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Review Article

SOLID STATE AND SUBMERGED FERMENTATION FOR THE PRODUCTION OF BIOACTIVE SUBSTANCES: A COMPARATIVE STUDY

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ABSTRACT
Fermentation has been widely used for the production of a wide variety of substances that are highly beneficial to individuals and industry. Over the years, fermentation techniques have gained immense importance due to their economic and environmental advantages. Ancient techniques have been further modified and refined to maximize productivity. This has also involved the development of new machinery and processes. Two broad fermentation techniques have emerged as a result of this rapid development: Submerged Fermentation (SmF) and Solid State Fermentation (SSF). Discovery of the beneficial activity of several secondary metabolites produced by microorganisms (bioactive compounds) has resulted in the further exploration of fermentation as a production technique for these compounds. At the research level, both SSF and SmF have been used; however, some techniques yielded better results than others. Much work still needs to be done to identify the best fermentation technique for each bioactive compound. This paper reviews different fermentation techniques for the production of bioactive compounds. Comparison of these techniques for the identification of the better technique is also dealt with.

KEYWORDS: Enzymes, Antibiotics, Hypercholestrolemic agents, Antihypertensive agents

INTRODUCTION

Fermentation is the technique of biological conversion of complex substrates into simple compounds by various microorganisms such as bacteria and fungi. In the course of this metabolic breakdown, they also release several additional compounds apart from the usual products of fermentation, such as carbon dioxide and alcohol. These additional compounds are called secondary metabolites. Secondary metabolites range from several antibiotics to peptides, enzymes and growth factors (Balakrishnan and Pandey, 1996; Machado *et al*.,.2004; Robinson *et al*., 2001). They are also called 'bioactive compounds' since they possess biological activity. Recently, researchers have demonstrated that several of these secondary metabolites are industrially and economically important. They have been used in a variety of industries such as pharmaceuticals (Demain, 1999) and food (Rossi, 2009; Daverey and Pakshirajan, 2009), especially in the field of probiotics (Dharmaraj, 2010) and prebiotics (Wang, 2009). The emergence of these industries has led to the amplification of techniques used in the laboratory on a large scale. This has presented a plethora of problems, since the creation of a controlled environment for microorganisms needs to be carried out with utmost adherence to parameters and processes. Adverse conditions may result in the production of unwanted compounds instead of the bioactive compound of interest. The development of techniques such as Solid State Fermentation (SSF) and Submerged Fermentation (SmF) has lead to industrial-level production of bioactive compounds. These techniques have been further refined based on various parameters such as the substrates used, environmental parameters and the organisms used for fermentation. Based on research, certain bioactive compounds have found to be produced in higher quantities in SSF, whereas other compounds have been extracted using SmF. Fermentation has been classified into SSF and SmF mainly based on the type of substrate used during fermentation.

Solid-State Fermentation (SSF)

SSF utilizes solid substrates, like bran, bagasse, and paper pulp. The main advantage of using these substrates is that nutrient-rich waste materials can be easily recycled as substrates. In this fermentation technique, the substrates are utilized very slowly and steadily, so the same substrate can be used for long fermentation periods. Hence, this technique supports controlled release of nutrients. SSF is best suited for fermentation techniques involving fungi and microorganisms that require less moisture content. However, it cannot be used in fermentation processes involving organisms that require high a_w (water activity), such as bacteria. (Babu and Satyanarayana, 1996).

Submerged Fermentation (SmF)/Liquid Fermentation (LF)

SmF utilizes free flowing liquid substrates, such as molasses and broths. The bioactive compounds are secreted into the fermentation broth. The substrates are utilized quite rapidly; hence need to be constantly replaced/supplemented with nutrients. This fermentation technique is best suited for microorganisms such as bacteria that require high moisture

content. An additional advantage of this technique is that purification of products is easier. SmF is primarily used in the extraction of secondary metabolites that need to be used in liquid form.

Substrates used for fermentation

The outcome of fermentation highly varies for each substrate; hence, it is extremely important to choose the right substrate. Fermentation techniques have to be optimized for each substrate. This is primarily due to the reason that an organism reacts differently to each substrate. The rates of utilization of various nutrients differ in each substrate, and so does productivity. Some of the common substrates used in solid state fermentation are wheat bran, rice and rice straw, hay, fruit and vegetable waste, paper pulp, bagasse, coconut coir, and synthetic media (Pandey *et al*., 1999). Some common substrates used in submerged fermentation are soluble sugars, molasses, liquid media, fruit and vegetable juices, and sewage/waste water.

