AN OVER VIEW ON SOLID STATE FERMENTATION

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1. Introduction

It was Pasteur who, in the late Nineteenth Century realized that microbes were responsible for fermentation and his work laid down the foundation for subsequent development of industrial fermentation processes for the production of desired substances. The term fermentation is derived from the Latin word, fever, to boil, thus describing the appearance of the action of yeast on extracts of fruit or malted grain. The boiling appearance is due to the production of CO_2 bubbles caused by the anaerobic catabolism of the sugars present in the extract. However, fermentation has come to have different meanings to biochemists and to industrial microbiologists. Its biochemical meaning relates to the generation of energy by the catabolism of organic compounds, whereas its meaning in industrial microbiology tends to be much broader. The industrial microbiologists have extended the term fermentation to describe any process for the production of product by the mass culture of microorganisms (Stanbury and Whitaker, 1984).

2. Types of Fermentation

Fermentation processes can be broadly divided into submerged fermentation and surface fermentation.

2.1 Submerged fermentation

In submerged fermentation, carbon substrate and other nutrients are solubilized in water and used in deep fermentation. In this process microorganisms are grown dispersed through a liquid medium. The fermentation vessel usually consists of sterilizable tank (Stainless steel fermentors). Fermentor vessel has several hundred liters capacity and is equipped with a mechanical agitator. Sterile air can be introduced into fermentor or air lift type is used. Fermentation is carried out with auto control of pH, temperature, dissolved oxygen and foam.

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2.2 Surface fermentation

Surface process refers to the growth of the organism on the surface of either solid state material or liquid nutrients solution.

3. Solid State Fermentation

Solid State Fermentation (SSF) deals with the utilization of water insoluble materials for microbial growth and metabolism. Solid state fermentation refers to the growth of microorganism on solid materials without the presence of free liquid. In practice, solid substrate fermentation is commonly carried out in dense slurry systems in the absence or near absence of free liquid, resulting is so called 'semi solid' or solid state fermentation systems. However, dilute slurry solid substrate fermentation also finds many applications. It is not easy to establish an exact line of demarcation with respect to free liquid in fermentation. Solid state fermentation means the growth of microorganism on solid materials not in a liquid phase. The moist solid material may be stationary, that is, the material may be put in a tray or flask and inoculated, the microorganisms then develop on the substrate as they do in nature. A second fashion of carrying out fermentation on a solid substrate is to occasionally shake or turn the moist substrate so that part of the bottom is moved to the top. The third method is to use solid substrates, usually in the form of kernels of grain, which are inoculated and continuously agitated so that each inoculated kernel is constantly being moved in the fermentation vessel, thus preventing sporulation and surface mould growth. Since biological activity ceases below a moisture control of about 12% (Golueke, 1960), this establishes the lower limit at which solid substrate fermentation can take place. As the moisture content of the substrate approaches this value, the microbiological activity is retarded more and more. The upper limit for solid state system is a function of absorbency and hence moisture content which varies with the substrate material type. For example, free water becomes apparent in maple bark at 40% moisture but in wheat straw it becomes noticeable at about the 75% level (Cannel & Moo – Young, 1980a & b). For most solid substrate materials, free liquid becomes apparent above the 80% moisture content.

Because of the low amounts of water available in solid state fermentation, the class of microorganism that most commonly used is fungi. This

makes sense because fungi naturally grow on solid materials (e.g. press of wood, leaves, roots etc.). In fact, some fungi are able to grow and reproduce on wheat having a moisture content of only 14%. One exception to this rule is in composting, where thermophilic bacteria are the most important microorganism present.

This article is intended to be a review of solid state fermentation under the following headings.

A historical sketch of the development of SSF.
A discussion of the advantages and disadvantages.
Different processing techniques and products.
Some experience with SSF at Department of Biochemistry, Faculty of Medicine, University of Jaffna.

4. Historical Sketch

Have you ever been confronted by a mouldy piece of bread or mould covered lime ? If you have, then you have witnessed solid state fermentation occurring spontaneously in nature. And as with many other natural processes man learned early in his history how to put solid state fermentation to work for him. He did this without knowing about microorganism or biochemical processes.

There are three traditional solid state fermentation processes which are still in use today and whose histories reach far back in time. They are Oriental food fermentation, mould – ripened cheese and composting. Since their development was independent of one another, a short historical sketch will be presented for each.

