# SINGLE CELL PROTEIN FROM RICE STRAW

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**Abstract**

*Studies were carried out to standardise a medium for the production of single cell protein from alkalitreated straw (ATS) by a strain of Penicillium chrysargenum. Ammonium dihydrogen phosphate and a combination of urea and ammonium sulphate were found to be the best sources of nitrogen. The optimum were 500, 200, 200, 30, and 5 mg per litre of medium. Addition of trace elements Fe, Mn, Co, Zn and Cu did not show any significant increase in protein yield. Substrate concentration of 1 to 1.5% (W/V) was found to be most suitable for maximal production of protein. No significant effect was observed when tap water was used in the place of distilled water for media preparation. The efficiency of the optimised medium was compared with 3 other standard media used to grow cellulolytic organisms. The production of SCP was scaled upto 40 litre fermentor level. The product obtained from the fermentar after 72 hr growth contained 34% crude protein whereas that from the shake flasks contained 38-42% protein. 93-96% of the 74-76% cellulose originally present in the substrate (ATS) was utilized by the organism. The conversion of ATS to crude protein was about 19%.*

## Introduction

Rice straw is a major agricultural by-product in the tropics and is used mainly as a cattle feed besides many other minor uses. The digestibility of straw in ruminants, however, is only 40% due to its high ligni and silicate contents and its deficiency in many essential nutrients[6]. To feed to ruminant animals it is to be delignified and supplemented with urea. Conversion of it by fermentation to single cell protein (SCP) widens the scope of its utilization.

Fermentation of cellulosic materials for the production of SCP using bacteria, actinomycetes, yeasts, as well as fungi has been reported by a number of workers 1, 2, 4, 5, 7, 8. Filamentous fungi have been shown to provide an acceptable source of edible protein 9, 11. Use of Penicillium mycelium as a protein supplement in animals has been reported[3].

The present investigation involves studies to develop a simple, low cost, submerged fermentation process for the conversion of rice straw to SCP by using a strain of *Penicillium chrysogenum*. Scale up of fermentation up to 40-litre level has been carried out.

## Materials and Methods

*Penicillium chrysogenum* St-F-3B used in this study was obtained from National

Collection of Industrial Microorganisms, Poona, India and was maintained on a modified potato-dextrose-agar medium containing 0.01% yeast extract and 0.5% dextrose (instead of 2%) at 27°C and stored at 4°C. Monthly subcultures were made.

*Inoculum*: A 48 hr old culture grown on alkali-treated straw (ATS)—mineral medium was used as inoculum. This was prepared by inoculating the medium (ATS, 1%) with spores from a PDA slant and incubating on a rotary shaker (230 rpm) at room temperature (23-28°C).

*Substrate:* For the purpose of improving the enzyme digestibility and for delignifying, the rice straw was washed and pretreated with 4% sodium hydroxide (1: 50 W/V) and steamed for 60 min. The straw was then washed free of alkali, dried at 55°C and ground. The substrate treated as above was used at 1% (dry weight) level in shake flask experiments. In the trials with 40-litre fermentor, the substrate was added in wet condition itself, after washing it free of alkali, at 10% level. This corresponded to approximately 2% dry weight of the substrate in the medium.

*Mineral medium:* The basal medium used for all the studies was of the following composition:

| *Chemicals* | *g/l* | *Chemicals* | *mg/l* |
|---|---|---|---|
| $KH_2PO_4$ | 0.904 | $FeSO_4.7H_2O$ | 4.90 |
| NaCL | 0.127 | $MnSO_4.H_2O$ | 1.86 |
| $CaCl_2.2H_2O$ | 0.110 | $CoCl_2.6H_2O$ | 2.01 |
| $MgSO_4.7H_2O$ | 0.101 | $ZnSO_4.7H_2O$ | 1.68 |
| | | $CuSO_4.5H_2O$ | 0.20 |

For selecting a suitable source of nitrogen the various nitrogenous compounds tested were $NaNO_3$, $Ca(NO_3)_2$, $NH_4NO_3$, $NH_2Cl$, $(NH_4)_2HPO_4$, $NH_4H_2PO_4$, $(NH_4)_3PO_4.3H_2O$, $(NH_4)_2SO4$, Urea. and a combination of urea and $(NH_4)_2SO_4$. In all cases, the nitrogen concentration was kept constant, the amount of N present in 0.2% $(NH_4)_2SO_4$ (i.e. 423 mg N per litre). In the case of the combination of urea and $(NH_4)_2SO_4$ they were incorporated in such a way so as to give equal amounts af N.

