The Influence of Kudzu Root Starch on the Growth and Metabolism of Baker's Yeast During Aerobic Semi-Solid Fermentation

Park, D.H*., C.S*. Sun Woo, R.D. Tanner and G.W. Malaney****

*Chemical Engineering Department, Chonnam National University

**Civil and Environmental Engineering Department, Vanderbilt University

반고상 발효에서의 빵 효모 증식과 신진대사에 대한 갈근 전분의 영향

박돈희* · 선우 창신* · 로버트 디 태너** · 죠지 밀러니**

전남대학교 화공과*, 밴더빌트대학교**

Abstract: In a study of the aerobic growth of Baker's yeast (*Saccharomyces cerevisiae*) on Maxon-Johnson medium (with glucose as substrate) solidified with kudzu root starch, it was observed that, between 8 and 24 hour incubation, 10 and 12% solids stimulated greater cell production than did 6 and 8% solids. The concentration of solids also affected the secretion of protein from the yeast cells with the highest content of extracellular protein at 10-24 hour incubation stimulated by 10% starch solids.

Key words: *Saccharomyces cerevisiae*, semi-solid fermentation, Kudzu Root Starch.

It seems probable that the concentration of solids (undissolved materials) is an important operational parameter in semi-solid or solid (SSF) fermentations; however, apparently no single value or range of values has been established as optimal for homogeneous semi-solid fermentations by workers in this field.

Experimentally determined standards exist for heterogeneous systems. Han and Anderson (1975) studied the fermentation of pretreated ryegrass straw at a moisture level of 75% (25% solids). Rolz *et al.* (1979) found it necessary to add 1.4 parts of water to one part of sugar cane pieces, i.e., ca. 42% solids, to achieve a reasonable yield of ethanol. Balasubramanya *et al.* (1984) in their "dry" fermentation of willow dust used 16-17% solids as substrate. Huang *et al.* (1984) investigated the influence of initial moisture content of steamed rice serving as substrate for the aerobic growth of *Aspergillus koppan*. Of the moisture levels examined (28.12, 30.83, 33.51, 37.54, and 40.01%), 37.5% gave the maximum growth of the fermentation organism when growth was estimated by monitoring CO_2 evolution. Using wheat bran as substrate, a moisture content of 41.5% gave optimal growth of the mold.

Park *et al.* (1984), using gelatin as principal solid present in the aerated fermentation mash (but not serving as substrate), observed at 9 hours into the batch run that the maximum level of yeast cells was at 8% (wt/vol) gelatin. The highest yeast level was reduced by one-third at 10% and 12% and by one-half at 0% and 16% gelatin. Maximum utilization of

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the glucose substrate also occurred at 8% gelatin, with use at 12% gelatin content almost as great. The highest production of extracellular protein and L-lysine was observed at 16% gelatin, wherease 12% (the highest value measured) promoted maximum L-lysine concentration.

This is a report of SSF studies on the effects of solids concentration on the semi-solid fermentation of glucose by aerated Baker's yeast (*Saccharomyces cerevisiae*) when the gelatin was replaced by kudzu (*Pueraria lobata*) root starch as solidifying agent and as the principal solid in the system (The Korean name for kudzu is kuzu.). The primary response variables monitored were biomass and extracellular protein. A starch such as kudzu starch would not be expected to interfere with the protein assay as did gelatin and strach could also eventually serve as an inexpensive carbon substrate if treated with amylase. Amylase treatment was beyond the scope of this study.

MATERIALS AND METHODS

A description of the fermentation organism, aerobic fermentor and preparation of slide cultures is given in the accompanying paper by Park *et al.* (1985a).

Growth medium; The basic culture medium was the Synthetic Medium C of Maxon and Johnson (1953) with 10% (wt/vol) glucose as substrate. The MJ medium was sterilized in the autoclave (15 min at 125-126℃). A 200 m*l* portion was placed in a boiling water bath. Then 5 to 12% (wt/vol) dry kudzu starch was added and the mixture held at the boiling point for about 3 min with constant agitation. This treatment caused the starch to gelatinize and the mixture assumed a semi-solid consistency. Practical sterility was achieved by the boiling. The mixing was completed in a 100 m*l* blendor for 1 min during which time weighed yeast cells were added. The dry kudzu starch (kuzu arrowroot starch) was manufactured by the Japanese firm of Yoshino Zakura Industries and was imported into the U.S. by Erewhon Natural and Organic Food Co., Boston, MA.

Sampling; At selected time intervals, one or more slide cultures were removed from the fermentor.

Cell growth monitoring; A small amount of fermentation mash was scooped from the surface of the slide culture with a spatula and transferred to the biomass sample chamber described in Park *et al.* (1985b).

Measuring yeast growth; The growth of yeast biomass in the fermentation mash was monitored by the biomass sample well method described in Park *et al.* (1985b).

Chemical analysis; The fermentation mash was scraped from a culture slide into a small beaker using another microscope slide. This mash was diluted with deionized water at ca. 40℃. The mixture was stirred thoroughly, then was analyzed for extracellular protein.

Assay of extracellular protein; The Coomassie Blue dye-binding analysis for proteins developed by Bradford (1976), as modified by Franklin *et al.* (1984), was used. A 0.4 m*l* portion of sample was pipetted into a 13×100 mm Bausch and Lomb Spectrophotometer cuvet. Protein dye reagent (3.8 m*l*) was added and the contents were mixed carefully by inversion to avoid protein loss. After 10 min, absorbance (OD) of the solution was read on the B & L Spectronic 20 spectrophotometer. A standard curve was prepared by treating a series of solutions of sonicated yeast cell protein (0.0 to 10.0 g/*l* of MJ medium) in a similar fashion. A basic assumption was that 45% of the yeast cell was protein. A standard curve was prepared with *Aspergillus niger* α-amylase (Miles Laboratories) and was found to be essentially the same as that for yeast cell protein. Therefore, this fungal α-amylase was used to prepare subsequent protein calibration curves.

