. Carbon or nitrogen concentration could define the cell growth characteristics (low or high cell density, pellet or hyphae development etc.), but the right concentration of the microparticle and fermentation parameters provide just benefits to control cell overgrowth with higher productivity values. Therefore, researchers had to try different microparticles and their concentrations to find out the optimum microparticle type and concentration for its product. In general, researchers use different concentrations of microparticles (from 0.05 to 25 g/L) to find out the best concentration for cell viability, cell growth morphology, and productivity.

As can be easily seen from the literature, MPEC can be affected by the medium, fermentation parameters, fermentation strategy, microorganisms, type of microparticle and microparticle concentration. Therefore, it is important to choose the most appropriate microparticle and its concentration for each microorganism.

5.2. Mechanism of Microparticles in Controlling Fungal Cell Growth

The first question of the MPEC strategy is how the process works. There are different studies that give an explanation by comparing the morphology and productivity of microorganisms. The addition of microparticles intentionally at the beginning of the incubation promotes the growth type of a dispersed form instead of a pelleted one. It may be due to cell-particle collisions and shear stress exerted while shaking with microparticles. Furthermore, using the smaller particles ($\leq$42 μm) prevents the cell immobilization on microparticles, and microparticles may hamper the interactions of hyphae with each other [10]. It is also reported that adding microparticles prevents spore aggregation in the initial phase of fermentation. Observed effects could be from interactions of filamentous fungi spores and microparticles with chemical or physical mechanisms. These phenomena were carried out by 3D photographs using a stereomicroscope and spatial distribution of GFP fluorescence within cellular aggregates using a confocal laser scanning microscopy [48]. Active zone theory was also done to solve this phenomenon by comparing the green fluorescent

protein production by micrographs using a stereomicroscope and a confocal laser scanning microscopy. Research shows that different concentrations of titanate microparticles provide smaller pellets and occur inside the biomass. This means that microparticles can be inside the filamentous fungi pellets and a better biomass filling occurred by a loose interior structure [73]. It is also shown that the smaller pellets are composed of a higher cell concentration than the bigger pellets. The loose interior structure improves the mass transfer and penetration capability of cells with higher active zone values. The smaller pellets provide higher active zone values and enhance the productivity of any filamentous fungi microorganism [73]. In summary, a control mechanism is needed for filamentous fungi systems to limit cell concentration and enhance productivity. Conventional techniques (temperature, pH, dissolved oxygen concentration, agitation, impeller type, reactor type and media composition) were not enough because of high energy requirement and non-feasible large-scale applicability. Microparticles get into filamentous fungi mass by the intentional addition at the inoculation phase, enhancing the cell growth characteristics and productivity by the proper microparticle type and concentration (Figure 1). Especially, microparticle concentration determines the cell growth characteristics. The optimum concentration of microparticles provides a limited reuniting of free mycelium, smaller pellets (higher active zone values), limited cell growth (no cell leakage), controllable fermentation conditions (agitation, pH, O_2, etc.), and good mass transfer for any type of filamentous fungi. So, this technique can also be feasible for large-scale experiments.

The other studies are all about the relationship between microparticles usage and the pellet size or morphology difference of microorganisms. Therefore, there is still a need to find out the mechanism of microparticles on filamentous fungi fermentations, morphology and productivity, because available information about the mechanism of microparticles is not enough to explain totally of microparticle strategy. Although there are various studies about the mechanisms of microparticles in controlling fungal cell growth, the mechanism has not been fully explained or understood. Therefore, different techniques such as image analysis and modeling tools can be used to elucidate the mechanism of microparticle-enhanced fermentations.

6. Applications of Microparticles in Liquid Fermentation for the Productions of Value-Added Products

Different types of microparticles were used for overcoming morphological problems in fungal fermentation processes, such as the production of enzymes, lipids, pigments, organic acid, etc. Various products and microparticles, were summarized below as well as in Table 2.

6.1. Enzyme Production

There is already a significant number of research about improving fungal enzyme production by using microparticles and, accordingly, various enzymes, such as β-mannanase, polygalacturonase, phytase, cellulase and β-D-glucuronidase, were produced. Yatmaz et al. [7] researched the effects of microparticle adding on β-mannanase production by *Aspergillus sojae* transformant 1 (AsT1). In that research, talcum and aluminum oxide were separately added to the broth, which includes glucose or carob extract, to improve β-mannanase activity. They reported that the highest β-mannanase activities were reached in glucose media with 1 g/L aluminum oxide (514.0 U/mL) and 5 g/L of talcum-added carob extract media (568.7 U/mL), respectively. The same research group carried out β-mannanase production in a bioreactor by different fermentation strategies in fed-batch fermentation (suspended, immobilized cell, biofilm, and microparticle-enhanced bioreactor) [54]. Results showed that the addition of microparticles was significantly improved enzyme activity compared to other fed-batch fermentation strategies. Moreover, they also reported that fungal growth could be controlled by microparticle adding in large-scale (30-L bioreactor) β-mannanase fermentation (Scale-up processing with different microparticles) [53].