Bioactive compounds extracted

Various bioactive compounds such as antibiotics (Maragkoudakis *et al*., 2009; Saykhedkar and Singhal, 2004; Ohno, 1995), pigments (Dharmaraj *et al*., 2009), enzymes (Aguilar *et al*., 2008; Kokila and Mrudula 2010), hypercholestrolemic agents (Xie and Tang 2007; Pansuriya and Singhal, 2010), antioxidants (Tafulo *et al*., 2010), antihypertensive agents (Nakahara *et al*., 2010), antitumor agents (Ruiz-Sanchez *et al*., 2010), biosurfactants and bioactive peptides (Pritchard *et al*., 2010) have been extracted using fermentation. There has been little information regarding the comparative study of these fermentation techniques with respect to the production of bioactive compounds. This has become a necessity since productivity must be maximized to meet demand. This review makes an attempt to list the fermentation techniques for the production of these bioactive substances, and compare them for identification of the better technique.

Enzymes

Fermentation is the primary technique for the production of various enzymes. Both fungi and bacteria yield an invaluable array of enzymes when fermented on appropriate substrates. Both solid-state and submerged fermentation are used for enzyme production. SmF is usually implemented in case of bacterial enzyme production, due to the requirement of higher water potential (Chahal, 1983). SSF is preferred when enzymes have to be extracted from fungi, which require lesser water potential (Troller and Christian, 1978). More that 75% of the industrial enzymes are produced using SmF, one of the major reasons being that SmF supports the utilization of genetically modified organisms to a greater extent than SSF. Another reason why SmF is widely used is the lack of paraphernalia regarding the production of various enzymes using SSF. This is highly critical due to the fact that the metabolism exhibited by microorganisms is different in SSF and SmF, and the influx of nutrients and efflux of waste materials needs to be carried out based on these metabolic parameters. Any slight deviation from the specified parameters will result in an undesirable product.

Fungal Enzymes

Several enzymes of industrial importance have been extracted from the fungi belonging to the genus *Aspergillus*. The importance of this genus is so much, that it has been studied as a model organism for fungal enzyme production (Holker *et al*., 2004). In fact, *A.niger* is by far the single largest fungal source of enzymes. The metabolic differences between SSF and SmF have a direct impact on the productivity of the fungus. This can be clearly illustrated from Table 1 (Holker *et al*., 2004). Phytase was obtained using submerged fermentation from *Thermoascus auranticus* (Nampoothiri *et al*., 2004) and its activity was found to be 468.22 U/mL.

TABLE 1. Enzyme production by Aspergillus species

Enzyme	Microorganism	Substrate		Productivity		References
		SSF	SmF	SSF	SmF	
Estrase	*Aspergillus niger* I-1472	Sugar beet pulp	Media containing cinnamic acid	20 nkat/mg dry wt.	0.4 nkat/ml	Asther *et al*., 2002
Cellulase	*Trichoderma viride* ATCC	Wheat bran	Mandel's liquid media	60.5 FPU	28 FPU	Vintila *et al*., 2009
Invertase	*A. niger* (mutant)	Polyurethane Foam	Basal media	Higher	Lower	Montiel-Gonzalez *et al*., 2002
Lipase	*A. niger* NCIM1207	Wheat bran and olive oil	Synthetic oil based media	630 IU/g dry wt.	18 IU/ml	Mahadik *et al*., 2002
Phytase	*A. niger*	Wheat bran and soybean milk	M1 medium (semisynthetic)	884 U/g	N/A	Krishna and Nokes (2001), Papagianni *et al*., 2001
Polygalactouronase	*A. niger*	Wheat bran, coffee pulp	Pectin-based production media	2.28 U/l	0.48 U/l	Maldonado *et al*., 1998
Tannase	*A. niger* Aa-20	Polyurethane foam	Production media	12,000 IU/l	2500 IU/l	Aguilar *et al*., 2001

Bacterial Enzymes

Bacteria have been used to produce various enzymes such as amylase, xylanase, L-asparaginase, and cellulase. It was earlier believed that that the best method of production of enzymes from bacteria is by using submerged fermentation. However, recent studies have shown that SSF is more efficient than SmF for bacterial enzyme production. The main reason can be attributed to the metabolic differences. In the case of

SmF, the accumulation of a variety of intermediate metabolites results in lowered enzyme activity and production efficiency. An overview of bacterial enzyme production has been illustrated in Table 2.