In the case of optimising the concentration of various elements such as P, Na, Ca and Mg, the nitrogen concentration in the medium was kept at 600 mg of N per litre contributed by the combined addition of urea and $(NH_4)_2SO_4$.

In the media optimization studies glass distilled water was used for media preparation, whereas in the fermentor trials tap water was used eliminating the addition of trace elements. Initial pH of the medium in all the studies was adjusted to 5.0.

For comparsion of growth of the organism on different mineral media (with ATS as substrate), standard media like VRS-medium[12], Reese's medium[10] and NCL-medium* (personal communication) were used.

*Cultivation and harvesting*: In all the cases 200 ml medium and 1 g of the substrate (ATS) were taken in 500 ml Erlenmeyer flasks. Fo optimization of substrate concentration the substrate was added at levels ranging from 1 to 10 g/100 ml medium. The mineral concentration also was increased correspondingly. The flasks were plugged with cotton wool and autoclaved at 15 psi for 20 min (In the case of each variable flasks were taken in triplicates and the average values were taken). After cooling the flasks were inoculated

*Composition of NCL Medium

| *Chemical* | *g/l* | *Chemical* | *mg/l* |
|---|---|---|---|
| $(NH_4SO_4$ | 5.0 | $FeSO_4.7H_2O$ | 5.00 |
| $KH_2PO_4$ | 1.0 | $MnSO_4.H_2O$ | 1.56 |
| $MgSO_4.7H_2O$ | 0.5 | $ZnSO_4.7H_2O$ | 1.40 |
| $CaCl_2.2H_2O$ | 0.1 | $CoCl_2.6H_2O$ | 2.00 |
| $CaCO_3$ | 1.0 | | |

with the seed culture (5% V/V) and were incubated on a rotary shaker (230 rpm) at ambient temperature (24-28°C).

After cultivating for 72 hr the contents of the flasks were harvested by filtering and washing through a previously dried and weighed filter paper (Whatman No. 1). Then the product (biomass+residue, if any) was dried at 55°C, weighed and powdered. The powdered sample was used for various analyses. pH of the culture filtrate was determined using a Toshniwal pH meter.

*Cultivation in 40-litre fermentor*: 40-litre medium was sterilized at 121°C for 20 min and cooled. Inoculated with 48 hr old seed culture at 5% (V/V) level.

Medium was aerated with sterilized air at the rate of 1 litre of air per litre of medium per minute (1 VVm) and was agitated at 500 rpm. Temperature was maintained at 25±1°C. Foaming was controlled by the addition of diluted and sterilised Silicone 21 Defoamer (Metromark Private Ltd., Calcutta) as and when required.

The 72 hr old mycelia in the fermented broth was harvested by centrifuging using a basket type centrifuge. This was dried at 55°C, weighed and powdered.

*Sample analyses*: Estimation of crude protein was done by determining the total nitrogen by micro-Kjeldahl method and multiplying it by the factor 6.25.

Non-protein nitrogen was estimated by the same method after removing the protein by trichloroacetic acid precipitation.

Cellulose content was determined by Updegraff's method.[13]

Total fat content was estimated by standard AOAC method.