Polygalacturonase, one of the important industrial enzymes, were produced by *Aspergillus sojae* in microparticle-enhanced shake flask fermentation. Accordingly, aluminum oxide microparticles were added into the medium in the range of 0.05 to 25.0 g/L. Results indicated that the addition of 20.0 g/L aluminum oxide microparticle doubled polygalacturonase activity and pellet size was decreased in comparison with non-microparticle fermentation [76]. Coban et al. [9] investigated the effect of adding talcum and aluminum oxide microparticles on phytase activity and pellet size of *Aspergillus ficuum* in shake-flask and bioreactor fermentation. The fermentation medium was supplemented with 5, 10, 15, 20 and 25 g/L talcum and aluminum oxide. According to the results of shake-flask fermentations, the addition of 15 g/L of talcum and aluminum oxide increased phytase activity by 185% and 97%, respectively. However, when microparticle concentrations were higher than 15 g/L, enzyme activity decreased to 1.69 U/mL (talcum) and 1.58 U/mL (aluminum oxide), respectively. On the other hand, the average pellet radius of Fungi was decreased by the addition of talcum and aluminum oxide from 800 μm to 200 μm and 500 μm, respectively. They also reported that the maximum phytase activity was measured as 6.49 U/mL in bioreactor fermentation supplemented with 15 g/L talcum. In addition, Coban et al. [50] carried out phytase production in fed-batch and continuous fermentations with and without the addition of talcum microparticles. Accordingly, 10 g/L phytate and glucose in different concentrations were added to the fermentation medium. They indicated that the maximum enzyme activity in fed-batch fermentation increased from 4.9 U/mL to 9.6 U/mL with the addition of 15 g/L of talcum. In continuous fermentations, phytase activity was determined as 6.3 U/mL with talcum addition, while phytase activity was 3.3 U/mL without talcum addition. Furthermore, the average pellet radius was decreased from 500 μm to around 100 μm in fed-batch fermentation.

Aluminum oxide, which is widely preferred microparticle by researchers, was used to improve laccase production [66]. Laccase production trials were carried out in shake-flask fermentations by using *Cerrena unicolor* Murr. strain 137 and *Pleurotus sapidus* (DSM 8266) separately and aluminum oxide microparticles were added to the medium in different concentrations (5, 10, 15, 20, 30 g/L). They reported that laccase activity was increased 3.5-fold by adding microparticle for *C. unicolor* and reached 2233 U/L. In addition, there is no significant change in laccase activity, when microparticle concentration was increased above 15 g/L. On the other hand, laccase activity, produced by *P. sapidus*, was increased by increasing microparticle concentration and reached maximum activity as 835 U/l by adding 30 g/L aluminum oxide. Moreover, although the pellet size of both species decreased, the structure of species was different. When *C. unicolor* pellets had dispersed morphology, *P. sapidus*, pellets were more compact [66]. Besides, various microparticles were tried to control morphology and improve enzyme activities. For example, aluminum oxide, talcum powder, silica, and glass bead were added to fermentation broth and the effect of these microparticles on β-D-glucuronidase production with *Chaetomium globosum*. The results showed that silica microparticle greatly affected the morphology relative to the other microparticles [57]. Apart from these, glucoamylase and fructofuranosidase production carried out with addition of magnesium silicate and aluminum oxide [48], and cellulase production were improved and pellet size controlled with aluminum oxide [64].

6.2. Organic Acid

Except from enzyme production, there are many research benefited from microparticles to improve production of value-added products, such as lactic acid, lipids, and polysaccharides. Coban & Demirci [51] used talcum and aluminum oxide to increase lactic acid production by *Rhizopus oryzae*. Determination of microparticle concentration (0, 1, 3, 5, 8, 10, 15, 20, and 25 g/L) was carried out in shake-flask fermentation, firstly. They reported that lactic acid concentration increased from 6.02 g/L to 24.01 g/L and 13.88 g/L, when 10 g/L of talcum and 15 g/L of aluminum oxide was supplemented, respectively. They indicated that the reason of talcum addition resulted in higher lactic acid production is that smaller pellet aggregation in shake-flask. After determination of microparticle type

concentration, bioreactor conditions were optimized within microparticle-enhanced media. Accordingly, lactic acid production increased around 4-fold and 2.3-fold by addition of 10 g/L of talcum and 15 g/L of aluminum oxide in shake flask, respectively. Moreover, the maximum lactic acid concentration was obtained as 75.1 g/L, when supplemented with 10 g/L talcum.