4.1 Oriental food fermentation

The Oriental people have been using solid state fermentation for several millennium. In contrast to the Western World, the Oriental people do not regard moulded food as only fit for the garbage can. Indeed they take pride in an art of food preparation in which moulding is an integral part. In the book of Chau Lai (one of the thirteen classics of Confucius) written before 1000 BC, one can read how the King's cook used 20 jars of soy sauce for the ceremonial rites of the Chau Dynasty (Youg & Wood, 1974). The first step towards the preparation of soy sauce is the fermentation of steamed rice by SSF.

Until about the 18th Century, the preparation of fermented solid

foods remained in the home. Family recipes were closely guarded secrets that were handed down from generation to generation. Some families formed businesses and in time the fermented food industry of the Orient developed into what it is today – a thriving, modern and highly successful enterprise.

4.2 Mould ripened cheese

As with oriental food fermentation, the exact origins of the cheese making process is unknown. Undoubtediy it was an important step for man as it allowed him to store the perishable proteins found in milk in the form of cheese, while today there are over 500 varieties of cheese, this study is only concerned with two basic types; blue vained cheese (e.g. Roquefort) and surface moulded cheese (e.g. Camembert) and both of these classes are examples of solid state fermentation by moulds.

The first record of mould ripened cheese is found in the writings of Pliny in the first century AD where he praises the quality of Roquefort cheese (Gray, 1970). This cheese was made in caves in the south of France from sheep's milk. The actual record of its production goes back 1000 years. Camembert cheese also originates from France but its history is even more obscure than that of Roquefort.

4.3 Composting

For many centuries farmers and gardeners throughout the world have practiced composite by placing vegetable matter and animal manure in piles or into pits for decomposition prior to use. However, it was not until 1925 that composting as a systematized process for treating wastes was proposed. At this time, Sir Albert Howard developed the Indoor Process in India (US Environmental Protection Agency, 1971). Since 1944 the Indian government has promoted composting and as a result there were 2,500 small composting plants operating by 1969. Throughout the rest of the world only 100 plants in operation between the years 1960 – 1969 (US Environmental Protection Agency, 1971). Most of the European plants have been built since 1950, the most successful being in the Netherlands when 19% of all municipal wastes are composed (Teensma, 1961).

To conclude this brief historical sketch we see that solid state fermentation has developed in three separate lines. By far the most successful is the oriental food fermentation industry which is found primarily in the East. Mould

– ripened cheeses are largely a product of Europe. Composting on the other hand is practiced on a large scale only in India.

With the advent of deep-tank fermentation, the bran process was neglected in the Western World and research was devoted exclusively to the design of fermentors which contained the nutrients of the fermentation in a liquid phase. The drug houses developed equipment for producing antibiotics from *Streptomycetes*, using the deep-tank equipment, which had been developed for the penicillin fermentation. Since the discovery of antibiotics, practically all new fermentor equipment and new plants are based upon the conventional deep tank fermentation for producing microbial products.

5. Advantages and Disadvantages of Solid State Fermentation

As discussed in the previous section, traditional solid state fermentation came about as a result of two primary reasons. a) the desire for more fasting foods as with oriental fermented foods and mould-ripened cheese and b) the need to dispose of garbage and waste (as in composing).

However, for today's engineer looking for alternative processes, solid state fermentation offers many attractive features that perhaps were taken for granted or were not considered important in ages gone by. In comparison to converted slimed or aerated liquid media fermentation, solid state fermentation offers the following **advantages**.

a.) Because no free water is present, the volume of the media per gram of substrate is drastically reduced. This leads to many obvious benefits:

-The space occupied by the fermentation equipment is small relative to the yield of the product (yields in SSF are at least as high as in Continuous Stirred Tank Reactor (CSTR), (Hesseltine, 1977).

-there are no enormous amount of liquid waste to deal with.

-in many cases there is no need for filtration as the product is concentrated in the substrate and can be used directly (e.g. Oriental foods).

-if it is necessary to extract the product from the substrate (e.g. enzyme production) then much less solvent is needed

than in CSTR.