## Results and Discussoin

Among ten different compounds tested as nitrogen source, $NH\ H_2PO_4$ and a combination of urea and $(NH_4)_2SO_4$ were found to yield high percentage of protein in the final product. However, the total protein produced was higher in the latter case (Table 1). Utilization of nitrates were found to be very poor and it could be further

TABLE 1. *Effect of different nitrogen sources on the production of protein*

| *Nitrogen source* | *Final pH* | *Total yield of the product g/l g substrate* | *Protein yield* | |
|---|---|---|---|---|
| | | | *Percentage* | *Total mg/l g of substrate* |
| Sodium nitrate | 7.20 | 0.820 | 4.19 | 34.37 |
| Calcium nitrate | 7.15 | 0.782 | 7.04 | 55.05 |
| Ammonium nitrate | 2.05 | 0.593 | 11.33 | 67.19 |
| Ammonium chloride | 1.95 | 0.692 | 12.56 | 86.92 |
| Urea | 7.00 | 0.562 | 16.61 | 93.35 |
| Ammonium phosphate dibasic | 2.10 | 0.557 | 17.81 | 99.20 |
| Ammonium sulphate | 2.10 | 0.457 | 19.87 | 90.81 |
| Ammonium phosphate | 2.15 | 0.505 | 22.00 | 111.10 |
| Urea + Ammonium sulphate | 3.50 | 0.496 | 31.56 | 156.53 |
| Ammonium dihydrogen phosphate | 2.40 | 0.432 | 34.48 | 148.95 |

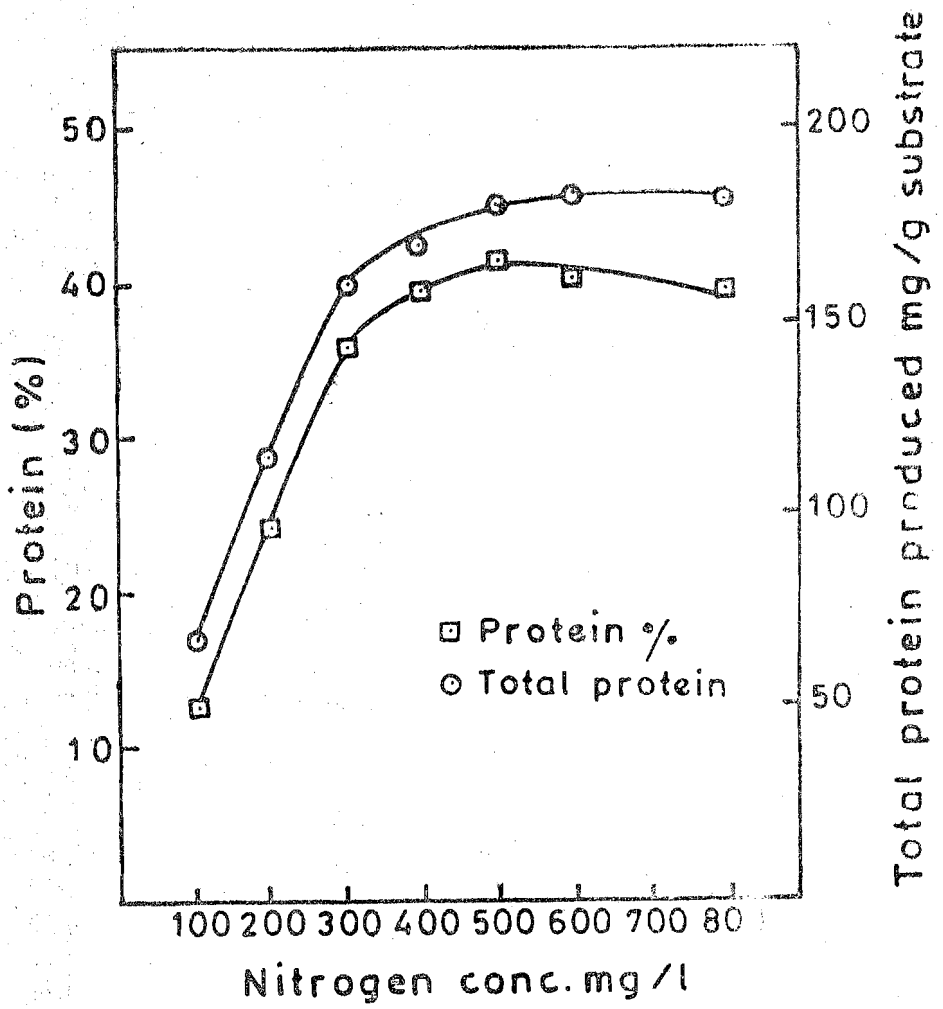


Fig. 1. Effect of Nitrogen concentration on the production of protein.

observed (Table 1) that where the pH in the final medium was either very high or very low the growth of the culture was poor. So a pH somewhere between 3 and 6 should be maintained in the medium for better growth. This can be attained by adding a combination of urea and $(NH_4)_2SO_4$ as nitrogen source.