6.3. Pigments

Productivity of yellow pigments from *Monascus purpureus* ZH106-M was investigated with addition of different microparticles in shake-flask fermentation. For this reason, talcum, aluminum oxide, SiO_2, and TiO_2 in three concentrations (2, 10, and 20 g/L) were added into media. The results of study showed that the maximum yellow pigment production was obtained with talcum (554.2 U/mL). Moreover, it was also reported that morphological changes, such as like including smaller mycelial size, rougher hyphae, and decreased cell integrity, were induced by talc addition [60]. On the other hand, Huang et al. [74] was evaluated sodium starch octenyl succinate (OSA-SNa) microparticle combined with Triton X-100 for improving of *Monascus* red pigments. They reported that the yield of extracellular red pigments production, which were increased by addition of microparticle, was 82.6% higher than controls.

6.4. Lipids

Talc powder was also used to enhance lipid production in shake-flask fermentation with *M. isabelline*. They tried seven different concentration (from 0.1 to 10 g/L) to improve lipid production by controlling morphology. They reported that pellet diameters decreased by adding microparticle until 6.0 g/L, but pellet population get larger. When microparticle concentration was 6 g/L and higher, formation of mycelial was determined. Except from morphological effects, lipid content, which consist of palmitic acid, oleic acid, linoleic acid and polyunsaturated fatty acid, improved by increasing of microparticle concentration and reached highest level (0.75 g lipid/g cell biomass) by the addition 10 g/L talc powder [49].

6.5. Polysaccharides

Tong et al. [62] studied to improve cordyceps polysaccharides production from *Paraisaria dubia* by using talc in shake-flask fermentation. For this approach, they added talc into media in concentration from 1 g/L to 20 g/L with four different particle sizes in range of 400–5000 mesh. The highest intracellular polysaccharides and exopolysaccharides production in shake-flask fermentation was measured by adding 15 g/L of 2000 mash talc as 69.2 mg/g and 478.2 mg/L, respectively. They also reported that talc addition, determined concentration and size, promoted to growth of mycelial pellets [62].

Accordingly, the yield of production could be improved by using various microparticle in fungal fermentation processes. However, the use of microparticle to control fungal morphology is not widely preferred in the industry although some promising scale-up studies were carried out [35]. On the other hand, kind of cheaper and bio-friendly microparticles may be used by industrial manufacturers. For example, talc could be one of the preferable microparticle owing to its price in the range of 10 EUR cents per kg. Moreover, it could be evaluated in feed additives and fertilizers by co-harvested with biomass after fermentation process [77].

7. Conclusions and Future Trends

The recent trends of submerged fermentation for the scale-up of filamentous fungal product formation have gained interest due to the promising high production rates for a large variety of products. However, several fungal morphology-related problems must be solved for the efficient design of industrial bioreactors. Microparticle-enhanced cultivation (MPEC) is one of the novel approaches to control the size and structure of mycelial clumps in submerged fermentation. Various research reports have shown the prospect of using microparticles for the production of enzymes and other metabolites of fungal producers. In

addition to the comparison of productivity and yield with the control, these research reports have also reported the effect of microparticle addition in the media on the size and structure of mycelial clumps. It has been established in various reports that fungal morphological properties such as size have an effect on the secretion of fermentation products in the media. In almost all of the studies, the addition of microparticles increased the overall productivity and yield of various products. However, the optimum values correspond to the optimum size of the pellets or clumps of fungal mycelia. Therefore, more research is needed to analyze the mechanism of microparticles in controlling fungal cell growth. Microscopic analysis of their effect has already been conducted but more research is needed on various mechanisms of their action on fungal morphology and cell growth.

An increase in the concentration of the microparticles decreased the pellet size. However, smaller than optimum pellet size also had a negative effect on productivity. Therefore, future studies should focus on finding the optimum amount of microparticles in the media that corresponds to maximum productivity for each product and strain. In addition, other morphology engineering techniques such as the optimization of aeration and agitation can be combined with the addition of microparticles to get the maximum productivity levels of the desired products. Many fungal strains can produce higher amounts of desirable fermentation products but have not gained industrial interest due to the production of large mycelial clumps. Such fungal strains and microbial products can benefit from the addition of microparticles, and thus more research is needed on the effect of different microparticles on such products. Such strategies can lead to the optimization of industrial processes with the addition of microparticles for increased productivity and lower costs of downstream processing.

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