-storage area required immediately following fermentation is greatly reduced.

b) Since most bacteria require high moisture levels to survive, solid state fermentation excludes or reduces greatly the problem of bacterial contamination. Most of these processes require no sterilization measures whatsoever.

c.) The media is relatively simple as usually a natural as opposed to synthetic media is used.

d.) Aeration is easily achieved as there are air spaces between the substrate particles. (Continuous mixing is often not needed, an occasional stirring being sufficient.)

e.) In solid waste management not only is the volume of material reduced but pathogenic microorganism are killed and insect breeding and vermin harborage is prevented through SSF. The product is also physically more stable than the original materials. SSF is adaptable to either continuous or batch processes and the complexity of equipment is no greater than that required for CSTR.

However, to be fair, there are some important **disadvantages** to SSF that should be brought to light.

a.) The types of microorganism that can be used in SSF are limited to those that can grow at reduced moisture level, namely fungi including *Rhizophus, Aspergillus Mucor* and *Penicillium*; some yeast and *Streptomyces*. If the organism requires free water, then either a still or agitated deep tank liquid fermentor must be used.

Typically, bacteria can only grow when in a liquid phase or at least the nutrient medium must have free water present. For this reason, bacterial fermentation is carried out usually in liquid media or in semi liquid substrates such as curdled milk to make acid products and cheese. Likewise, most yeast grow in fruit juices or simple sugars made by the enzymatic digestion of cereal grains, although some exceptions do exists.

b.) In large scale operations, the heat generated by the respiring microorganism must be

CFTRI.

Dehairing is one of the first steps associated in the series of processes leading to the production of finished leather. Use of chemicals such as lime and sulphide leads to the problems in the disposal of tannery effluent. As an alternative enzymes can be used. But high cost of the currently available enzymes is the problem stalling the progress of enzymatic dehairing on a commercial scale. *Aspergillus flavus* can grow luxuriantly on a solid support and elaborate copious amounts of extracellular protease.

6.3 Organic acids

Solid state fermentation is industrially exploited for the production of citric acid, itaconic acid, lactic acid and gallic acid (from *Aspergillus niger*).

6.3.1 Citric acid

Citric acid is produced in large scale in Thailand by SSF. About 1/5 of the total citric acid produced in Japan is by SSF (Lockwood, 1975) it amounted to about 2500 tonnes annually in as early as 1965 (Lockwood, 1979). SSF was also used in earlier years to produce gallic acid by *Aspergillus niger* on gallnuts (Van Tieghem, 1867). The large scale production of itaconic acid in Japan by using modernized koji fermentation system (Viniegra Gonzalez, 1988).

6.3.2 Lactic acid production from cucumbers and sauerkraut

- The pickling of cucumbers

The phase normally requires 2 or 3 days and during this time the number of lactic bacteria increases rapidly and there is steady increase in total acidity and a corresponding decrease in pH. *Leukonestoc mesenteronotes, Lactobacillus brevis, Pediococcus cerevisiae* and *Lactobacillus plantarum.*

- Production of Sauerkrat

Sauerkrant is obtained by lactic fermentation of properly prepared and shredded cabbage in the presence of not less than 2% and not more than 3% salt. The fermentation is a natural one induced by bacteria resident on the leaves of the cabbage. *Leuconostoc mesenteroides, R. planlanim* and *L. brevis* are used (Underkofler and Hickey, 1954; Rose, 1982; Desrosier, 1970).

6.4 Mushrooms

Over the last two decades the production and consumption of mushrooms world wide has increased from about 250,000 tonnes per annum in 1960 to over 1 million tonnes in 1980. Traditionally, they have been

regarded as a luxury food consumed only by people in the lower income groups and increasing attention is being given to their value as a food. Mushroom production at farm and commercial scales is an example of SSF which is used in all parts of the world. As the mushroom produced by submerged fermentation lacks typical aroma / flavour. The major varieties of commercially produced mushroom in high quantity include; button mushroom, paddy – straw mushroom, shiitake, oyster mushroom, truffles and morel mushroom.

6.5 Mould-ripened cheeses

Another major application of SSF, practiced through out the would is the ripening of cheeses by fungi to import distinct flavours or to soften the *cheese Penicillium camembertill* is grown on the surfaces of curd cake in the manufacture of soft cheeses. In Camembert and Brie types, *Penicillum roquefortill* allowed to grow in the body of the raw pressed curd in the production of marbled cheeses such as green – or blue – veined varieties of the type Roquefort, Gorgonzola and Stiltos.