A steady increase in the protein level in the final product was observed as the concentration of N was increased from 100 through 500 mg per litre in the medium. Further addition of N did not show any marked effect (Fig. 1).

Concentration of phosphorus which was supplied in the form of $KH_2PO_4$ was varied from 50 to 400 mg per litre and 200 mg P/l was found to be sufficient to give maximum yield of protein (Fig. 2).

Addition of Na in the form of NaCl did not have a significant effect. But addition of 200 mg Na per litre showed a slight

TABLE 2. *Protein yield at different concentrations of sodium in the medium*

| Concentration of sodium mg/litre | Total yield of product g/l g substrate | Protein yield | |
|---|---|---|---|
| | | Percentage | Total mg/g substrate |
| 0 | 0.471 | 34.12 | 158.32 |
| 25 | 0.457 | 34.68 | 160.40 |
| 50 | 0.449 | 36.19 | 162.49 |
| 100 | 0.443 | 37.85 | 167.66 |
| 200 | 0.432 | 38.88 | 167.96 |
| 400 | 0.425 | 38.40 | 163.20 |

TABLE 3. *Protein yield at different concentrations of calcium in the medium*

| Concentration of calcium mg/litre | Total yield of product g/l g substrate | Protein yield | |
|---|---|---|---|
| | | Percentage | Total mg/l g of substrate |
| 0 | 0.474 | 32.48 | 154.04 |
| 15 | 0.427 | 34.52 | 147.40 |
| 30 | 0.414 | 39.51 | 163.57 |
| 45 | 0.442 | 36.74 | 162.28 |
| 60 | 0.435 | 36.48 | 158.69 |

TABLE 4. *Protein yield at different concentrations of magnesium in the medium*

| Concentration of magnesium mg/litre | Product yield g/l g substrate | Protein yield | |
|---|---|---|---|
| | | Percentage | Total mg/l g substrate |
| 0 | 0.450 | 31.20 | 140.40 |
| 5 | 0.445 | 39.15 | 174.22 |
| 10 | 0.452 | 37.98 | 171.67 |
| 15 | 0.443 | 39.27 | 175.07 |
| 30 | 0.455 | 39.14 | 178.09 |

increase in the protein percentage from 34 to 38 (Table 2).

Fortification of the medium with 30 mg Ca per litre was found to be optimum.

TABLE 5. *Effect of trace elements on the protein production when tap water and glass distilled water were used for media preparation*

| Water used | Trace elements | Total yield of product g/l g substrate | Protein yield | |
|---|---|---|---|---|
| | | | Percentage | Total mg/l g substrate |
| Glass distilled water | Added | 0.450 | 38.57 | 173.57 |
| | Not added | 0.423 | 37.87 | 186.70 |
| Tap water | Added | 0.499 | 35.57 | 158.29 |
| | Not added | 0.450 | 36.75 | 165.38 |

TABLE 6. *Effect of different media on the production of protein*

| Medium | Product yield g/l g substrate | Protein yield | |
|---|---|---|---|
| | | Percentage | Total mg/l g substrate |
| V R S [12] | 0.652 | 15.18 | 98.97 |
| N C L | 0.518 | 30.68 | 158.92 |
| Reese's [10] | 0.494 | 31.43 | 155.26 |
| Formulated in this laboratory | 0.440 | 41.40 | 182.16 |

Further increase in Ca tended to slightly lower the protein percentage (Table 3).

Incorporation of Mg in the medium at 5 mg per litre level gave rise to maximum yield of protein (Table 4).

Even though our substrate, the alkali-treated straw which is not a pure cellulosic material, may have various elements in itself fortification of the medium with the above mentioned elements was required for a better growth of the organism.

