Solid-State Fermentation of Rice by *Monascus Purpureus*

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Abstract

The concept of Solid-State Fermentation is briefly explained in comparison to other fermentation principles, and several types of fermenters are presented. A recently developed "Swing Reactor" for SSF is shown. When inoculated on rice, the mould *Monascus purpureus* forms red pigments, Which can be used as food colors (Ang-kak, Red Rice). By Response Surface Methodology, serveral factors have been optimized for maximal red colour formation. Showing that presoaking time of rice, pH of soaking water, age of preculture and inoculum size were not of importance within the observed limits. For a fermentation time of 7 days, start humidity is optimal at 34% and temperature is optimal at 28.8 C. These results of small scale fermentation could be transferred to the Swing Reactor.

Introduction

The concept of Soild-State Fermentation (SSF) has only been developed in the last decade[1,2], although there has a broad range of applications in conventional food biotechnology for centuries (Table 1)[3]. The applications range from cheese, meat products like raw sausage and ham to fermented fruit and vegetables.

Principle of SSF

The principle of SSF shall be explained by comparing it to other fermentation systems[2]. In submerged fermentation, i.e. fermentation of alcoholic beverages like beer and wine, The microoranisms and the substrate are homogeneously distributed within the surrounding free aqueous phase. There is a good mass transfer between the microorganisms and the water phase providing the substrate.

An example for a solid substrate fermentation of food is the production of meju balls or surface mould ripened cheeses like Camembert. The microorganisms are in an outer layer around the substrate, there is thus an heterogeneous distribution of microorganisms and substrate. There is no free aqueous phase, as all water present is bound either to the microorganisms

Table 1. Examples for the Application of Solid-State-Fermentation in the Food Industry

Product Range	Product	Microorganisms involved
Dairy Foods	Cheese	Penicillium spp.
		Propionibacterium freudenreichii
		Brevibacterium linens
	Butter (Nizo)	Lactobacillus helveticus
	Lactococcus lactis subsp. lactis	
Meat and Fish	Raw sausage	Lactococcus lactis subsp. lactis
	Ham	Lactococcus lactis subsp. cremoris
	Katsobuoshi	Penicillium candidum
		Lactobacillus spp.
		Aspergillus glaucus
Bakery goods	Sour dough	Acetobacter spp.
		Streptococcus spp.
		Lactobacillus spp.
		Saccharomyces spp.
Fruit and vegetable	Pickles	Lactobacillus spp.
	Sauerkraut	Streptococcus spp.
	Meju/Miso	A. oryzae
	Kanjang/Shoju	Yeasts
	Red Rice/Ang kak	Monascus purpureus
Chocolate	Cocoa fermentation	Lactobacillus spp.
		Acetobacter spp.
		Yeasts

(After KUNZ 1988)

or to the substrate.

Solid-State-Fermentation can be seen as a combination of both previously described priciples. In rice fermentation by *M. purpureus*, the fungus is to be found as an outer layer on the rice grains. The substrate and the microorgaanisms thus being heterogeneously distributed seen from a microscopic view. Seen as a whole within the fermenter, however, microorganisms and substrate seem to be homogeneously distributed. This distribution is thus called "pseudo-homogeneous". The microorganisms are in equilibrium with the gas phase streaming between the substrate particles and are controlled by water activity, as there is no free water phase.

The substrates for SSF can be solid, liquid, or gaseous. A solid substrate serves for the immobilization of the microorganism, which are surrounded by a layer of bound water. Solid substrates are applied in starch degradation, colour and flavour formation. In the case of a liquid substrate, the microorganisms agglomerate or are immobilized on an inert supporter. Around these particles, a film of liquid substrate forms. This principle is found for example

in traditional vinegar production. If a gaseous substrate is present, i.e. in biofilters, the microorganisms are also surrounded by a layer of bound water which the substrates and products are diffusing through.

