# S-8-5 Microbial Production of Vitamin $B_2$

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## I. INTRODUCTION

Riboflavin (Vitamin $B_2$), is a water soluble vitamin which can be produced on a commercial scale by various fermentation processes using different microorganisms. It is essential for the growth and normal health of animals as well as of man. Riboflavin is used as pure form for human nutrition and therapy and in the crude concentrated form for animal feed supplements. Although it is produced by both chemical synthesis and fermentation processes, it is believed that the microbial synthesis should be able to compete quite successfully with the synthetic chemical processes.

Riboflavin can be synthesized by many various types of microorganisms. Bacteria, yeasts, moulds, algae and protozoa are the microorganisms which can potentially produce riboflavin [1].

Three groups of microorganisms have been found to synthesize riboflavin in significant amounts: (1) bacteria of butanol-acetone group, of which representative is *Clostridium acetobutylicum*; (2) certain *Candida* yeasts; and (3) the two closely related *Ascomycetes, Eremothecium ashbyii* and *Ashbya gossypii*. Among these *E. ashbyii* and *A. gossypii* are capable of producing very large amounts of this vitamin under appropriate conditions.

Different natural sources have been used for the production of riboflavin by fermenting microorganisms.

The changes in the culture media and the selection of high riboflavin-producing mutant cultures cause the most important increases in riboflavin productivity.

## II. FERMENTATION PROCESSES

Commercial fermentation processes for riboflavin production are relatively recent, having been developed since about 1940. The *Ascomycete* processes by using *E. ashbyii* and *A. gossypii* are now assuming the most important position in the riboflavin manufacturing field.

1. Culture medium. Cultures of *E. ashbyii* and *A. gossypii* are grown on solid agar media in slant tubes and Petri dishes. The composition of the medium is as follows (g/liter): glucose, 20.0; peptone, 5.0; yeast extract, 5.0; malt extract, 5.0; $MgSO_4.7H_2O$, 0.2; $K_2HPO_4$, 0.2 [2]. The pH of the medium is adjusted to the desired value with 0.5 M $H_2SO_4$. Both liquid and solid media were sterilized at 121°C in an autoclave before inoculation.

2. Fermentation Parameters. General fermentation conditions that affect microorganism growth and productivity are pH, temperature, substrate type

and initial substrate concentrations, initial growth factor concentrations, and agitation and aeration rates.

A. Effect of initial pH. The optimum initial pH value is 6.5 for *E. ashbyii* and *A. gossypii*. Riboflavin production by these two *Ascomycetes* fits the third group of Gaden classification. In riboflavin fermentations the pH of the medium decreases with time and drops from its initial value of 6.5 to approximately 4.5. At this point the microorganisms grow rapidly, but no significant amount of riboflavin are synthesized. Then both riboflavin production and pH increase and riboflavin formation terminates at approximately pH 9.5 [2].

B. Effect of temperature. For *E. ashbyii* and *A. gossypii* maximum specific growth and riboflavin production rates are obtained at 30°C in all medium compositions. At lower temperatures both growth and riboflavin yield reduce similar to observed at higher temperatures.

C. Effect of initial substrate concentration. For the growth of both microorganisms the main carbon source is glucose. Maximum yields are obtained with an initial glucose concentration of 20.0 g/l for both microorganisms.

Glycerol, sunflower oil, whey and several combinations of these substrates can also be used for riboflavin production. Table 1 shows the effects of different substrates and their initial concentrations on the specific growth rates and riboflavin yields.

Maximum specific growth and riboflavin production rates and vitamin yields are obtained in the media containing both glucose and sunflower oil as substrate [2].

TABLE 1

Effects of various substrates on specific growth rates ($\mu$) and riboflavin yields ($Y_{P/S_{Co}}$) for *Eremothecium ashbyii* and *Ashbya gossypii* (30°C, stirring rate=100 rpm, initial pH=6.5).

| SUBSTRATES | Initial concentration (g/liter) | *E. ashbyii* | | *A. gossypii* | |
|---|---|---|---|---|---|
| | | $\mu^a$ ($h^{-1}$) | $Y_{P/S_{Co}}$ | $\mu^a$ ($h^{-1}$) | $Y_{P/S_{Co}}$ |
| Glucose (G) | 20.0 | 0.1854 | 3.6 | 0.1538 | 3.1 |
| Glycerol (Gl) | 20.0 | 0.1733 | 3.1 | 0.1315 | 1.3 |
| Sunflower oil (S) | 20.0 | 0.1669 | 3.0 | 0.2245 | 2.9 |
| G + Gl | 5.0 + 15.0 | 0.2044 | 4.5 | 0.1979 | 1.7 |
| G + Gl | 7.5 + 12.5 | 0.2006 | 3.7 | 0.2153 | 1.9 |
| G + S | 5.0 + 15.0 | 0.2438 | 9.2 | 0.2323 | 5.2 |
| G + S | 10.0 + 10.0 | 0.2358 | 6.8 | 0.2635 | 8.8 |
| Whey (W) | 12.5 | 0.1379 | 6.5 | - | - |
| W | 30.0 | - | - | 0.1529 | 2.2 |

a Calculated in the region of exponential growth (Monod kinetic model).

