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LABORATORY-SCALE PRODUCTION OF CELLULASE, GLUCOAMYLASE AND ALPHA-AMYLASE

Ernesto J. del Rosario

Institute of Chemistry and National Institutes of Biotechnology and Applied Microbiology
University of the Philippines at Los Baños, College, Laguna, Philippines

ABSTRACT

The laboratory-scale production of the enzymes cellulose, glucoamylase and alpha-amylase was optimized in terms of substrate composition and concentration, inoculum size, pH and other parameters.

A local isolate of *Penicillium* was found to produce a higher cellulase activity when grown on nutrient-supplemented sugarcane bagasse compared to *Trichoderma reesei* strains QM 9414 and NRRL 11485. Batch culture of the *Penicillium* in an airlift fermenter at pH 4.5 for two days resulted in maximum activities on filter paper and carboxymethylcellulose.

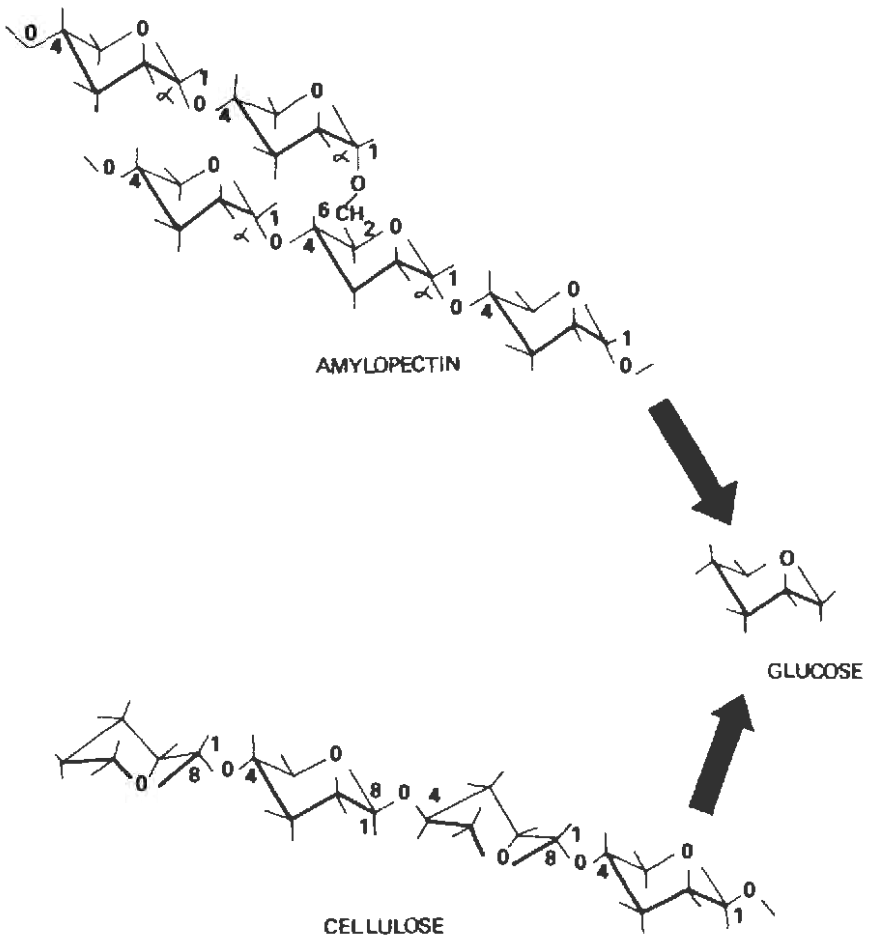
Aspergillus awamori NRRL 3112 produced the highest glucoamylase activity after four days of batch growth in an airlift fermenter using as substrate a mixture of cassava root flour and rice bran (1:2 weight ratio). The optimal conditions were pH 5.5, 30°C, 20% solids level of substrate and 10% inoculum size.

Alpha-amylase was produced by *Bacillus subtilis* NRRL 3411 in an airlift fermenter using as substrate a mixture of cassava root flour, rice bran, fish meal and soybean meal. After three days of fermentation the enzyme was harvested, concentrated and stabilized. Alternatively a solid enzyme powder was prepared.

Introduction

Plant biomass, which is produced by photosynthesis, is the world's most important renewable material and energy resource. It could serve as partial substitute for fossilized biomass such as petroleum, coal and natural gas whose reserves are continually being depleted. Being dependent on land area, plant biomass production is limited and its utilization for food or fuel is governed by socio-economic and geo-political factors.

The important constituents of plant biomass are carbohydrates and lignin. Included in the former are cellulose, hemicellulose, starch and sugars. Cellulose and starch are polymers of glucose, while hemicellulose includes pentosans or polymers of pentoses (fine-carbon sugars) and pentose derivatives as well as hexans or polymers of hexose (six-carbon sugars) other than glucose. On the other hand, lignin is a complex material of high molecular weight consisting of phenylpropane units linked together by a variety of bond types. Cellulose, which is usually tightly complexed with lignin and hemicellulose, is the most abundant organic substance in the world. It makes up approximately 50% of the cell wall material of wood and



Scheme 1. Chemical structures and complete hydrolytic reactions of amylopectin and cellulose.

plants and between 25 to 50% (dry basis) of wood, sugarcane bagasse, rice straw, corn cobs and other lignocellulosic materials. Starch is the primary carbohydrate in cereal grains and staple crops such as cassava and sweet potato. Approximately 25% of fresh cassava and sweet potato is starch.