6.6 Dough fermentation

Bread and other similar products are produced daily in enormous quantities by infinite number of commercial concerns through out the world. The fermentation of the dough, prior to baking by the action of yeast is vital for obtaining acceptable quality product and involves an application of SSF.

6.7 Ethanol

SSF is suitable for the production from fibrous and pulping substrates. Kao- Liang liquor from sorghum grains is also produced in high quantities in Taiwan by the application of SSF.

6.8 Coffee, cocoa & vanilla

The coffee beans are subjected to SSF in the so called dry fermentation where the beans are piled in a heap for removing sticky mucilage layer for facilitating quick drying of the perchment coffee and improving the raw appearance of the beans. Similarly, cocoa berry and vanilla beans are also fermented by the application of SSF for the formation of flavour precusors.

6.8.1 Coffee

Coffea arabica and *C. robasta* are the two main species used in the production of coffee. Two methods are available – Wet and Dry. In the wet method the cherries are passed through a

pulping machine which removes all the extraneous material and leaves the beans surrounded by the parchment and a layer of mucilage. These are then immersed in water contained in concrete or wooden vats. Fermentation occurs and during this period mucilage surrounding the beans is digested. The length of time for digestion to occur can vary from 6 – 72 h depending upon the temperature, thickness of the mucilage layer and the concentration of pectic enzymes present. The mucilage digestion may be accelerated by the addition of pecteolytic enzymes or by alkaline treatments with sodium or calcium hydroxide. Several types of moulds, yeast and bacteria have been isolated from fermenting beans where lactic acid producing pectiolytic organisms are involved. Full flavour of coffee develops on roasting, when amino acids react with sugars in Maillard non-enzymatic browing giving many flavour compounds. The super grade coffee contains about 1% of the stimulant caffeine, an alkaloid dry trimethylxanthine.

6.8.2 Cocoa (*Theobroma cacoa*)

Harvested pods are split open and allowed to ferment at ambient temperature (25 – 35°C). The fermented beans are then sun dried to lower their moisture content from 50 to 7.8%. The major organisms occurring during fermentation are yeast, lactic acid and acetic acid. The yeast (*Kloeckera, Hansenula, Tonalopsis* and *Saccharomyces* spp.) become active first and covert the sugars in the adhering pulp to ethanol and CO_2. The majority of yeast, strong fermentors are active for about 24h. *Kluyveromyces fragilis* break down pectin. The predominant aroma in cocoa fermentation is that of acetic acid which is brought about by the oxidation of ethanol to acetic acid. The production of acetic acid and its diffusion in to the cotyledons is essential to the development of true chocolate flavour. West African cocoa beans are regarded as top quality (pH 5.1 – 5.3), while South East Asian beans are of generally lower quality (pH 4.5 – 4.8).

6.8.3 Vanilla (*Vanilla planifolia*)

Two methods of processing are available and they are the Mexican curing method and Malagasy Republic curing method.

The Mexican curing method is the oldest one and is still in use. The beans are first kept in sheds for few days to shrink. They are then spread at on mats in the sun for a few hours, after which they are covered over. The beans are collected at nights and placed in a wooden containers lined with

blankets in which they are allowed to sweat. The alternative process is repeated until the beans change to dark brown colour. Beans are dried without disturbing the fine aromatic balance and without allowing the bean to split.

In Malagasy Republic curing method, the stored beans are first immersed in hot water (65°C) for a few minutes to rupture the inner cell walls and possibly stimulate the enzyme systems present. Then the beans are left to sweet for days and then laid out in trays, converted with cloth. Air is dried to 30 – 50 % moisture content. The glucoside glucovanillin in the beans is hydrolysed by a p-glucosidase to glucose and vanilla or methyl protocatechnic aldehyde which is the major flavour component present at 1 – 5 % levels. The other flavour compounds produced during fermentation are vanillyl alcohol, helitropin and anisinic acid.

6.9 Starter inoculum

Innumerable small and large fermentor plants commercially produce a variety of starters by the application of SSF, for use in different fermentation processes through out the world. Few major example include: Spores for mushroom cultivation, ragi inoculum, Chinese yeast, baker, *Ectomycorrhize* starter inoculum, peat – based legume inoculants and inoculum for blue – veined cheeses. In addition large quantity of fungal spores used in transformation of various organic compounds are also produced by SSF method.