D. Effect of growth factors. Effects of some growth factors such as meso-inositol, D(+)-biotin and thiamine for riboflavin production by *E. ashbyii* and *A. gossypii* were investigated in various media. In order to determine initial concentration effects of these growth factors, glucose was used as substrate at the optimum growth conditions.

While, D(+)-biotin and thiamine are ineffective in stimulating either growth or flavinogenesis, maximum specific growth and riboflavin production rates and riboflavin yields are obtained in the media containing 0.2 g/l inositol for *E. ashbyii* (Table 2).

It is also observed that all of these three compounds are effective in supporting growth and product formation with *A. gossypii*. Optimum initial growth factor concentrations are found as 0.5 g/l inositol, 0.4 μg/l D(+)-biotin and 0.04 g/l thiamine for this *Ascomycete*. The maximum specific riboflavin production rate is observed with thiamine in glucose-based medium.

Addition of these growth factors to a medium containing either glucose or whey increases both the growth rate of the microorganisms and riboflavin production. In these experiments, the concentrations of substrates used are kept constant at their optimum values obtained for *E. ashbyii* and *A. gossypii*.

It is observed that the addition of these three growth factors to a medium containing glucose or whey increase the specific growth rates by 34% and 97% respectively for *A. gossypii*.

TABLE 2
Effects of various growth factors on specific growth and riboflavin production (ν) rates and riboflavin yields for *E. ashbyii* and *A. gossypii* (30°C, initial pH=6.5, stirring rate=100 rpm).

| SUBSTRATES | *E. ashbyii* | | | *A. gossypii* | | |
|---|---|---|---|---|---|---|
| | μ ($h^{-1}$) | ν.$10^3$ (g R/g mo per h) | $Y_{P/S_{Co}}$ | μ ($h^{-1}$) | ν.$10^3$ (g R/g mo per h) | $Y_{P/S_{Co}}$ |
| G + I[a] | 0.1957 | 4.00 | 3.8 | | | |
| W + I | 0.1386 | 1.78 | 7.6 | | | |
| G+I+B+T[b] | | | | 0.2068 | 3.92 | 7.5 |
| W+I+B+T | | | | 0.3015 | 0.48 | 4.0 |

Initial substrate concentrations are (g/l):
[a] G, 20.0; W, 12.5; inositol (I), 0.2.
[b] G, 20.0; W, 30.0; I, 0.5; D(+)-biotin (B), $4.10^{-7}$; thiamine (T), 0.04.

E. Fermentor experiments. The *Ascomycete* processes for riboflavin production are aerobic and it is necessary either to aerate or shake the cultures [3].

Effects of agitation and aeration rates on riboflavin fermentation by *Ashbya gossypii* were investigated in three batch, sparged, and mechanically agitated fermentors having the working volume of 0.42, 0.85, and 2.5 liters respectively. An 18-22 h grown 4% (v/v) of inoculum was used for

inoculation. The ultimate objective through this analysis is to relate the specific riboflavin production rate to the gassed power input per unit volume, the superficial air velocities and the maximum microorganism concentrations.

Stirring rate effects were investigated at the range of 150-350 rpm at constant air flow rate of 1/4 vvm at 30°C. Specific growth rates and riboflavin production rates increase by increasing the agitation rate and reach maximum values at 250-300 rpm, and then decrease at 350 rpm for all fermentor sizes. The results show that maximum microorganism concentrations and riboflavin production are low at low stirring rates, vitamin synthesis increases at 250-300 rpm and riboflavin yield decreases because of the mechanical disintegration of cells at 350 rpm for all fermentor sizes.

Effect of aeration rate on microorganism growth and riboflavin synthesis were investigated at the range of 1/6-3/8 vvm at 250 rpm constant agitation rate at 30°C. Optimum specific growth and riboflavin production rates are obtained at 1/4 vvm. Microorganism concentrations and vitamin yields decrease at low aeration rates. The results showed that the aeration rate is also an important kinetic parameter for riboflavin production. At high aeration rates, an inhibitory effect of air is observed for riboflavin fermentation.

Assuming that, the agitation and aeration rates are important parameters in riboflavin production by *A. gossypii*, both these factors have, therefore, been included when correlating the experimental results. Mechanical power inputs per liquid volume in aerated and stirred fermentors were calculated [4]. The specific production rates obtained in this study were correlated to gassed power inputs per unit volume ($P_g/V$), the superficial air velocities ($v_s$), and maximum microorganism concentrations ($X_m$) in the form

$$\nu = (3.5*10^{-5})(P_g/V)^{-0.131}(v_s)^{-2.4*10^3}(X_m)^{1.001} \quad (1)$$

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