The chemical structure of cellulose and starch and the reaction scheme for their complete hydrolysis into glucose, through the action of acids or enzymes, are shown in Scheme 1. Starch consists of two types of polymers, namely amylose and amylopectin. The former is a linear polysaccharide linked by alpha-1,4 bonds while the latter, which is shown in Scheme 1, is a branched polymer containing both alpha-1,4 and alpha-1,6 glucosidic bonds. On the other hand, cellulose is a

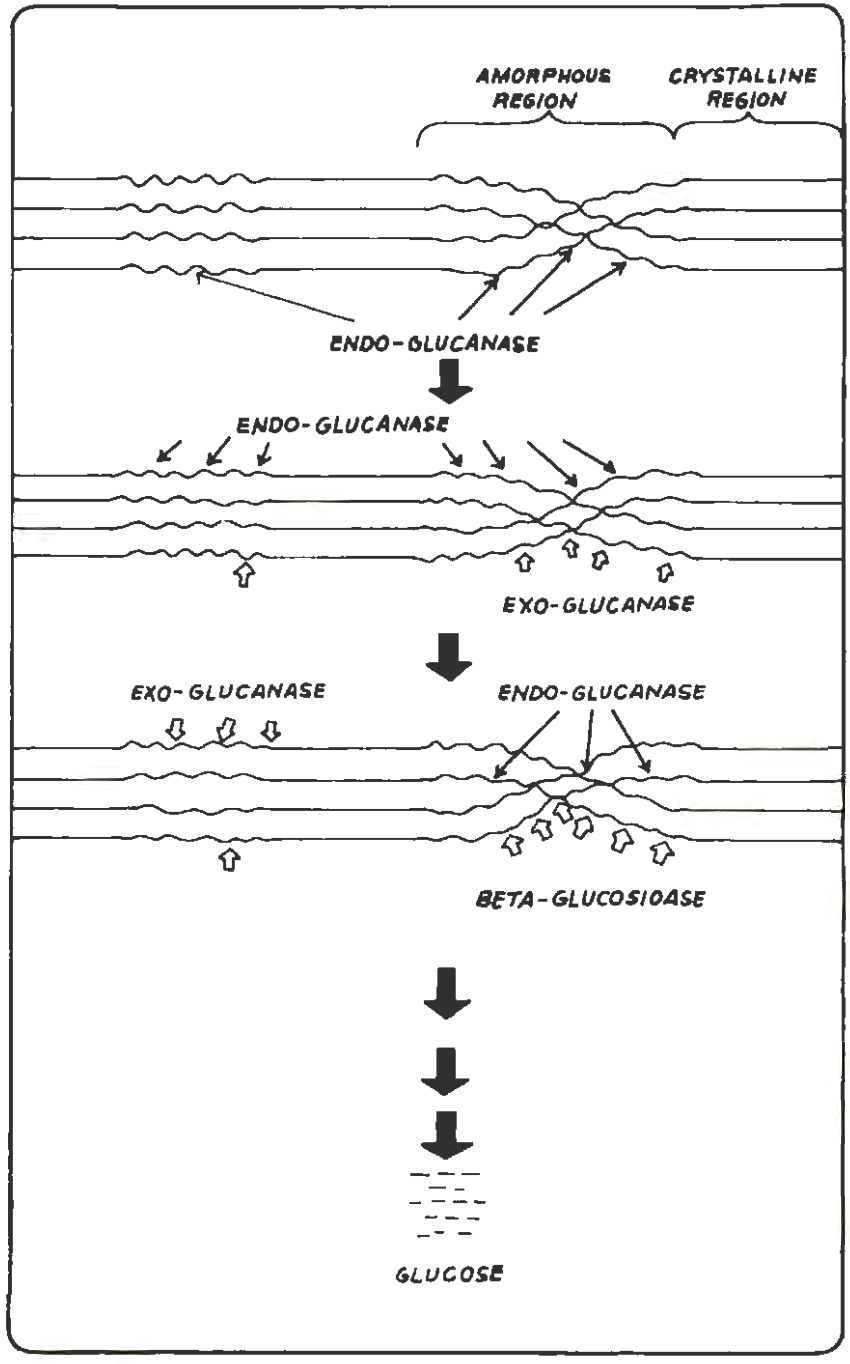


Figure 1. Enzymatic hydrolysis of cellulase (schematic).

linear polysaccharide linked by beta-1,4 glucosidic bonds (Roberts and Caserio, 1964).

The enzymatic hydrolysis of cellulose, as schematically shown in Figure 1, is generally believed to occur through the action of at least three components of the cellulase enzyme complex, namely exo beta-1,4-glucanase (or 1,4β-glucan cello-biohydrolase), endo beta-1,4-glucanase (or 1,4-β-glucan glucano-hydrolase) and β-glucosidase. The endo-glucanase attacks the amorphous region of the cellulose fibrils by splitting internal glucosidic bonds thereby increasing the number of non-reducing ends. In turn, the exo-glucarase hydrolyzes a penultimate glucosidic bond of the exposed non-reducing ends and releases cellobiose which is further degraded to glucose by β-glucosidase (Fägerstam *et al.*, 1977). Detailed studies on the mechanism of action of the cellulase enzymes have been described in the proceedings of several symposia (Hajny and Reese, 1969; Wilke, 1975; Dailey *et al.*, 1975; Gaden *et al.*, 1976; Ghose, 1977; Ghose, 1981).

