The Effect of Light on Baker's Yeast Cell Growth and Protein Secretion

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효모의 증식과 단백질 분비에 대한 빛의 효과

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ABSTRACT: It has been observed that white light can suppress both cell growth and protein secretion in Baker's yeast. This effect was explored in batch liquid fermentations. Possible applications of this phenomenon are (a) use as a tool for pre-concentrating excreted enzymes prior to subsequent purification and (b) an engineering variable for regulation yeast fermentations.
KEY WORDS □ *Saccharomyces cerevisiae*, protein secretion, white light.

In biology there have been many studies on the photobiology of microorganisms, including non-photosynthetic species. This paper reports on background literature and preliminary research on light as an engineering variable which may prove helpful in the control of non-photosynthetic yeast fermentation.

In 1914 Buchta (1914) reported on the influence of white light, as well as light of various spectral ranges, on the growth of yeast. He found inhibition of growth by white and blue light, but acceleration of growth by red light.

Ehrenberg (1966) studied the effects of three spectral ranges (blue, 400-500mμ; red, 600-700 mμ; far-red, 700-1200mμ) on the growth of *Saccharomyces cerevisiae*. Only the blue range was shown the inhibitory effect.

Woodward, Cirillo and Edmunds (1978) found the yeast cells grown at 12 °C. were slightly slowed in further growth decreased rate by cool-white fluorescent light below 1,250 lux intensity. As the intensity increased beyond this level, there was increasing inhibition of growth.

Edmunds (1980) showed that different strains of *S. cerevisiae* varied in their growth response to cool-white fluorescent light at 5400 lux. One strain showed little susceptibility to light.

Anderson and Roth (1983) suggested the resistance to light inhibition was genetically controlled.

Sulkowski *et al.* (1964) reported that yeast cells being adapted from anaerobic to aerobic growth were inhibited by artificial daylight both in growth and in respiration as measured by rate of oxygen uptake. Cell which had previously been grown aerobically were not affected by light.

Preliminary trials were made in the laboratory to explore the inhibitory effect of light on Baker's yeast (*S. cerevisiae*).

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MATERIALS AND METHODS

Organism

The fermentation organism was Baker's yeast (*S. cerevisiae*) purchased at a local grocery in the form of Fleischmann's brand active dry yeast.

Fermentor and Medium

The fermentor (see Fig. 1) consisted of two parts: (a) polymethylmethacrylate irradiator and (b) a glass feed reservoir. The irradiator had the tube and shell configuration with a 15 W fluorescent white bulb as center tube and the irradiated yeast culture flowing between the tube and the shell. The bulb emitted a light intensity of roughly 8000 lux. About 40% of the yeast culture was irradiated at a given moment, therefore, as a first approximation, the entire yeast culture volume was exposed continuously to about 3000 lux. During the "dark" experiments the bulb was removed and irradiator and feed reservoir were covered with foil.

500m*l* Maxon-johnson (Maxon and Johnson 1953) culture broth containing 10% glucose (pH = 5.0) inoculated with 2 g/*l* Baker's yeast was pulled by peristaltic pump at 43m*l*/min from the feed reservoir through the irradiation cylinder (volume ca 200m*l*) and then recycled back to the feed reservoir. The reservoir contents were stirred at 400 rpm with a 1" magnetic stirring bar. There was no external aeration. During the "light" experiments the reservoir were dimly lit by the room ceiling light bulbs. The temperature of the fermenting mass held at 32°C + 2°C.

Measurement of Yeast Cell Mass

A 1.0m*l* portion of the 1:4 dilution of sample in the beaker was diluted with 9 volumes of deionized water. The concentration of yeast cell mass was estimated by the method described by Wei, *et al.* (1982), then making the dilution correction. Color problems developed with this method, so the effect of washing the cells was determined. The yeast cells were washed by mixing 1.0m*l* of the 1:4 dilution with 9 volumes of deionized water, followed by centrifuging at about 1300 rpm. The supernatant liquid was discarded and the cell pellet diluted with 10m*l* of deionized water. Spectrophotometer readings were made as above.

Assay of Extracellular Protein Concentration

The Coomassie Blue dye-binding analysis for proteins developed by Bradford (1976) was used.

Assay of L-Lysine Concentration

The microbiological method for measurement of L-lysine is described by Wei, *et al.* (1982).