However, the addition of trace elements such as Fe, Mn, Co, Zn and Cu did not show any significant effect of enchancing

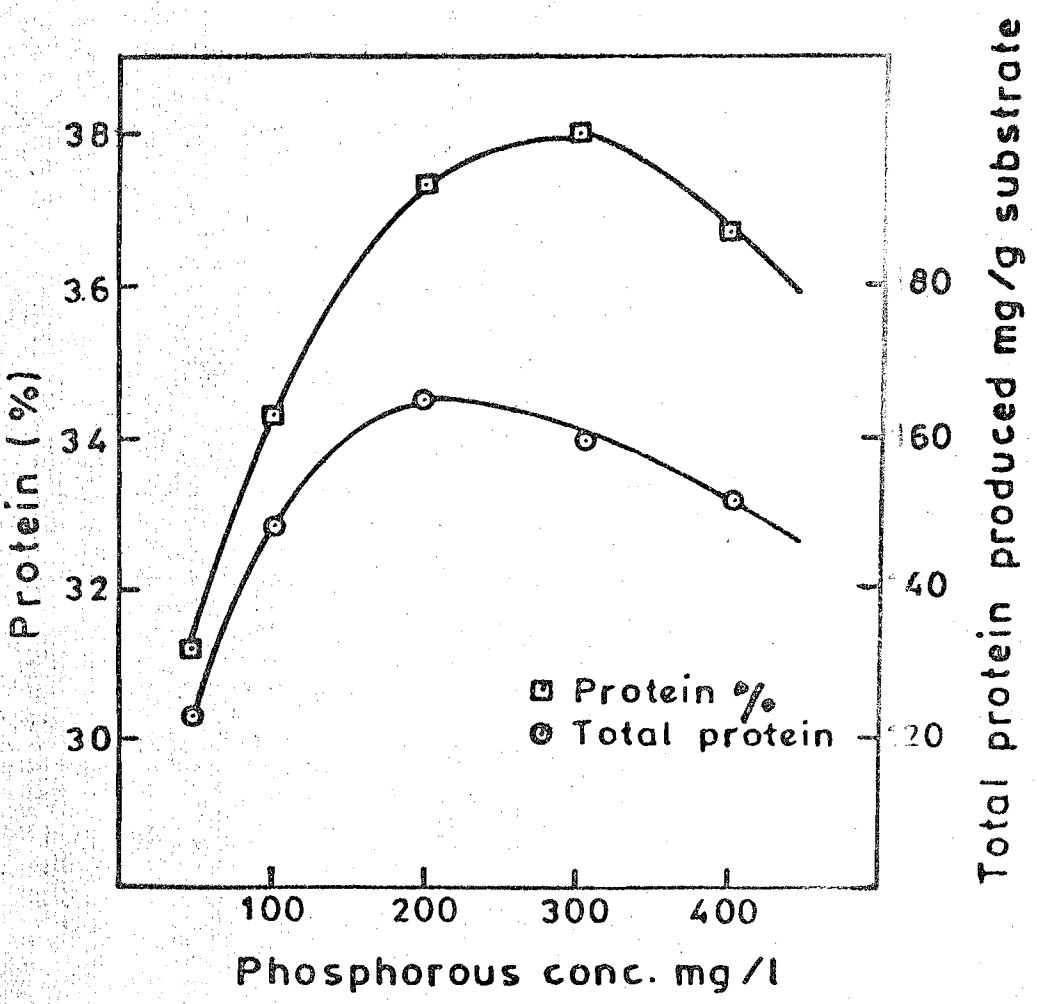


Fig. 2. Effect of phosphorus concentration on the production of protei

the protein yield. Use of tap water for media preparation showed only a slight effect (Table 5).

Effective utilization of substrate was found only when the substrate concentration was in the range of 1-3% (W/V) and when it was above 5% the growth was found to be negligible (Fig. 3).