The enzymatic action of amylases on starch is schematically shown in Figure 2. After gelatinization or swelling in water, starch is dextrinized or converted into smaller fragments through the action of alpha-amylase and beta-amylase. The latter is an exo-glucanase and splits off maltose from the non-reducing end of starch, while the former randomly hydrolyzes an internal alpha- 1,4 glucosidic bond. The resulting dextrins are hydrolyzed by glucoamylase into glucose.

Cellulase Production

Previous work done by the author on cellulase production dealt with semisolid cultures of several strains of the cellulolytic mold *Trichoderma viride* or *reesei*) using, as carbon substrate, mixtures of rice bran with either rice straw or rice hulls (Vilela *et al.*, 1977). Although reasonably high enzymatic activities were obtained in the culture filtrates, at least seven days of culturing were needed and the semisolid technique for enzyme production was found difficult to scale-up in later studies.

Our more recent work has concentrated on cellulase production in a locally-fabricated laboratory-scale fermenter using as substrate sugarcane bagasse or rice straw, which was supplemented with other nutrients (Bondoc, Bondoc, 1982; E.J. del Rosario and D.M. Marquez, unpublished data). An airlift fermenter, which is shown in Figure 3, was used in the fermentation studies. Aeration and agitation of the culture medium in the fermenter was simultaneously brought about by filtered air through an 'airlift effect'. The latter refers to the upward flow of the air-entrapped liquid inside the inner tube of the fermenter followed by the downward liquid flow in the outer annular region after air release such that a continuous loop motion of the liquid is obtained.

Using as substrate a mixture of NaOH-treated sugarcane bagasse and rice bran (1: 1 w/w) at 5% solids level and initial pH of 5, two strains of *Trichoderma reesei* and a local isolate of *Penicillium* (kindly given by Dr. Ireneo Dogma, Jr.) were compared in terms of cellulase production. Although slightly different initial spore

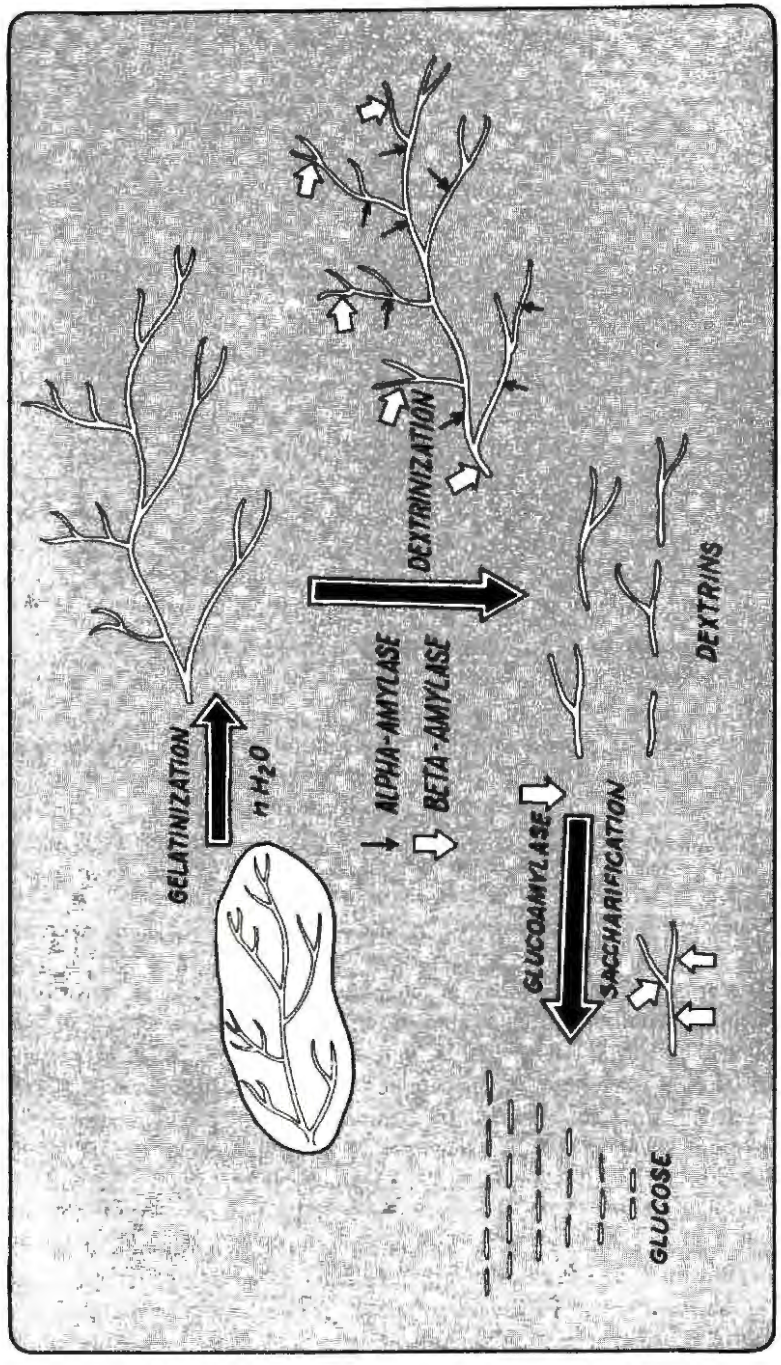


Figure 2. Enzymatic Starch Saccharification (Schematic).