Advantages of SSF are:
- optimal substrate utilization
- high yield
- no autinhibition
- simple downstream processing
- less environmental stress (no waste sater)

Disadvantages of SSF are:
- difficult monitoring of pH, oxygen, carbon dioxyde
- empirical process regulation
- complicated heat transfer
- lack of technical design principles

Engineering Systems for SSF

There are three basic engineering principles for SSF[3]: Fixed Bed, Moved Bed, and Fluidized Bed Fermentation (Fig. 1). In Fixed Bed Fermenters, the microorganisms are immobilized on a solid support, which can be inert (wooden chips in traditional vinegar production) or the substrate (cocoa fermentation). Substrate and water are led to the microorganisms by the conditioned gaseous or liquid phases, which are blown upwards or percolate downwards through the supporter layer. The problems arising are the limited layer height and the agglomeration

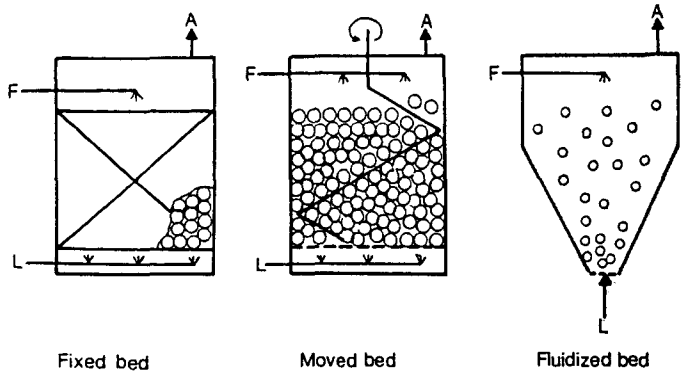

Fig. 1. Basic Engineering Principles for Solid-State-fermentation

of microorganisms because of metabolic water formed and mycelial growth, which lead to diffcult substrate transport,irregular reaction conditions and insufficient removal of metabolic heat, which can lead to overheating and dying-off of the biomass.

Moved Bed Fermentation is just a modification of Fixed Bed Fermentation, as the substrate or inert supporter is mixed by mechanical impellers or by moving the reactor itself, shich can be provided with baffles. These fermenters are just modified mixers. A problem arising is the irregular mechanic stress, which can lead to agglomeration on the one side and to destruction of particles and microorganisms on the other side.

Fluidized Bed Fermentation is another system for SSF. The microorganisms are immobilized on a solid support and fluidized by a pumped gaseous phase. The metabolic water formed can lead to agglomeration of the partickes resulting in larger particles and thus greater sedimentation velocity, rendering fluidization more diffcult. A reduction of the water layer, however, makes metabolic power and thus yield decrease.

Solid-State-Reactor Development

There are four criteria a reactor for SSF should meet:

- periodic mechanical stress on each of the particles
- no mechanical stress on all of the particles at a time
- combination of heat removal and oxygen supply by a conditioned air stream
- process regulation by several metabolic parameters

A "Swing-Reactor" has been developed which is derived from a new concept in mixing technology[3]. There are no shearing not mixing inlets in the fermenter, but the vessel is moved like