EXPERIMENTAL RESULTS AND DISCUSSION

The influence of solids concentration on production of yeast biomass; It can be seen in Fig 1 that at 10-12 hr (the time of maximum cell

production in this batch run) the higher concentrations of kudzu starch solids examined (10 and 12%) stimulated greater biomass development than did 6 and 8% solids. Park *et al.* (1984) observed greater cell production at 8-16% solids (gelatin) than at 0% solids, with highest production at 8% solids. These workers suggested that a primary causal factor in increased cell production in the gelatin solids case is CO_2 entrapped in the fermenting solid mash. Presumably, CO_2 has the same role in the kudzu root starch system as well. Preliminary tests with elevated CO_2 levels in the air stream does stimulate cell growth in submerged culture systems, Park *et al.* (1984).

The influence of solids concentration on production of extracellular protein; The results given in Fig 2 show that the amount of extracellular protein secreted at the 10-12 hr interval was significantly increased by solids levels, especially at 8 and 10% after 12 hr run time. The highest content of extracellular protein at 24 hr was produced in the 10% solids case.

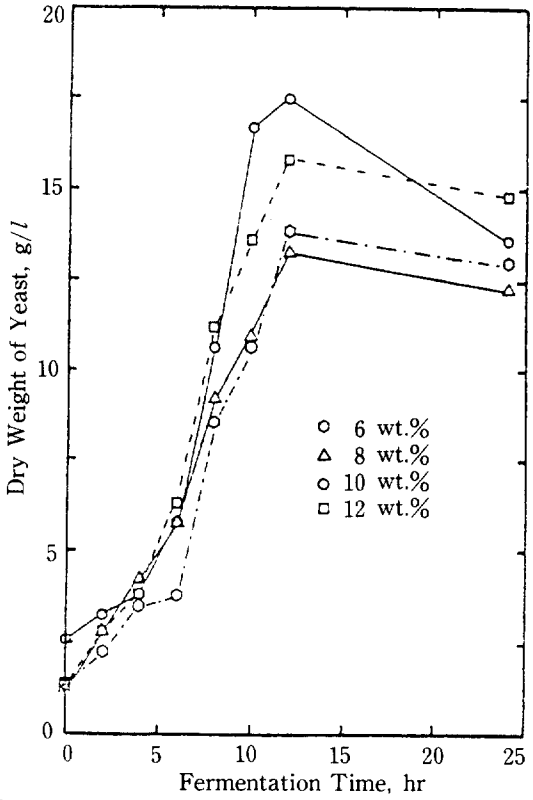

Fig.1. *The effect of concentration (wt/vol) of solids (kudzu starch) in the growth medium on the production of yeast cells during aerobic growth of Baker's yeast in Maxon-Johnson medium with 10% glucose as substrate.*

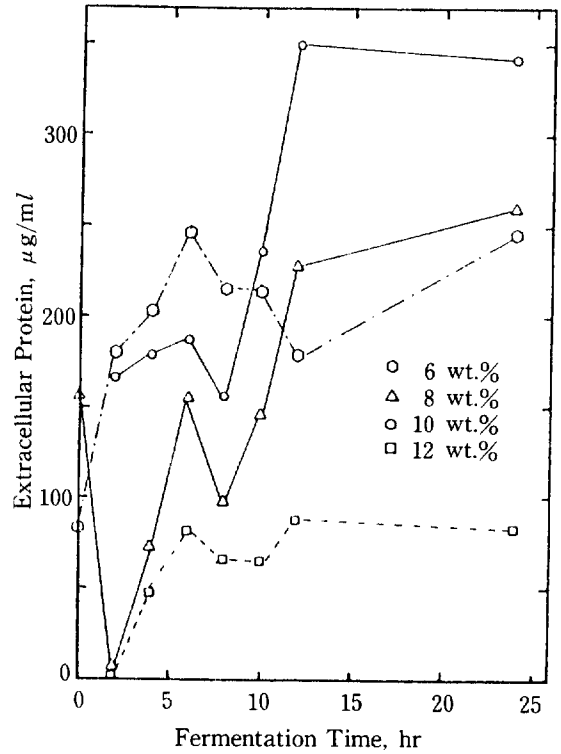

Fig.2. *The effect of concentration (wt/vol) of solids (kudzu starch) in the growth medium on the production of extracellular protein during aerobic growth of Baker's yeast in Maxon-Johnson medium with 10% glucose as substrate.*

When gelatin was the principal solid present, Park *et al.* (1984) found that the concentration of protein excreted by the yeast cells into the growth medium was essentially the same at 16% solids as at 0% solids but much lower at 12% solids. Unfortunately, in the Park's study, gelatin, the solidifying agent, is also a protein and introduced interferences in the protein assay, so the findings were not conclusive. In the present study, the essentially protein-free starch solidifying agent did not interfere in the protein determination.

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적 요

고상 발효의 기초적인 매커니즘을 규명하기 위하여 고형화된 배지속에 빵 효모를 증식시켰다. 이때 실험 배지는 Maxon-Johnson 배지 이었으며, 고형화 물질로는 갈근에서 얻은 전분을 무게비로 6-12%를 첨가하였다. 전분이 6-8% 첨가 될때 보다도 10-12% 첨가될 때 효모의 증식이 8-24시간에서 훨씬 빨랐으며, 단백질의 분비량도 전분을 10% 첨가 하였을때 10-24시간에서 가장 높았다.

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