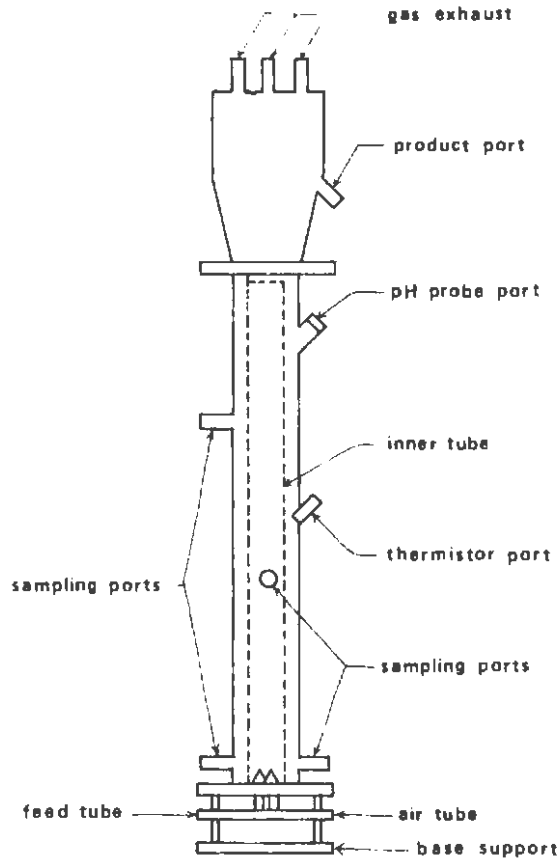


Figure 3. The airlift fermenter

concentration of the three microorganisms were used, much higher carboxymethylcellulose (CMC) volumetric activities (abbreviated as CMCVA) were obtained with *T. reesei* NRRL 11485 as shown in Figure 4. The effects of substrate composition on cellulase production by this microbial strain using either alkali-treated or untreated bagasse are summarized in Table 1. It is seen in the table that untreated bagasse is a better substrate than alkali-treated bagasse for cellulase production in terms of filter paper (FP) and CMC enzymatic activities in the fermentation medium. This result could be explained by the property of untreated bagasse to induce cellulase production by *T. reesei;* apparently, the highly resistant nature of the substrate forces the microorganism to secrete a more active enzyme for cellulose breakdown. In the case of alkali-treated bagasse, the partially delignified material is less efficient in inducing cellulase production by the microorganism.

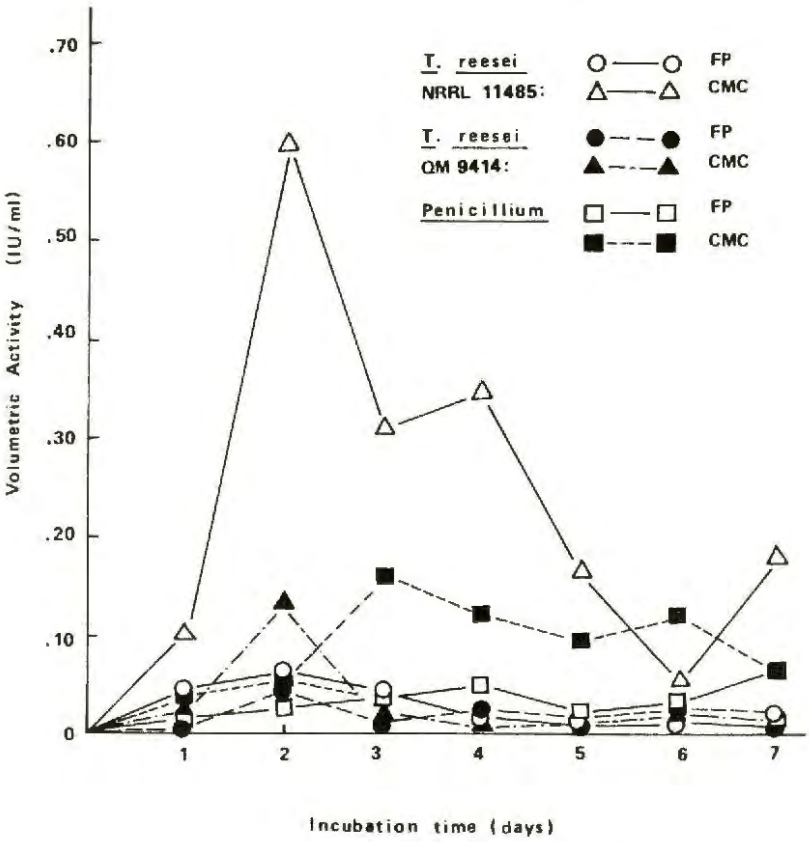


Figure 4. Volumetric activity on filter paper (FP) and carboxymethylcellulose (CMC) of culture filtrates of *T. reesei* NRRL 11485, *T. reesei* QM 9414 and *Penicillium* sp. 86.

Using untreated bagasse as carbon substrate, the production of cellulase by *T. reesei* QM 9414 was optimized in terms of substrate composition. The experimental results, which are presented in Table 2, show that the highest FP activities were obtained for a medium containing equal amounts of bagasse and rice bran while the lowest activities were observed using bagasse only. Corresponding results for the *Penicillium* isolate (Code No. 86) are given in Table 3 and show that the highest FP and CMC activities were obtained for medium E which contained untreated bagasse as carbon substrate to which were added $(NH_4)SO_4$, KH_2PO_4, urea, $Cacl_2$ and $MgSO_4$.