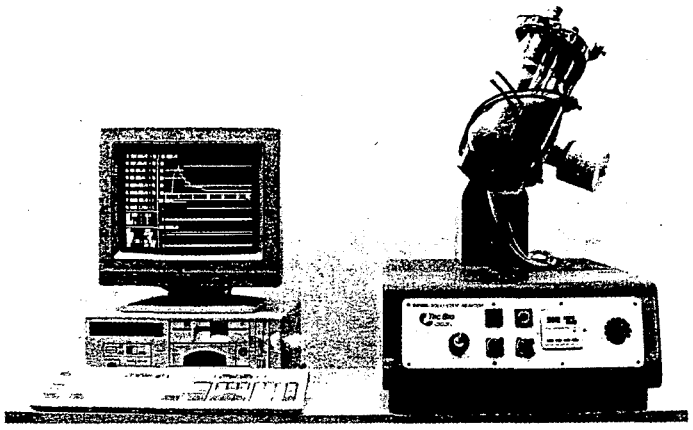

Fig. 2. Swing-Solid-State Fermenter

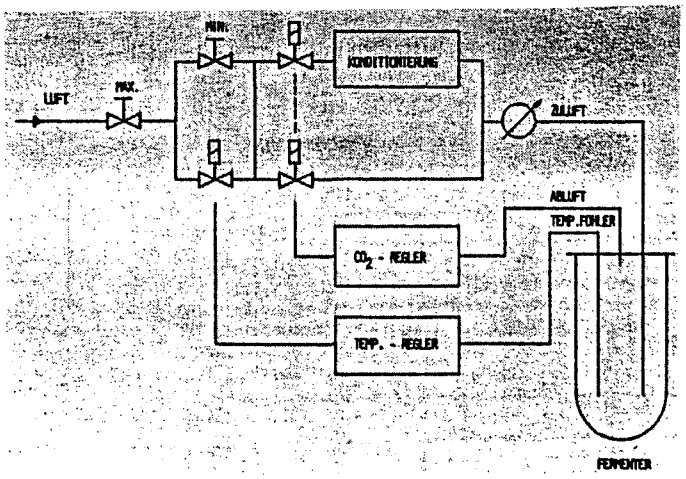

Fig. 3. Scheme of the Swing-Reactor Control

an ocean wave resulting in threedimensional reversion kinematics. Because of a special movement succession. supply pipelines can be connected to the fermenter without the danger of drilling. Fig. 2 shows this Swing-Solid-State-Fermenter with a 2 L vessel. Fig. 3 shows the scheme of reactor control: the air supply is conditioned after the exhaust air is analyzed for carbon dioxide and temperature.

For operation, particles and microorganisms in the vessel are brought on working temperature by regulating the temperature of the container the fermenter is standing in. Air streams through the filling. Temperature rises because of metabolism of the microorgaisms. If the temperature rises above a set value. the air supply increases by the factor 5···10, removing heat and water until the temperature is blow the set value leading to a normal air supply again. If the aw decreases below a set value, the air supply is humidified, until the aw is in the acceptable range again. As a third control feature, carbon dioxide can be used. There is thus a quasi-on-line analysis and automatic regulation.

Production of red rice

Rice is fermented with *Monascus purpureus* went in Solid-State-Fermentation.
Fig. 4 shows the production scheme[5]. Downstream processing is by drying and grindiding, giving a dark red powder which can be used as a colourant for foods like fish, rice wine, red soybean cheese, pickled vegetables and salted meats. There has also been found a bacteriostatic priciple in methanol extracts of *M. purpureus* called Monascidin as well as a principle decreasing blood pressure and regulating lipid metabolism (Monacolin).