TABLE 2. Bacterial enzyme production using fermentation

Enzyme	Bacterium	Substrate		Productivity		References
		SSF	SmF	SSF	SmF	
L-Asparaginase	*Streptomyces spp.*, *Serratia marcescens*	Soybean meal	Yeast extract medium	49.23 U/ml	24.61 U/ml	Basha et al., 2009
Amylase	*Bacillus spp.*	Oil cakes, wheat bran, bagasse	Starch broth	Around 50000 U/g	400 U/ml	Singh *et al*., 2010, Gangadharan *et al.*, 2006
Xylanase	Thermotolerant *Bacillus sp.*	Corn cob and wheat bran	Corn cob and yeast extract peptone	6.18 U/g	16.13 U/ml	Gupta and Kar, 2009
Cellulase	*Bacillus sp.*	Banana waste	Carboxy Methyl Cellulose/ glycerol	9.6 IU/gds	1.2 U/ml	Sukumaran *et al*., 2005

Hypercholestrolemic Agents

Hypercholestrolemic agents are those substances that block the overproduction of cholesterol in the liver. They are of great medical importance as high blood cholesterol levels (hypercholesterolemia) are believed to be one of the primary causes of arthrosclerosis which leads to coronary heart disease. The first hypercholestrolemic agent to be discovered was compactin. Over the years, research has led to the discovery of other hypercholestrolemic agents such as lovastatin, mevastatin, pravastatin, and simvastatin (Manzoni and Rollini, 2002). Compactin, Lovastatin, and Pravastatin are direct products of fermentation; they are also called natural statins. Natural strains possess a polyketide part and a hydroxyl-hexahydro naphthalene part to which side-chains are attached (Manzoni and Rollini, 2002). Simvastatin is a semi synthetic statin obtained by the bioconversion of Lovastatin. Simavstatin differs from natural statins in the possession of an additional methyl group. The change in human lifestyle has led to the sudden spurt in cases of arthrosclerosis over the years, which has driven the demand for statins. This has resulted in the need to scope out newer and more efficient ways to produce them. Fermentation is a cost-effective way of mass production of statins. Both SSF and SmF have been used for this purpose. Fungi are widely used for producing statins. The various strains used are elucidated in Table 3

TABLE 3. Statin production using fermentaion

Statin produced	Fungal strain	Substrate		Productivity		References
		SSF	SmF	SSF	SmF	
Compactin	*Penicillium spp*	Wheat bran, soybean meal and groundnut oil cake	Water soluble soybean meal with glucose, peptone and $MgSO_4$	725 µg/gds	456 mg/l	Shaligram *et al*., 2009, Chakravarti and Sahai, 2002
Lovastatin	*Aspergillus terreus*	Wheat bran, rice husk, paddy straw	Yeast extract media	1500 µg/ml	400 µg/ml	Manzoni and Rollini, 2002, Szakacs *et al*., 1998
Mevastatin	*Penicillium citrinum*	Wheat bran	Seed medium containing glucose, glycerol, peptone and $MgSO_4$	0.0554 mg/ml	490 mg/l	Manzoni and Rollini, 2002, Chakravarti and Sahai, 2002, Ahmed *et al*., 2006
Pravastatin	*Streptomyces spp.*	Rice bran and rice husk	Nutrient broth containing yeast extract and glucose	15 mg/l/h	80-85%	Park *et al*., 2003, Kostova *et al*., 2004

Antibiotics

Antibiotics are the most important category of bioactive compounds extracted from microorganisms using fermentation. The first antibiotic to be commercially extracted using fermentation was penicillin from *Penicillium notatum*. This was done as early as the 1940s using SSF and also SmF. Now, there are a multitude of antibiotics that have been produced using fermentation. This includes cyclosporins, tetracyclins, surfactins, streptomycin, and cephalosporin. Table 4 shows the list of antibiotics produced using fermentation. The early methods of production relied on the utilization of submerged fermentation. In recent times, the development of suitable substrates has led to the widespread use of solid state fermentation over submerged fermentation. However, comparison of results shows that certain strains are more suited to SSF and some are more suitable for SmF. Therefore, the fermentation technique must be decided based on the microorganism that is being used for production.

Recent advances have suggested that antibiotics produced through SSF are more stable and produced in higher quantities than SmF. This can be attributed to lesser production of

intermediate compounds in SSF. However, the implementation of SSF is limited by the quality and characteristics of the substrate material used. Due to this reason, it is necessary to test the production capacity of a wide variety of substrates before optimization of the fermentation process.