A summary of the FP activities and protein content of the culture medium for the three fungal strains tested is given in Table 4. It is apparent that the *Penicil-*

Table 1. Effect of substrate composition on filter paper (FP) and carboxymethylcellulose (CMC) volumetric activities of culture filtrates of *T. reesei* NRRL 11485 grown on untreated or alkali-treated bagasse*.

*Substrate Composition***	*Protein Concentration %*	*FP Volumetric Activity IU/ml*	*CMC Volumetric Activity IU/ml*
Untreated bagasse:			
Bagasse only	6.7	0.000	0.025
98: 1RB	6.9	0.008	0.041
88: 2RB	6.2	0.033	0.044
58: 5RB	4.9	0.064	0.185
Medium E	6.7	0.058	0.496
Alkali-Treated bagasse:			
Bagasse only	7.0	0.009	0.034
98: 1RB	6.5	0.010	0.039
88: 1RB	7.0	0.015	0.033
58: 5RB	5.5	0.036	0.132
Medium E	4.4	0.049	0.170

*Substrate: 5% (w/v) solids: initial inoculum count-3 x 10^8 spores per shake flask culture spores per shake flask culture; incubation time: 2 days.

**9B: 1RB (bagasse: rice bran 9: 1 w/w); 8B: 2RB (bagasse: rice bran 8:2 w/w); 5B 5RB (bagasse: rice bran 5:5 w/w); Medium L (constituents in g per 100 ml: bagasse, 5.00; $(NH_4)_2SO_4$, 0.14; KH_2PO_4, 0.20; urea, 0.03; $Cacl_2$, 0.03 $MgSO_4 \cdot 7H_2O$, 0.03).

Table 2. Effect of substrate composition on filter paper (FP) and carboxymethylcellulose (CMC) volumetric activities of culture filtrates of *T. reesei* QM 94 14 grown on untreated bagasse*

*Substrate Composition***	*Protein Concentration %*	*FP Volumetric Activity IU/ml*	*CMC Volumetric Activity IU/ml*
Bagasse only	8.1	0.011	0.045
9B: 1RB	7.9	0.012	0.012
8B: 2RB	7.8	0.022	0.092
5B: 5RB	9.2	0.045	0.111
Medium	9.4	0.038	0.116

*Substrate: 5% (w/v) solids; initial inoculum count -3.6x10^8 spores per shake flask culture; incubation time-2 days.

**Please see footnote of Table 1 for details.

Table 3. Effect of substrate composition on filter paper (FP) and carboxymethylcellulose (CMC) volumetric activities of culture filtrates of *Pencillium* sp. 86 grown on untreated bagasse*

*Substrate Composition***	*Protein Concentration %*	*FP Volumetric Activity IU/ml*	*CMC Volumetric Activity IU/ml*
Bagasse only	4.1	0.014	0.051
9B: 1RB	4.2	0.016	0.072
8B: 2RB	4.2	0.014	0.121
5B: 5RB	4.2	0.031	0.140
Medium	6.0	0.165	1.016

*Substrate: 5% (w/v) solids; initial inoculum count-8.0×10^8 spores per shake flask culture; incubation time – 4 days:

**Please see footnote of Table 1 for details.

lium strain produced the most active cellulase among the three. A comparison of our results with those reported in literature is also relevant. For example, when *Trichoderma longibrachiatum* was grown on 0.5% NaOH-and sodium chlorite-treated bagasse, the maximum FP Volumetric activity (FPVA), FP specific activity (FPSA), CMC volumetric activity (CMCVA), CMC specific activity (CMCSA), and percentage Lowry Protein (%LP) obtained on the seventh day of fermentation were ca. 0.111 IU/ml., 0.555 IU/mg P.O. 370 IU/ml. 1.851 IU/mg P, and 2.00%, respectively (Sindhu and Sandhu, 1980). For *Aspergillus terreus* GN1 cultured on 1% alkali-treated bagasse and 600 mg cornsteep liquor/1 of broth, maximum values of FPVA of 0.08 IU/ml and CMCVA of 1.00 IU/ml were obtained on the seventh day of fermentation (Garg and Neelakantan, 1982). In our study, the optimum FPVA, FPSA, CMCVA, CMCSA, and % LP obtained on the fourth day for *Penicillium* sp. 86 grown on 5% untreated bagasse and $(NH_4)_2SO_4$ and urea as N sources were 0.165 IU/ml, 0.275 IU/mg P, 1.06 IU/ml, 1.69 IU/mg P, and 6.00%, respectively. It is evident that the cellulase activities of *Penicillium* sp. 86 are comparable if not better than those of *T. longibrachiatum* and *A. terreus*. Moreover, it is economically advantageous to utilize *Penicillium* isolate for cellulase production because it requires less incubation time and uses untreated bagasse.

The above-mentioned results were obtained in 100-ml shake-flask cultures in order to select the best microorganism and medium for scaled-up cellulase production in the 3.5-liter airlift fermenter. In turn, optimization of the airlift fermentation process was done in terms of the solids level and pH of the medium. The effect of solids level on the enzyme activity of the culture medium at various culturing times is shown in Figures 5 and 6. Maximal FP and CMC volumetric activities were obtained using a 2.0% solids level after 48 hours incubation. Results of

Table 4. Composition of Filter Paper activities and protein concentration of culture filtrates of three fungal strains.