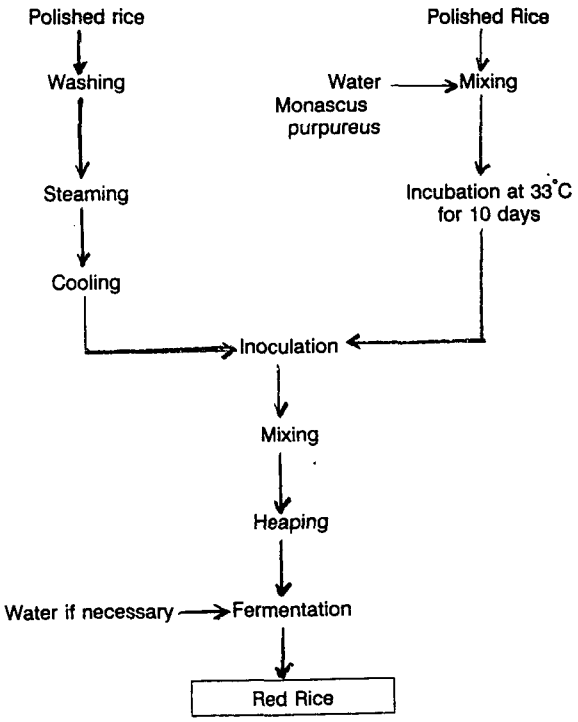

Fig. 4. Production of Red Rice (Ang-kak) 2

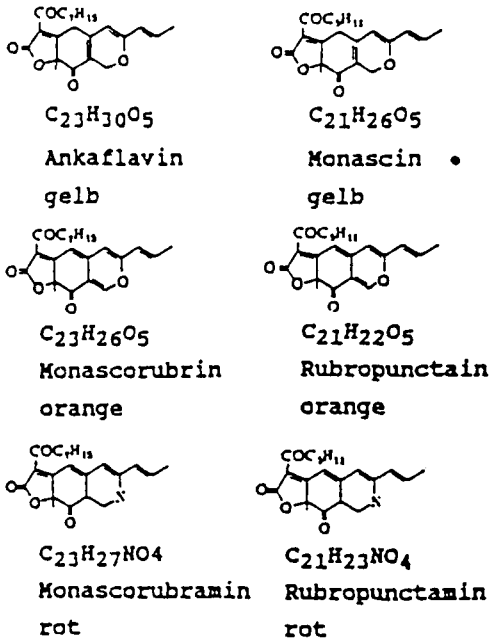

Fig. 5. Structures of the Pigments of *Monascus purpureus*

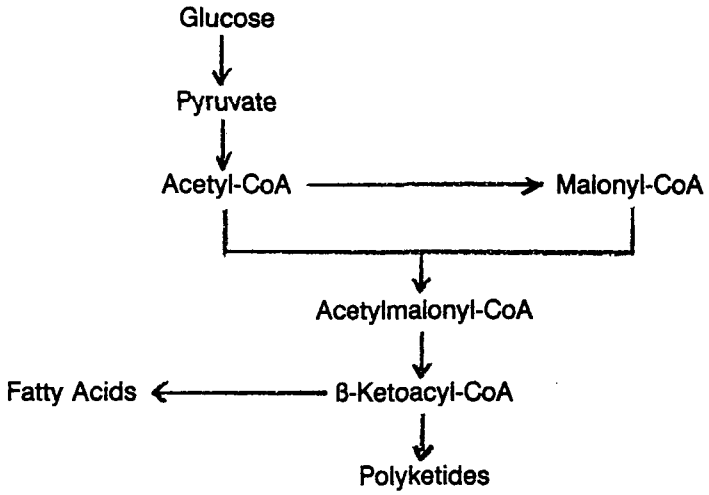

Fig. 6. Biosynthesis of Polyketide Pigments in Monascus purpureus

Fig. 5 shows the different pigments *M. purpureus* is able to form[6]. There are yellow, orange and orange pigments have a pyrenoid oxygen in the molecule, in the red pigments Monascorubramine and Rubropunctamine this oxygen is exchanged by nitrogen. The biosynthesis of these polyketide pigments is a side pathway of fatty acid synthesis, as can be seen from Fig. 6[7,8]. The mold only synthesizing the yellow and red forms, the nitrogen gets inside the molecule in secondary reactions supported by a pH around 6 and inorganic nitrogen (ammonium, nitrate) present.

Experimental part

Strain keeping was on presoaked and sterilized rice at 30℃ in simple glass beakers with aluminium top. New rice was inoculated every week, and the content of the beakers was mixed once a day by shaking.

There were three methods for fermentation. Most experiments have been done on a small scale in 250 m*l* glass beakers with about 50 g rice each resulting in a 2.5 cm layer. Some experiments have been done in a specially designed high layer fermenter and the previously described Swing Reactor of 2 L volume and four mixing cycles a day.

The quantitative determination of the pigments has been done after ethanol extraction of the dried and ground rice spectrophotometrically (500 nm).

Biomass determination could not be done by dry weight or optical density determination as in submerged fermentation. Ergosterol showed to be a good quantitative measure of growth. It was analyzed by HPLC in the non-hydrolyzable fraction.