6.10 Upgradation of ligno – celluloses

Ensiling which involves the growth of the naturally occurring lactic acid producing bacteria on agricultural products such as grasses is being practiced in most part of the world from ancient times for upgradation and preservation purposes (Woolford, 1984). Another type of upgradation for improving nutritive and digestive quantities of ligno-cellulosic agro-byproducts, such as straw, is also practiced (Lonsane and Ghildyal, 1989).

6.11. Mycotoxin

Mycotoxins, which are poisonous substances, causing diseases in man and animals, are produced by mould or fungi. Toxin producing fungi are divided into two groups, viz., field fungi and storage fungi. Important mycotoxins produced by fungi are ergoxins, fusarium toxins, T-2 toxins and aflatoxins (Detroy *et. al.*, 1971). Fusarium toxins reported to have been used in biological war in Afghanistan

and Kampuchea, in general, affect gastrointestinal system causing diarrhoea, bloody stools, etc. T-2 toxins are powerful skin irritants. The mycotoxins produced by mould *Aspergillus flavus* called aflatoxin are the most notorious and are distributed throughout the world in soil and air.

In UK, after out break of food poisoning due to aflatoxin produced by *Aspergillus flavus* on peanut, a large quantity of toxins was required for biochemical and animal feeding experiments. In order to produce these large quantities of toxin, different methods using solid and submerged fermentation were developed.

The production of mycotoxin by SSF leads to many times higher yield as compared to that by submerged fermentation. Large scale production was carried out which has paved a way for commercial production of mycotoxin in US by SSF.

6.12 Insecticides

Insects are controlled by using chemical or biochemical insecticides. Chemical insecticides with high efficacy and ease of use have disadvantages of polluting the environment and making insects resistant to chemicals. Microbial insecticides which are formulations containing toxins or spores or vegetative cells of pathogenic microorganisms have many adavantages and widely used over 400 species of fungi and 90 species of bacteria are known to attack insects (Quinlan and Lisansky, 1983).

The most economically important and widely produced bacterial insecticide is *Bacillus thuringiensis* (Bt). The toxins produced in the spores of Bt are high molecular weight proteins present in the form of crystals. When these crystals are taken up by the insects with their food, they become lethal after digestion by proteases in the mid gut of caterpillar (Quinlan and Lisansky, 1983). Fungi infect insects not only through the mouth part and the gut but also through the cuticle. The enzymes, lipases, proteases and chitinases, breakdown the cuticle. Nutrile products Inc. produced a product under the trade name Bitrol XK containing *Bacillus thuringiensis* Kurstaki HD1 markets the product obtained by SSF (Quinlan and Linsanky, 1983).

Table : Microbial insecticides produced by solid state fermentation (Misra, 1991).

Microorganism	Target	Development stage
Bacillus thuringiensis	Caterpillar, Mosquito, Black fly, Bud worm	Commercial
Beauvaria bassiana	Colorado beetle	Small scale
Hirsutella thompsonii	Citrus rust mite	Pilot plant
Metarhizium anisopliae	Spittle bug Mosquito Field cricket Rhinoceros beetle	Pilot plant Small scale Small scale Small scale
Nomurae rileyi	Caterpillar	Small scale

Metarhizium anisopliae is produced by SSF in Brazil. This is used against sugarcane froghopper and two pasture land froghopper species (Ferron, 1981). This is used in controlling rhinoceros beetle also (Lisansky and Hall, 1983). *B. bassiana* is produced in China in communes on solid state fermentation (McCoy, 1981). The conida is reported to be remarkably effective in controlling corn borers and pine beetles. *Hirsutella thompsonii* conida is used to control eriophyd and tetranychid mites of citrus and other plants.

6.13 Biodegradation

The application of SSF in biodegradation of undesirable and polluting chemicals under natural conditions have been practiced in specific cases. One of the major applications involve the sludge farming which involve soul as spoiled substrate on which organic biotransformations are effected either by promoting indigenous microbial population or by adding specific culture. For e.g., a mould culture systems have been used to degrade oil on railway tract, which other wise, accumulates as a result of frequent spillage from locomotives.