Fungal Strain	*Culture Substrate*	*Culture Time, days*	*Filter Paper Activity*		*Protein Concentration %*
			Volumetric Activity (IU/ml)	*Specific Activity (IU/mg protein)*	
T. reesei NRRL 11485	Medium E using alkali-treated bagasse	2	0.049	0.112	4.4
T. reesei NRRL 11485	Untreated bagasse: rice bran (5:5)	2	0.064	0.130	4.9
T. reesei QM9413	Untreated bagasse: rice bran (5:5)	2	0.045	0.049	9.2
Penicillium sp. 86	Medium E using untreated bagasse	4	0.165	0.275	6.0

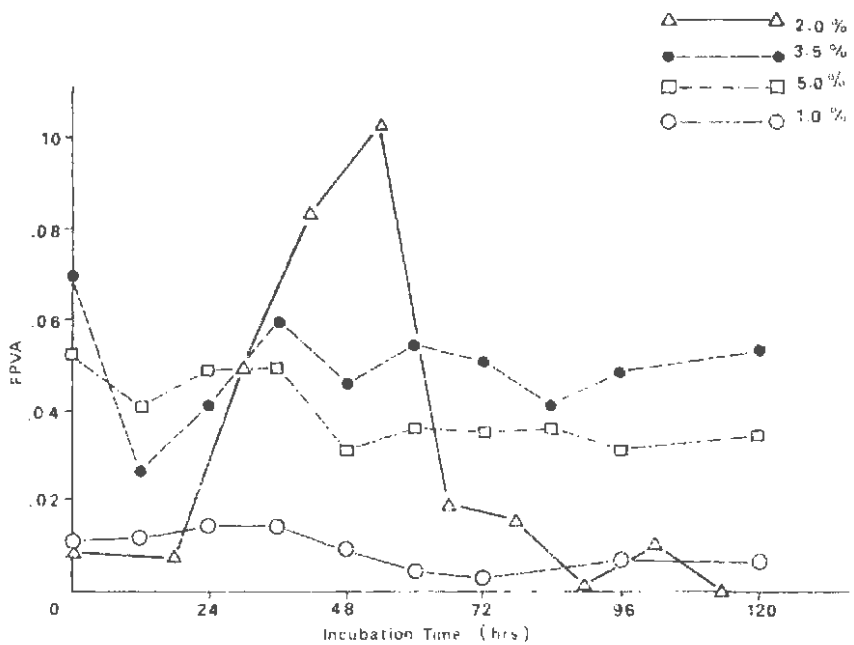


Figure 5. Plot of FPVA vs. incubation time for *Penicillium* sp. 86 grown on untreated bagasse at various solids levels tested using an airlift fermenter.

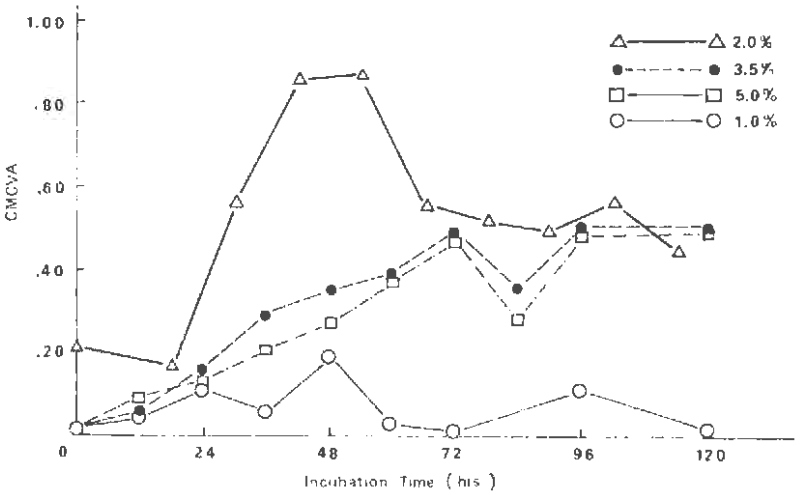


Figure 6. Plot of CMCVA vs. incubation time for *Penicillium* sp. 86 grown on untreated bagasse at various solids levels tested using an airlift fermenter.

the pH optimization studies, which are given in Figures 7 and 8, show that maximal cellulase production was achieved at pH 4.5 after 48 hours incubation using 2% untreated bagasse as carbon substrate. In summary, maximal production of cellulase was obtained by culturing *Penicillium* sp. 86 on 2% untreated sugarcane bagasse supplemented with 0.14% $(NH_4)_2SO_4$, 0.20% KH_2PO_4, 0.03% urea, 0.03% $CaCl_2$ and 0.03% $MgSO_4 . 7H_2O$ and with the pH controlled at 4.5 and aeration at 1 vvm (volume of air per volume of fermentation medium per minute) in an airlift fermenter at room temperature (ca. 30°C).

Glucoamylase Production

The batch production of the enzyme glucoamylase (or amyloglucosidase) has been optimized in our laboratory using the amyliolytic mold *Aspergillus awamori* NRRL 3112 (Acabal, 1983). The optimum temperature, pH, solids level, and inoculum size were determined for enzyme production in an airlift fermenter using a mixture of cassava root flour and rice bran (1:2 weight ratio) as substrate. The experimental results for the glucoamylase activity of the culture filtrate as a function of incubation time are presented in Figure 9. It is seen in the Figure that greater volumetric and specific activities of the enzyme were obtained at 30°C compared to 35°C.