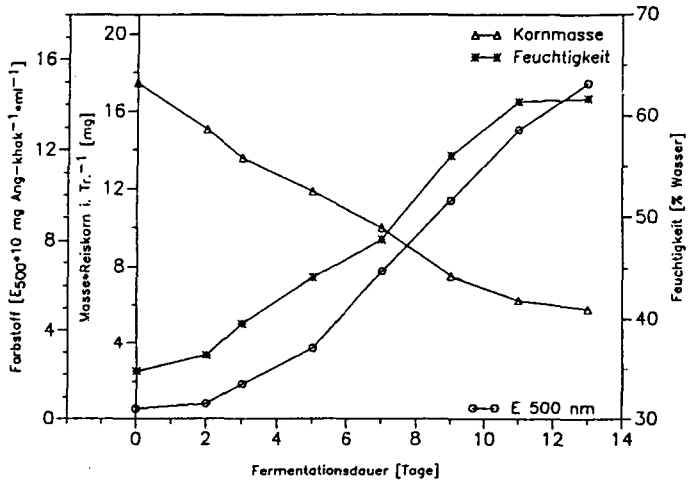

Fig. 7. Foramtion of Pigments, Grain Weight and Moisture in Dependence of Fermentation Time

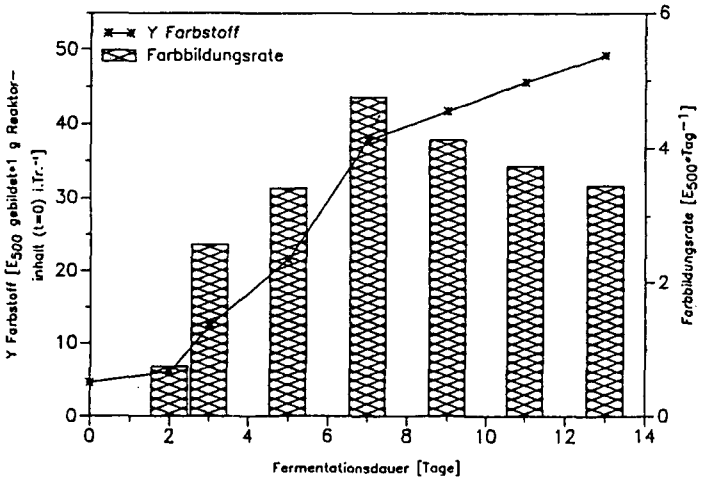

Fig. 8. Pigment Production Rate in Dependence of Fermentation Time

Fig. 7 shows the amount of pigments formed(expressed in extinction units; circles), the average grain mass(triangles)and the water content(asterisks) over a 14 days fermentation. A longer fermentation time would eventually lead to full metabolization, but there is a decrease of production rate with further decrease of dry matter. In Fig. 8, the maximum colour production rate (bars) can be seen to be on the 7th day of fermentation. All subsequent analyses have been done one the 9th day.

Fermentation optimization

The fermentation conditions have optimized on the small scale by an experimntal design

using two models and Response Surface Methodology:

– Multivariate examinations:
simultaneous variation of more than one variable

– Optimization by Response Surface Method

- 2 response variables: pigment production
  substrate consumption
- Model 1:
  2 factor variables (parameters) with 3 factor levels each:
  inoculum size(2, 10, 18%)
  age of preculture (2, 8, 14 days)
- Model 2:
  3 factor variables (parameters) with 3 factor levels each:
  pH of soakoing water (2, 5.5, 9); presoaking time had previously been found to be of no importance temperature (20, 30, 40℃)
  water amount added resp. moisture level at the start of fermentation: 0 ml/%
  3 ml/35%
  6 ml/39%

– Fractional Factorial Design

– Tests: Variance Analysis
Regression Analysis

Results

The inoculum size and the age of the preculture did not show a significant influence. Thus, a 2% inoculum and a 2 days preculture are sufficient.

