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, Hypercholesterolemic 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), hypercholesterolemic agents (Xie and Tang 2007; Pansuriya and Singhal, 2010), antioxidants (Tafulo et al., 2010), antihypertensive agents (Nakahara et al., 2010), antimutagen 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 reason that an organism reacts differently to 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.

**Table 1. Enzyme production by Aspergillus species**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Microorganism</th>
<th>Substrate</th>
<th>SSF Productivity</th>
<th>SmF Productivity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrase</td>
<td><em>Aspergillus niger</em> I-1472</td>
<td>Sugar beet pulp</td>
<td>Media containing cinnamic acid</td>
<td>20 nkat/mg dry wt.</td>
<td>0.4 nkat/ml</td>
</tr>
<tr>
<td>Cellulase</td>
<td><em>Trichoderma viride</em> ATCC</td>
<td>Wheat bran</td>
<td>Mandel’s liquid media</td>
<td>60.5 FPU</td>
<td>28 FPU</td>
</tr>
<tr>
<td>Invertase</td>
<td><em>A. niger</em> (mutant)</td>
<td>Polyurethane Foam</td>
<td>Basal media</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Lipase</td>
<td><em>A. niger</em> NCIM1207</td>
<td>Wheat bran and olive oil</td>
<td>Synthetic oil based media</td>
<td>630 IU/g dry wt.</td>
<td>18 IU/ml</td>
</tr>
<tr>
<td>Phytase</td>
<td><em>A. niger</em></td>
<td>Wheat bran and soybean milk</td>
<td>M1 medium (semisynthetic)</td>
<td>884 U/g</td>
<td>N/A</td>
</tr>
<tr>
<td>Polygalacturonase</td>
<td><em>A. niger</em></td>
<td>Wheat bran, coffee pulp</td>
<td>Pectin-based production media</td>
<td>2.28 U/l</td>
<td>0.48 U/l</td>
</tr>
<tr>
<td>Tannase</td>
<td><em>A. niger</em> Aa-20</td>
<td>Polyurethane foam</td>
<td>Production media</td>
<td>12,000 IU/l</td>
<td>2500 IU/l</td>
</tr>
</tbody>
</table>

**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...
Penicillium notatum extracted using fermentation was penicillin from fermentation. The first antibiotic to be commercially compounds extracted from microorganisms using antibiotics are the most important category of bioactive compounds extracted from microorganisms using fermentation. The early antibiotics produced using fermentation has been illustrated in Table 2.

### TABLE 2. Bacterial enzyme production using fermentation

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Bacterium</th>
<th>Substrate</th>
<th>Productivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylanase</td>
<td>Thermotolerant Bacillus sp.</td>
<td>Corn cob and wheat bran</td>
<td>6.18 U/g</td>
</tr>
<tr>
<td>Amylase</td>
<td>Bacillus spp.</td>
<td>Oil cakes, wheat bran, bagasse</td>
<td>Around 50000 U/g</td>
</tr>
<tr>
<td>L-Asparaginase</td>
<td>Streptomyces spp., Serratia marcescens</td>
<td>Soybean meal Yeast extract medium</td>
<td>49.23 U/ml</td>
</tr>
</tbody>
</table>

Hypercholesterolemic Agents

Hypercholesterolemic agents are 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 arteriosclerosis which leads to coronary heart disease. The first hypercholesterolemic agent to be discovered was compactin. Over the years, research has led to the discovery of other hypercholesterolemic 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. Simivastatin 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 arteriosclerosis 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 fermentation

<table>
<thead>
<tr>
<th>Statin produced</th>
<th>Fungal strain</th>
<th>Substrate</th>
<th>Productivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compactin</td>
<td>Penicillium spp</td>
<td>Wheat bran, soybean meal and groundnut oil cake</td>
<td>725 µg/gds</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>Aspergillus terreus</td>
<td>Wheat bran, rice husk, paddy straw</td>
<td>1500 µg/ml</td>
</tr>
<tr>
<td>Mevastatin</td>
<td>Penicillium citrinum</td>
<td>Wheat bran</td>
<td>0.0554 mg/ml</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>Streptomyces spp.</td>
<td>Rice bran and rice husk</td>
<td>15 mg/l/h</td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Organism</th>
<th>Substrate</th>
<th>Productivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporin A</td>
<td>Trichoderma cylindrosporium</td>
<td>Wheat bran, agro-industrial residue</td>
<td>1.4 mg/g, 0.08 mg/ml</td>
</tr>
<tr>
<td>Cephamycin C</td>
<td>Nocardia lactamidurans, Streptomyces clavuligerousNT4</td>
<td>Soybean flour</td>
<td>15.75 mg/g, N/A</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Streptococcus viridifaciens, Streptococcus clavuligerus</td>
<td>Sweet potato residue, Agro waste</td>
<td>2129 µg/g, 300 µg/g</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>Penicillium griseofulvum</td>
<td>Rice bran, Production media</td>
<td>9.732 mg/g, 0.1 mg/ml</td>
</tr>
<tr>
<td>Surfactin</td>
<td>Bacillus subtilis RB14</td>
<td>Soybean curd residue, Semisynthetic and synthetic media</td>
<td>200-250 mg/kg, 250 mg/l</td>
</tr>
<tr>
<td>Mycofenolic acid</td>
<td>Penicillium brevicompactum</td>
<td>Pearl barley, wheat bran and rice, Mannitol</td>
<td>6.1 mg/g, 1.2 mg/g</td>
</tr>
</tbody>
</table>

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 nutraceuticals. 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 (Haliselassie 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.

REFERENCES


Fermentation for the production of bioactive substances