The effect of pH on glucoamylase production by *A. awamori* NRRL 3112 at 30°C is shown in Figure 10. The optimal pH was found to be 5.5. A comparison of enzyme activity data at 20 and 25% solids level, which is drawn in Figure 11 shows the advantage of using the lower solids level. The results of this study are in

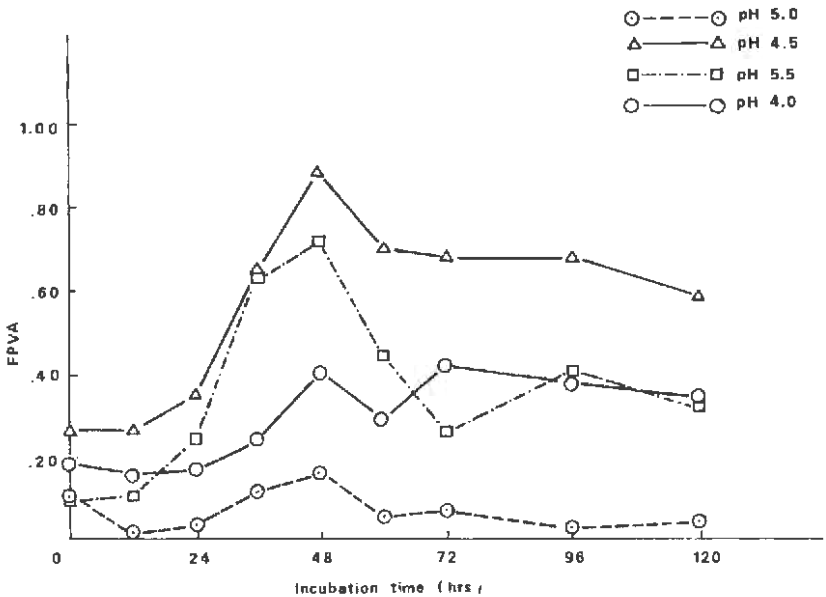


Figure 7. Plot of FPVA vs. incubation time for *Penicillium* sp. 86 grown on 2% untreated bagasse at various pH levels using an airlift fermenter.

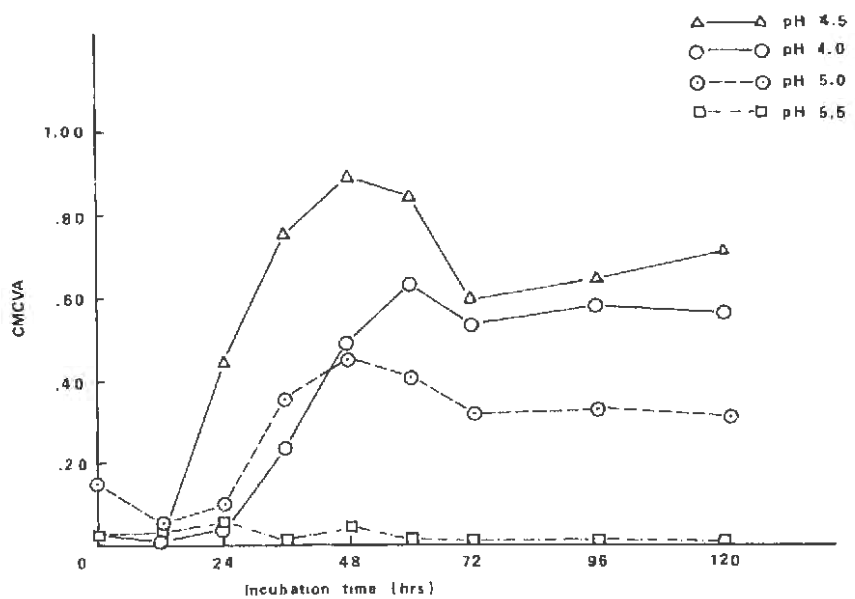


Figure 8. Plot of CMCVA vs. incubation time for *Penicillium* sp. 86 grown on 2% untreated bagasse at various pH levels using an airlift fermenter.