Depending on the above observations a media having the following composition was formulated which supported the best growth of the organism wh substrate (ATS) was added at 1% level.

| *Chemical* | *g/l* |
|---|---|
| $(NH_4)_2SO_2$ | 1.180 |
| $KH_2PO_4$ | 0.904 |
| Urea | 0.536 |
| NaCL | 0.508 |
| $CaCl_2.2H_2O$ | 0.110 |
| $MgSO_4.7H_2O$ | 0.050 |

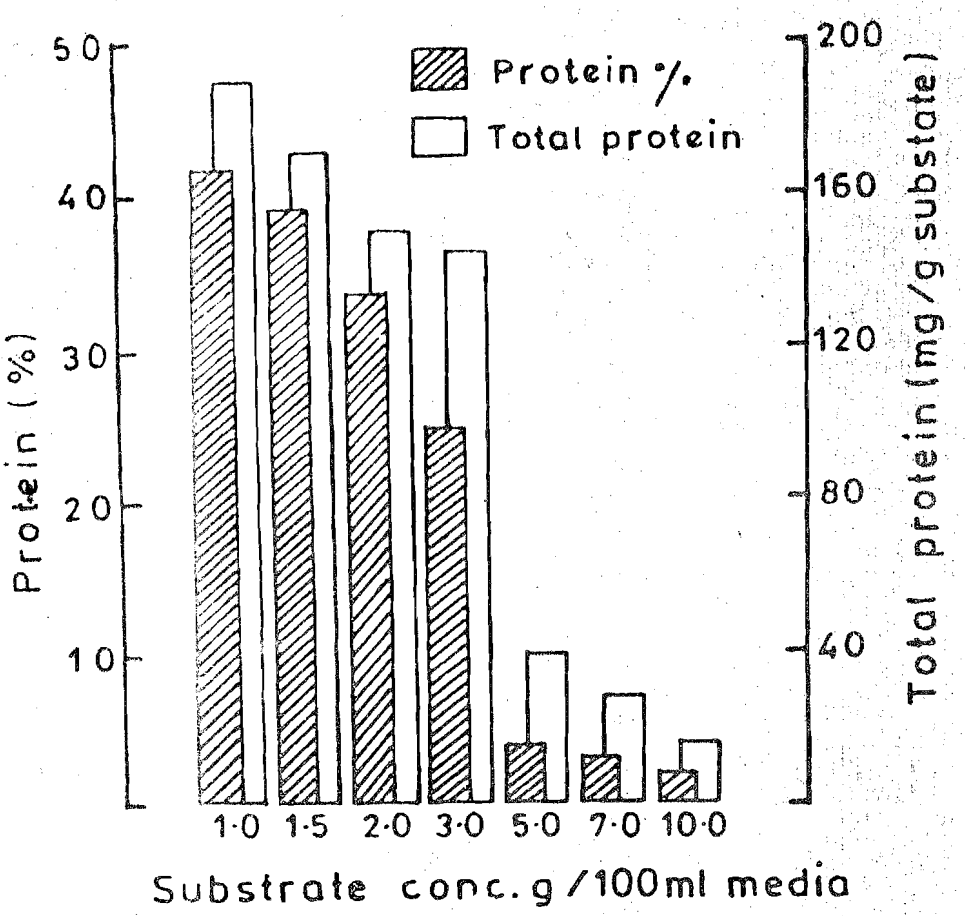


Fig. 3. Effect of Substrate (ATS) concentration on the production of protein

The protein yield was very much better in the above medium as compared to that in the VRS, Reese's, or NCL mineral medium, under the same cultural conditions tested in this work (Table 6). Typical fermentations in the formulated medium yielded a product having 38 to 42% crude protein only 5 to 6% of which was contributed by non-protein nitrogen. The total product yield ranged from 430 to 460 mg per gram of the substrate (ATS) (Tables 2,3,4, and 5). The total cellulose content of the substrate (ATS) was estimated to be 74 to 76%. From 93 to 96% of this cellulose was utilized by the organism 18 to 19% of the total substrate that disappeared was recovered as mycelial protein (Tables 2,3,4 and 5). In addition to protein the final product was found to contain 4.5 to 5.0% of fat.

The protein yield from the 40-litre fermentor was quite comparable to the yield from the shake flasks. The total yield of the final product was 562 per 1 g kg of ATS. The product was found to contain 34% protein. Thus, the total conversion of ATS to protein was about 19%.

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