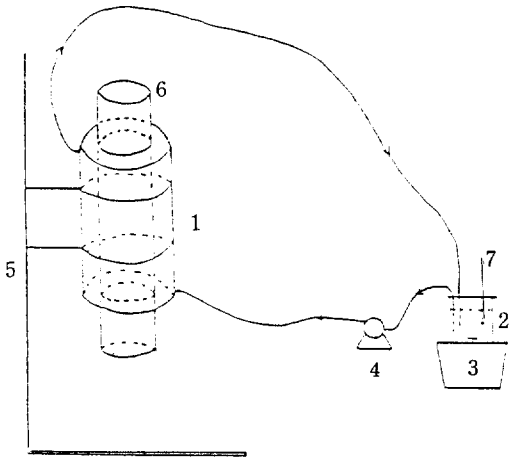

Fig. 1. *Diagram of apparatus*

1. Annular-type reactor
2. Beaker
3. Stirrer
4. Pump
5. Stand
6. Fluorescent bulb
7. Thermometer

RESULTS AND DISCUSSION

The growth results are shown in Fig. 2. For the first six hours, growth rates were similiar. There was an apparently negligible lag phase in the "with light" case, possibly followed by a period when light-growth was much slower than dark-growth. Later, light-growth may have become faster than dark-growth.

Effect of light on synthesis of protein

Sulkowski *et al.* (1964) reported research which suggested that the inhibitory effect of light was attributable to inhibition of protein synthesis. One piece of "evidence" they presented was failure of normal multiplication of cells, which, of course, contain a high content of protein. This must, however, be considered incomplete evidence since

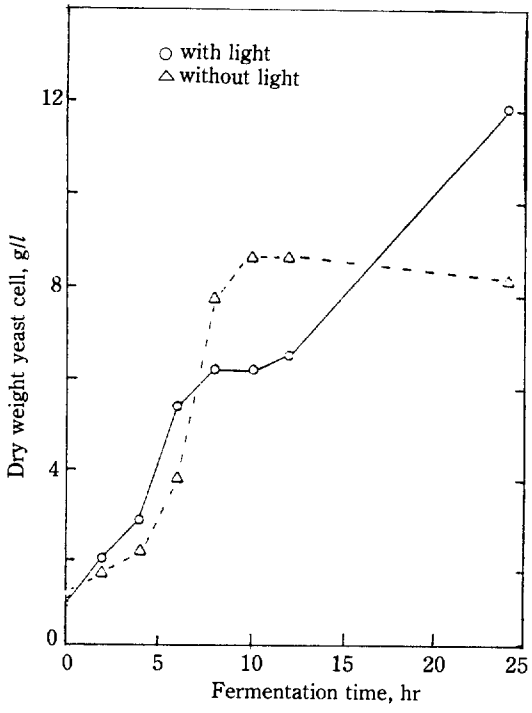

Fig. 2. *Effect of absence and presence of fluorescent white light on the growth of Baker's yeast in Maxon-Johnson medium with 10% glucose*

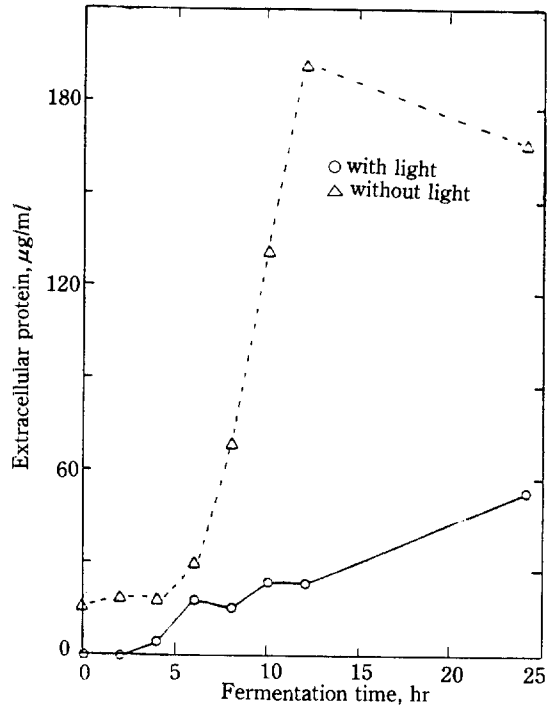

Fig. 3. *Effect of absence and presence of fluorescent white light on the secretion of extracellular protein by S. cerevisiae growing on Maxon-Johnson medium with 10% glucose*

growth inhibition could have been initiated by many other factors than lack of protein synthesis. The other piece of evidence offered was the failure of the yeast, in the presence of light, to produce intracellular galactose-metabolizing enzyme activity. However, galactozymase activity could be restricted by many other factors than failure of protein (enzyme) synthesis.