TABLE 4. Antibiotic production using fermentation

Antibiotic	Organism	Substrate		Productivity		References
		SSF	SmF	SSF	SmF	
Cyclosporin A	*Trichoderma cylindrosporium*	Wheat bran, agro-industrial residue	Medium containing glucose, casein acid hydrolysate, peptone and malt extract	1.4 mg/g	0.08 mg/ml	Sekar *et al*., 1997, Survase *et al*., 2009
Cephamycin C	*Nocardia lactamdurans*, *Streptomyces clavuligerus*NT4	Soybean flour	Production media	15.75 mg/gds	13.65 mg/ml	Kagliwal *et al*., 2009, Bussari *et al*., 2009
Tetracycline	*Streptococcus viridifaciens* *Streptococcus clauverigerus*	Sweet potato residue Agro waste	Cellulosic substrates	2129 µg/g 300 µg/g	N/A	Yang and Ling, 1998
Griseofulvin	*Penicillium griseofulvum*	Rice bran	Production media	9.732 mg/g	0.1 mg/ml	Saykhedkar and Singhal, 2004
Surfactin	*Bacillus subtilis* RB14	Soybean curd residue	Semisynthetic and synthetic media	200-250 mg/kg	250 mg/l	Ohno *et al*., 1995
Mycophenolic acid	*Penicillium brevicompactum*	Pearl barley, wheat bran and rice	Mannitol	6.1 mg/g	1.2 mg/g	Alani *et al*., 2009

Bioactive compounds obtained from fermented foods

Antihypertensive peptides

Antihypertensive peptides are a class of peptides that are used to inhibit the activity of the angiotensin-converting enzyme (ACE). This activity is also called Angiotensin-Converting Enzyme Inhibitory Activity (ACEIA). This enzyme is critical for blood pressure regulation, and is a major cause for hypertension. Due to the high stress levels among people today, it is now recommended that a portion of these antihypertensive agents must be included in a person's daily diet. This constitutes the Dietary Approaches to Stop Hypertension (DASH) plan for combating hypertension. The discovery of the production of antihypertensive compounds in fermented food products has been a giant leap in the field of neutraceuticals. Several microorganisms have been screened for the production of these compounds. Of these, the lactic acid bacteria have been the most successful. The usual technique used for production of these fermented foods is liquid fermentation.

According to a study carried out by Stefanova *et al*., strains of *Lactobacillus* such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii spp. bulgaricus* have been used for the production of fermented milk products with ACEIA. Strains of *Lactobacillus casei* and *Lactobacillus delbrueckii spp. lactis* have also been used for the same. The ACEIA activity was found to increase in the fermented product as the incubation time increased. The pH and temperature are usually maintained constant. It is to be noted that much work needs to be done regarding the extraction of the pure peptide from the fermented product. Antihypertensive peptides were also found in cheese (Pritchard *et al*., 2010). Enzyme-modified cheeses also contain some hypertensive peptides (Halieselassie *et al*., 1999; Tonouchi *et al*., 2008). Other fermented products such as soy sauce and fish sauce also contain hypertensive properties (Okamoto *et al*., 1995). The peptide-enriched Fermented Soybean Seasoning (FSS) was reported to have 2.7 times higher concentration of hypertensive peptides than normal soy sauce (Nakahara *et al*., 2010). Several traditional fermented foods such as douchi, natto, and nyufu also contain bioactive compounds with ACEIA. In such cases, both solid-state and liquid fermentation are used for production of these food products. It is to be noted that the quantity of antihypertensive peptides varies between fermented foods. Since deviation from traditional fermentation/production techniques may impact the authenticity of the product, a comparative study of different fermentation methods for each fermented food is yet to be performed.

β-Carboline Alkaloids

β-carboline alkaloids are derivatives of pyrido(b)indoles, and display a variety of anticarcinogenic, antimicrobial and antiviral activities. Their multifarious uses have rendered them extremely important pharmaceutically. Examples of these compounds are 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid. Until now, they have been produced using the conventional process called the Pictet-Spiegler reaction between indoethylamines and aldehydes or α-ketoacids (Brossi, 1993). This reaction yields very less β-carboline alkaloid and is also very expensive.

Due to this, there has been a need to discover alternate ways of production. Fermentation has the potential to naturally produce these compounds in a cost-effective way. Weber *et al*., (1993)

proposed the utilization of the fungus *Myrothecium verrucaria* for the production of β-carboline derivatives. Their method was mainly based on submerged fermentation and used a variety of substrates such as methanol and ethanol. They obtained 0.5 g of β-carboline derivative using this method. Recently, Singh *et al*., (2010) proposed the extraction of these compounds from *Trichoderma harzianum* by SSF. However a lot of work still needs to be done to explore the production of these alkaloids from alternate fermentation techniques.

CONCLUSION

The recent spurt in demand for natural medicine has made the discovery of alternate production methods the need of the hour. Fermentation, with its wide array of application and immense benefits, has proved to be a main contender to fill this void. However, due to the variations among different fermentation techniques, a lot of work still needs to be done in terms of comparison of these techniques. Also, a lot of exploration still needs to be carried out to identify sustainable substrates and processes to maintain productivity and quality. These can help in increasing production and reducing the cost of these compounds.

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