7. Solid State Fermentation at Department of Biochemistry

7.1 Citric acid

Citric acid production by *Aspergillus niger* UV2 (a strain isolated and mutated at the department) was carried at in production medium (containing gl^{-1}, glucose, 140., peptone, 7.0; NH_4NO_3 , 0.5; KH_2PO_4 , 0.5 ; $MgSO_4.7H_2O$ 0.1; $CuSO_4.5H_2O$, 0.06×10^{-3} and ferrous ammonium sulphate , 0.1×10^{-3} with (ml) methanol, 30 and gingili oil (2.0) using paddy husk as support. The citric acid productivity was higher in solid state fermentation (0.198 $gl^{-1}d^{-1}$) than in liquid fermentation (0.129 $gl^{-1}d^{-1}$). But the yield and efficiency of citric acid production were the same (This was a part of the Ph.D. work of Navaratnam, 1997).

7.2 Production of rennet

Rennet is the product of microbes while renin is from calf stomach, but both do the same function. Rennet is widely used in cheese production. *Aspergillus niger* N_4 was used for this studies. A solid state medium was formulated for rennet production, while optimizing the culture conditions. When the *Aspergillus niger* was cultivated on basal medium mixed with paddy husk (as support) 42.6 SU g^{-1} Dry Mouldy bran of clotting

activity and 14.2 SU g^{-1} DMM of proteolytic activity were obtained. After optimizing the culture conditions, nutrient, production time etc., 3473.4 SU g^{-1} DMM clotting activity was obtained. Here the proteolytic activity should be kept at reduced level because this may interfere with the clot formation which is one of preliminary steps in the cheese formation. The studies have led to 8.2 fold increase in clotting activity (This was a work for M.Sc. Biochemistry degree of Senthuran, 1997).

7.3 Production of (α- amylase)

α-Amylase from *Bacillus licheniformis* 6346 was produced using rice bran and other nutrients (Tambyrajah, *et. al.*, 1993). In the initial studies 29 U g^{-1} DBM of (α-amylase activity was obtained. By improving the nutrient content of the medium the enzyme production was increased to 1056 U g^{-1} DMB (Tambyrajah, *et. al.*, 1995) through 488 Ug $^{-1}$ DBM (Tambyrajah, *et. al.*, 1994) and the production time was reduced from 6 days to 4 days. As an alternative to the activation medium (where nutrient broth is used), fish extract prepared in the laboratory was used and the (α-amylase activity (1218U g^{-1} DBM) obtained was higher than that with the nutrient medium as the activation medium (1056 Ug^{-1} DBM) (Arasaratnam, *et. al.*, 1998 a). When fish extract was supplemented with different nutrients, the (α-amylase produced was further improved to 1426 U g^{-1} DBM. (Arasaratnam *et. al.*, 1998 b). Then we have substituted the commercial salts in solid medium with locally available fertilizers and obtained an α -amylase activity of 1075 1056 U g^{-1} DBM (Arasaratnam, *et. al.*, 1998c). Further studies are in progress.

7.4 Production of glucoamylase

Glucoamylase production by *Aspergillus niger* was studied using two different strains, viz. *Aspergillus niger* N4 and *Aspergillus niger* CFTRI 1105.

Aspergillus niger N4 was grown in wheat bran with additional nutrients in batch and fed batch modes, the extracts contained 44 and 64 U ml^{-1} glucoamylase activity respectively (Ramadas, *et. al.*, 1994).

Glucoamylase production with *Aspergillus niger* CFTRI 1105 in solid medium containing rice bran and other nutrients gave the glucoamylase production of 39.8 U g^{-1} DMM. This was improved by supplementing with soy powder to 243 U g^{-1} DMM (Arasaratnam, *et. al.*, 1994). Addition of paddy husk

to the medium improved the glucoamylase production to 1700 U g^{-1} DMM (Arasaratnam, *et. al.*, 1995). The continuous batch solid state fermentation was carried out for glucoamylase production (Arasaratnam, *et. al.*, 1998d) with extraction studies.

7.5 Production of fungal amylase

The *Aspergillus oryzae* obtained from ATCC culture collection was first selected for the production of fungal amylase and was not effective. Hence different strains were isolated from different environments. Among the isolated strains *Aspergillus oryzae* B12 was performing best and was selected for further studies. The optimization of medium and conditions led to an increase in amylase production from 417.9 to 2184 U g^{-1} DBM. Further work is in progress.

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