In this study, we set out to explore the effect of light on the production of extracellular proteins to see if light could act as a switch in producing extracellular products like invertase and genetically-engineered proteins made by and exported by yeast. During the fermentations described above, 5.0-m*l* samples of mash were collected periodically. These samples were centrifuged to remove the cells and the supernatant liquid was analyzed for extracellular protein by Bradford's method (1976).

The secretion of extracellular protein during the fermentations is shown in Fig. 3. Light dramatically reduced the amount of extracellular protein elaborated by *S. cerevisiae* to 1/3 to 1/4 that produced in the dark. This was the same reduction in magnitude as the inhibition of intracellular galactozymase activity reported by Sulkowski *et al.* (1964).

Effect of light on transport of nutrients into cells

Barran *et al.* (1974) demonstrated that amino acid transport in *Escherichia coli* is inhibited differentially by short periods of exposure to visible light. Koch *et al* (1976) showed inactivation of membrane transport of sugars into *E. coli* by near-UV light.

Woodward, Cirillo and Edmunds (1978) showed that sorbose transport into *S. cerevisiae* Y185 grown in cool-white fluorescent light at 3000 lux was only about half that into dark-grown cells. Their research suggested that the light-grown cells had undergone membrane damage which accounted for the changes in transport. These workers went on to demonstrate that increasing intensities of light progressively inhibited the ap-

parent transport into *S. cerevisiae* cells of amino acids such as histidine, glutamate, alanine, tyrosine, valine and leucine. Transport into the cell was inferred by reduction in concentration of the respective amino acid in the suspending liquid, although adsorption to the yeast cell wall also could have contributed to the reduction in extracellular amino acid concentration.

Sarthou, Gonneau and Le Goffic (1983) reported photoinhibition of peptide uptake in Candida albicans yeast cells by UV light.

Intracellular free lysine concentration are shown in Fig. 4. The two profiles are quite different with the content of intracellular lysine peaking early in the no-light run, while the highest level of lysine was achieved much later in the light run.

Perhaps lysine production in the no-light case was initially stimulated to make lysine available to serve as precursor for the production of high levels of extracellular protein (Fig. 3). Light, on the other hand, seemed to retard the formation of lysine in the early stages, but contributed to overproduction later, since the lysine was not needed because significant levels of protein were not being produced.

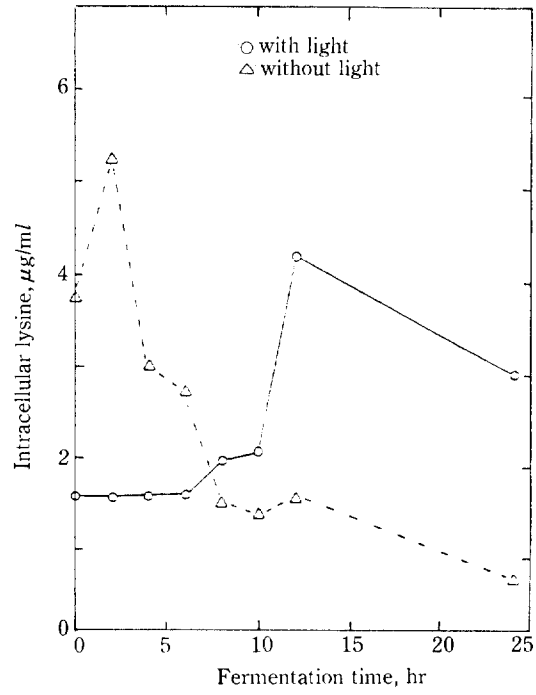

Fig. 4. *Effect of absence and presence of fluorescent white light on the formation of intracellular free lysine in Baker's yeast growing in Maxon-Johnson medium with 10% glucose*

Effect of light on respiration of exposed microorganisms

Sulkowski *et al* (1964) ascribed the inhibitory action of light to inhibition of the cytochrome system.

