PHOTOTROPHIC BREAKDOWN OF SOLID CELLULOSE FROM SILK COTTON TO HYDROGEN AND VOLATILE FATTY ACIDS BY *RHODOSPIRILLUM RUBRUM*

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ABSTRACT

Silk cotton, whose major constituent is cellulose, was utilized by *Rhodospirillum rubrum* as substrate for growth and hydrogen production. Some volatile fatty acids were detected as metabolic products.

INTRODUCTION

RHODOSPIRILLUM RUBRUM utilizes many organic compounds and produces hydrogen[1]. Recently, it was reported that *R. rubrum* could increase methane production from cattledung when introduced with heterotrophic bacteria[2,3], perhaps by breaking cellulose down to products easily taken up by methanogens. To test this hypothesis, silk cotton was used as substrate for growth of *R. rubrum*. Silk cotton contains 60–64% cellulose[4].

MATERIALS AND METHODS

Cultures of *R. rubrum* ATCC 11170 were maintained on a medium[5], with 0.1% DL-malic acid and 0.03% sodium L-glutamate as carbon and nitrogen sources respectively. For the present study, select populations of *R. rubrum*, which were found to grow on microcrystalline cellulose powder (MCCP), were repeatedly subcultured till an almost pure strain was isolated. This was subsequently maintained on the same medium with 0.1% MCCP as substrate.

Fresh silk cotton obtained from the buds of the *Ceiba pentandra* tree was cleaned and the seeds were removed. The cellulose content in silk cotton was determined and found to be 70 to 72% by weight. Cylindrical columns of 5 cm inner diameter, 40 cm long, fitted with glass stoppers having provisions for argon sparging and gas sampling at the top and liquid sampling from the bottom (figure 1) were used as culture vessels. The silk cotton (20 g) was stuffed in pieces of extruded polythene netting and one litre of basal medium[5] was added. The inoculum (1%) was a 10-day-old culture (O.D. 0.7). Incubation was at 28–34°C in diffuse sunlight as light source (4000–5000 lux), and under anaerobic conditions. Growth was measured at 660 nm with a Bausch and Lomb "Spectronic 20" spectrophotometer. Samples were centrifuged to remove cells and debris and the supernatant was analysed for reducing sugars colorimetrically[6,7] and by TLC[8]. The cellulose content at the end was also determined[6].

For analysis of volatile fatty acids (VFAs), the filtrate was acidified with 2 N orthophosphoric acid and analysed on a Chemito 3800 gas chromatograph (10% PEGA on Chromosorb W (HP) in an SS column (2 m); thermal conductivity analysis; hydrogen carrier (60 ml/min); temperatures: oven and injector 120°C, block 150°C; sensitivity 194 mA). Standard VFAs were found to have the following retention times (sec): acetic, 138; propionic, 210; butyric, 260; valeric, 310. Gas from the headspace was analysed using the same instrument (TCD: Porapak Q column; argon carrier (30 ml/min); temperatures: oven and injector 50°C, block 150°C; sensitivity 118 mA). The experiments were run for 45 days with duplicates and repeated 6 times. Experiments were also carried out with ammonium sulphate or ammonium chloride as nitrogen source instead of glutamate.

RESULTS AND DISCUSSION

R. rubrum was entrapped in the matrix of the silk cotton which was also used as substrate (figure 1). There was a change in the texture of silk cotton with shortening of the fibre length and reduction of tensile strength perhaps due to the breakage of cross-linking bonds; 50% of the cellulose in the silk cotton had been utilised. Figure 2 shows growth curves of *R. rubrum* grown on the silk cotton, with sodium glutamate, or ammonium sulphate or ammonium chloride as nitrogen source. The cell yield increased

Figure 1. Reactors for growth of *R. rubrum* on silk cotton: left, 14-day-old culture; right, culture on the day of inoculation.