Whereas the pH of the soaking water had no significant influence on pigment formation, the fermentation temperature and the water added had, as can be seen from Fig. 9a, b. Regression analysis has shown linear, quadratic and interaction influences. Partial deviation of the equations led to an optimal temperature of 28.8℃ and an optimal volume of added water of 2.2 ml, which equals a start humidity of 34%. Loss of total dry matter as a result of substrate consumption was maximal at 33℃ with 5 ml of water added (not shown).

The optimal factor combination for pigment formation applied, formation of ergosterol begins with the inoculation, but pigment formation shows a lag phase. On the fist day after inoculation, substrate and air mycelium can be seen. On the second day already, the rice grains are totally covered by mycelium, and a slight red colour can be seen. From the 7th day on, the mycelium

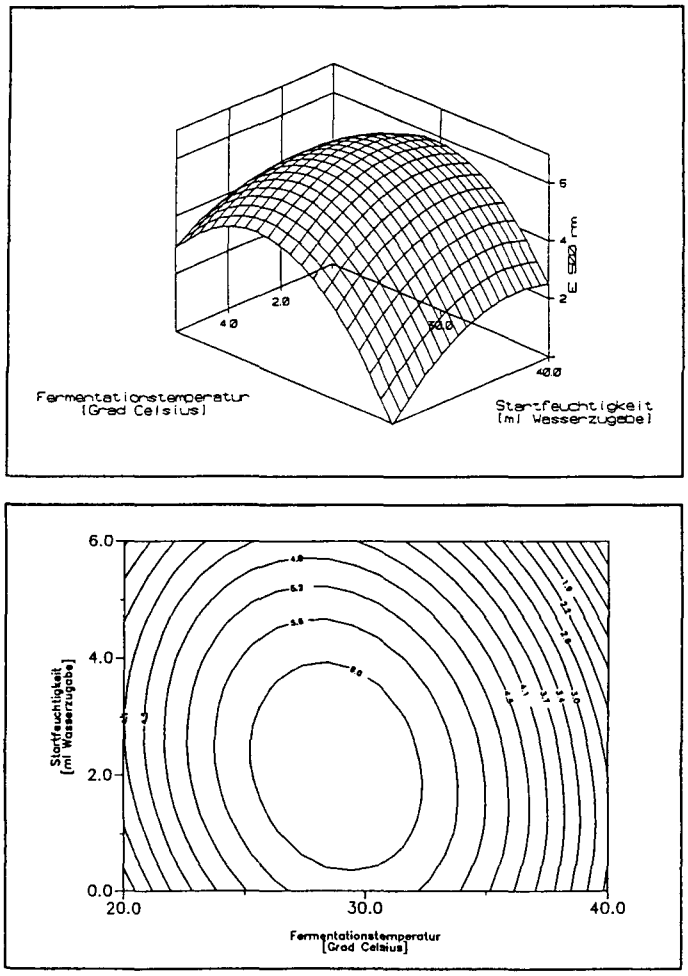

Fig. 9a, b. Influence of the Fermentation Temperature and Start Humidity on Pigment Formation

has grown through the whole grains, and a dark red colour formed is obvious.

Fermentation in high layer

A glass cylinder with a diameter of 7.5 cm has been filled with presoaked and inoculated rice up to a height of 26 cm. Air was supplied by a central tube provided with holes. Humidity and temperature were measured and manually regulated by the air supply. The fermenter has been shaken by hand once a day. With the optimal factor combination from the small size experiments applied, the results were similar.

In a large glass cylidder of 10 cm diameter and a height of substrate of 80 cm, however, problems with heat removal and moisture regulation could not be solved, resulting in a delayed pigment formation, which after 12 days was still incomplete.

Swing Reactor

The 2 L glass vessel was filled with 1400 g of presoaked and inocxulated rice. With four shaking intervalls of five minutes per day and control of moisture and temperature, the results obtained were similar to the small scale experiments.

This reactor has been used for other applications, too, for example starter culture production and composting. The next task in the development of the swing reactor is the through modelling of this fermenter type which is necessary for upscaling.

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