Ehrenberg (1966) suggested that light-inhibited respiration caused a partial reduction of the Pasteur effect which, in turn, stimulated fermentation (glycolysis) by the yeast cells.

Delbruck *et al* (1976) presented evidence that the effect of light on the fungus Phycomyces was the result of excitation of the riboflavin molecule in the fungal cell.

Edmunds *et al* (1979) reported that cells which were resistant to the inhibitory effect of white light lacked cytochromes a/a3, b and c. In particular, Edmunds (1980) offered evidence that respiratory pigments b and a/a3 are the photoreceptors for light inhibition of *S. cerevisiae* cells. Ulaszewski *et al* (1982), on the other hand, found no close relationship between light "resistance" and the cytochrome spectra of 12 different peptite strains of S. cerevisiae.

These light and dark effects recall the mode of fermentation of traditional Korean rice wine. This beverage is fermented in the dark which would encourage the development of a high content of extracellular protein which, in turn, perhaps contributes to the desirable flavor of the wine.

적 요

빵 효모의 회분식 배양에서 빛이 세포증식과 단백질 분비에 미치는 영향을 관찰하였다. 이 관찰된 현상으로 두가지 측면에 적용시킬 수 있는데 그중 하나는 배양물의 정제보다 배설된 효소를 농축시키는 방법으로서, 또 하나는 효모배양에서 규제를 위한 공학변수로 사용할 수 있다는 사실이다.

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REFERENCES

1. Anderson, J.M. and R.M. Roth, 1983. Photosensitivity in thipyronine-resistant yeast mutants. *J. Gen. Microbiol,* **129,** 63-72.
2. Barren, L.R., J.V. Daoust, J.L. Labelle, W.G. Marvin and H. Schneider, 1974. Differential effect of visible light on active transport in ecology. *Biochem. Biophys. Res. Commun.,* **56,** 522-529.
3. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* **72,** 248-256.
4. Buchta, L., 1914. *Zbl. Bakt., II, Abt.,* **41,** 340-355.
5. Delbruck, M., A. Katzir and D. Presti, 1976. Response of phycomyces indicating optical excitation of the lowest triplet state of riboflavin. *Proc. Natl. Acad. Sci. USA,* **73,** 1969-1980.
6. Edmunds, L.N., Jr., 1980. The blue light syndrome, Horst. *Springer-Verlag., Berlin.* 585-596.
7. Edmunds, L.N., Jr., R.I. Apter, P.J. Rosenthal, W.-K. Shen and J.R. Woodward, 1979. Light effects in yeast. *Photochemistry and photobiology.* **30,** 595-600.
8. Ehrenberg, M., 1966. *Arch. f. Mikrobiologie,* **54,** 358-361.
9. Koch, A.L., R.J. Doyle and Kubitschek, H.E. 1976. In activation of membrane transport in ecology by near ultra-violet light. *J. Bact.,* **126,** 140-151.
10. Maxon, W.D., and M.J. Johnson, 1953. Aeration studies on the propagation of Baker's yeast. *Ind. Eng. Chem.,* **45,** 2554-2560.
11. Sarthou, P., M. Gonneau and F.Le Goffic, 1983. Photoaffinty inhibition of peptide transport in yeast. *Biochem. Biophys. Res. commun.,* **110,** 880-892.
12. Sulkowski, E., B. Guerin, J. Defaye and P.P. Slonimski, 1964. Inhibition of protein synthesis in yeast by low intensities of visible light. *Nature,* **202,** 36-39.
13. Ulaszewski, S., J. Kolodynski and Z. Kotylak, 1982. Light effects in yeast. *Acta Microbiol. Polonica,* **31,** 227-237.
14. Wei, C.-J., R.D. Tanner and G.W. Malaney, 1982. Effect of sodium cholide on baker's yeast growing in gelatin. *Appl. Environ. Microbiol.* **43,** 757-768.
15. Woodward, J.R., V.P. Cillo and L.N. Edwards, Jr., 1978. Light effects in yeast: inhibition by visible light of growth and transport in S. cerevisiae grown at low temperature. *J. Bact.,* **133,** 692-703.

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