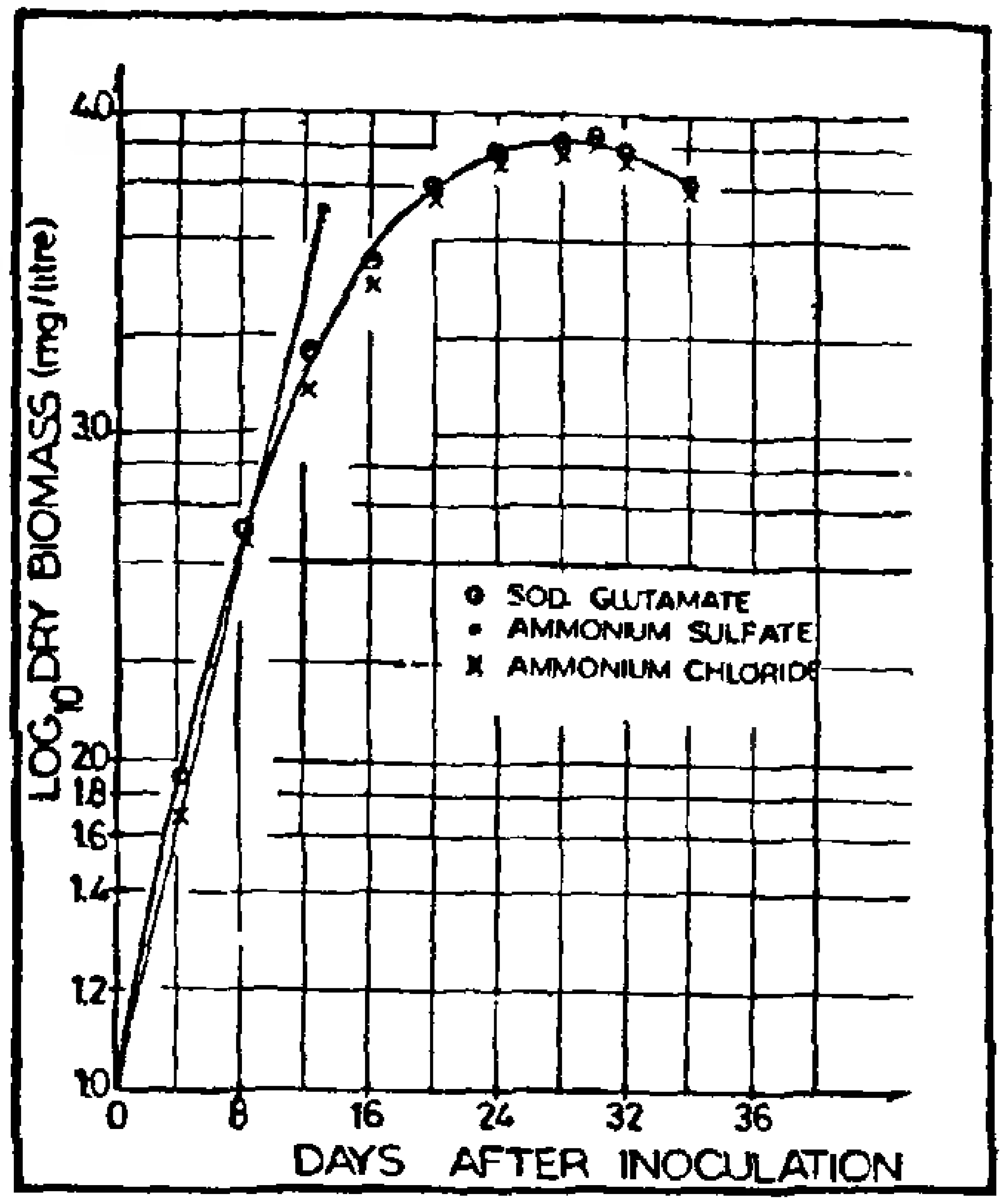

Figure 2. Growth curves of *R. rubrum* on silk cotton with different nitrogen sources.

from 10 mg dry weight/l on day zero to 6400 mg dry weight/l on day 24 to 7200 mg dry weight/l on day 30, after which the growth declined. The exponential phase lasted till the day 8, as indicated by the straight line in figure 2. Identical growth patterns observed with organic and inorganic nitrogen sources with silk cotton as the substrate show that the cellulose was indeed used as carbon source for growth.

It is interesting to note that there was no formation of reducing sugars during this conversion. All the mesophilic cellulolytic organisms tested so far have been shown to produce reducing sugars like cellobiose, glucose, etc. when grown on cellulose[9]. This report is the first one of a phototrophic bacterium degrading solid cellulose under anaerobic conditions in light. Moreover, one of the characteristics of *R. rubrum* is its inability to utilize glucose, unlike many rumen bacteria[10], and the only sugar on which it grows is fructose[11]. These characteristics indicate that *R. rubrum* is perhaps following a metabolic pathway different from the well-studied pathway of cellulolytic organisms[12].

During growth some VFAs were produced, and were detected in the filtrate. From the 1st till the 15th day acetic and propionic acids were produced at a level of 50 ppm. After the 15th day, only butyric acid (25 ppm) could be detected and the other two acids had completely disappeared. Hydrogen was detected in the headspace from the 1st day till the 40th day only with sodium glutamate as N source. Carbondioxide was present in the headspace in all the reactors. Though the photoproduction of these two gases by *R. rubrum* has been reported and reviewed[13] the fate of the remaining carbon and the different pathways of carbon substrate utilization have not been well studied. Our results seem to be the first report of some of the acidic metabolic products. Studies are continuing to elucidate the pathway(s) involved in the utilization of silk cotton.

CONCLUSION

1. *R. rubrum* breaks cellulose down to H_2 and Co_2.

2. Cellulose is broken down to products other than reducing sugars.
3. Volatile fatty acids are formed.

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NEWS

MODELS OF YOUNG GALAXIES

... "There is no reason why everything in the universe should shine. We should therefore not really be surprised that the dominant component of our galaxy (and other galaxies) seems to be 'dark' ... What a young galaxy would look like depends very much on what the dark matter in present-day galaxies actually is. For example, if black hole remnants of a bright and short-lived stellar population that formed early in galactic history constitute the dark matter, then young galaxies would be exceedingly luminous. If, on the other hand, weakly interacting photinos (or other such particles) are the gravitationally dominant constituent of our universe, then these particles would aggregate into galactic-scale self-gravitating swarms, into which the ordinary gas gradually sedimented before condensing into stars. According to the so-called 'cold dark matter' cosmogony, galaxies and clusters are dynamically dominated by weakly interacting particles left over from the early universe, and evolved from scale-independent initial fluctuations. The dynamical aspects of this model have been explored in great detail in recent years, with encouraging results; the next, much harder, step is to incorporate realistic gas dynamics dissipative effects, and star formation."

[Martin J. Rees (U. Cambridge, UK) in "Grappling With Galaxies: Basic Questions Persist," *THE SCIENTIST* 2(16): 15-7, 5 Sep 88, (Reproduced with permission from Press Digest, Current Contents®, No. 2, January 9, 1989, p. 9, Published by the Institute for Scientific Information, Philadelphia, USA.)]