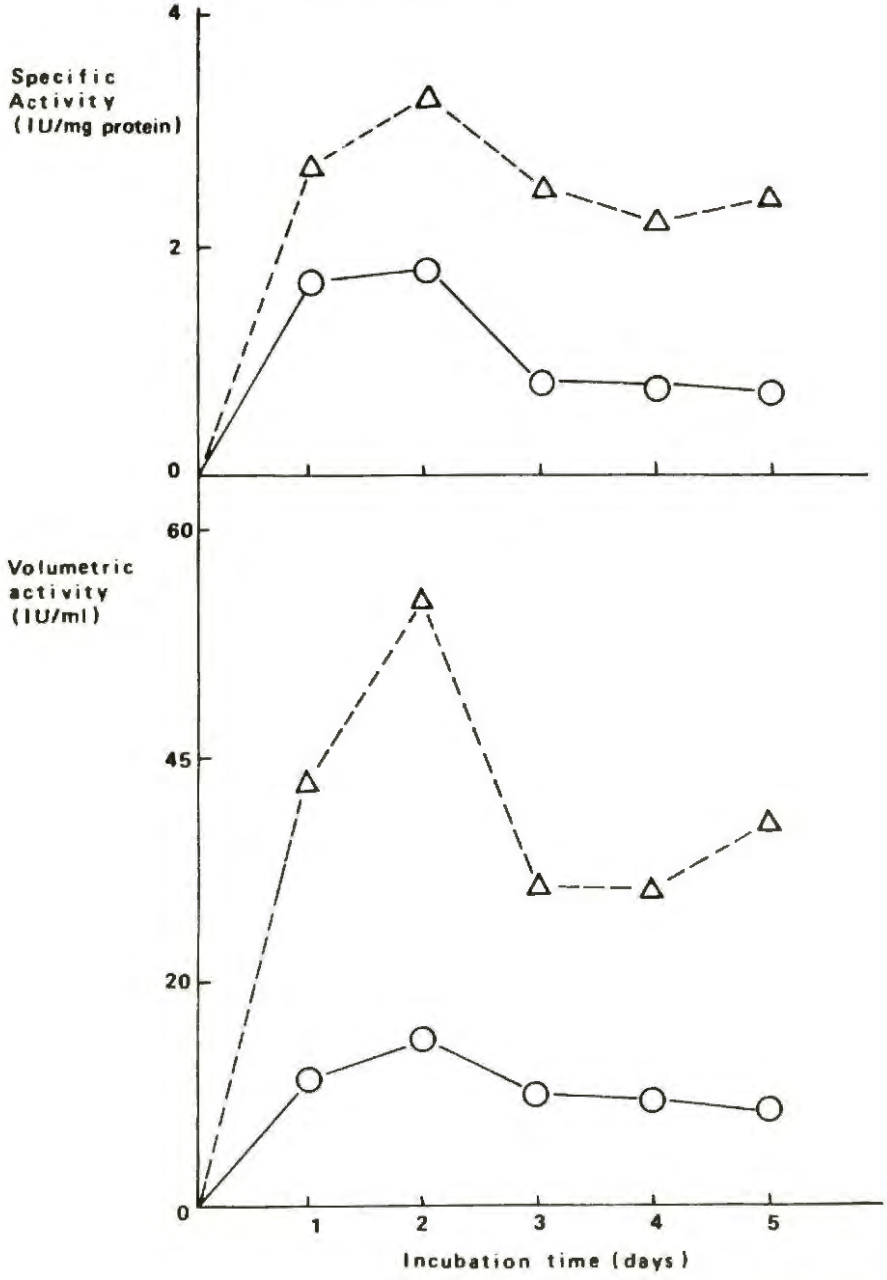


Figure 9. Volumetric and specific activities of glucoamylase produced by *A. awamori* NRRL 3112 at 30°C (Δ – Δ) and 35°C (0 – 0) with uncontrolled pH using 20% solids level of substrate and 10% inoculum size.

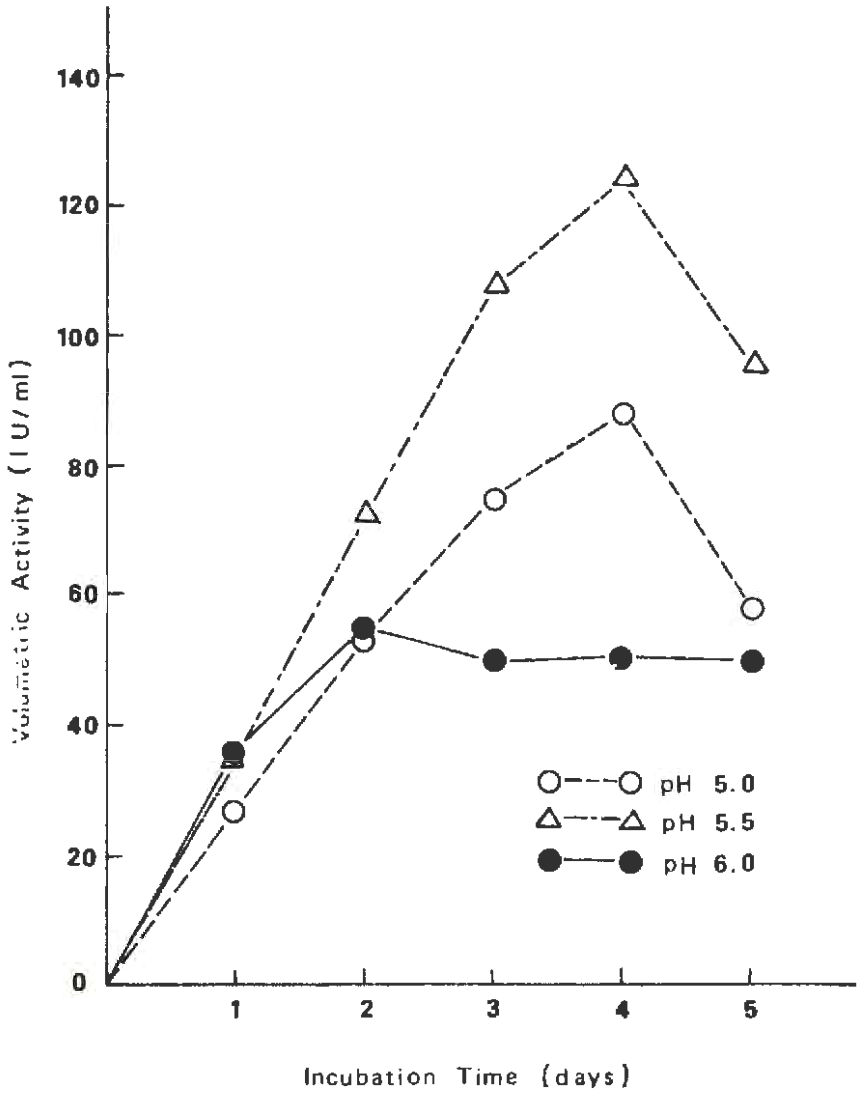


Figure 10. Volumetric activity of glucoamylase produced by *A. awamori* NRRL 3112 at 30°C as influenced by variation of pH using 20% solids level of substrate at 10% inoculum size.

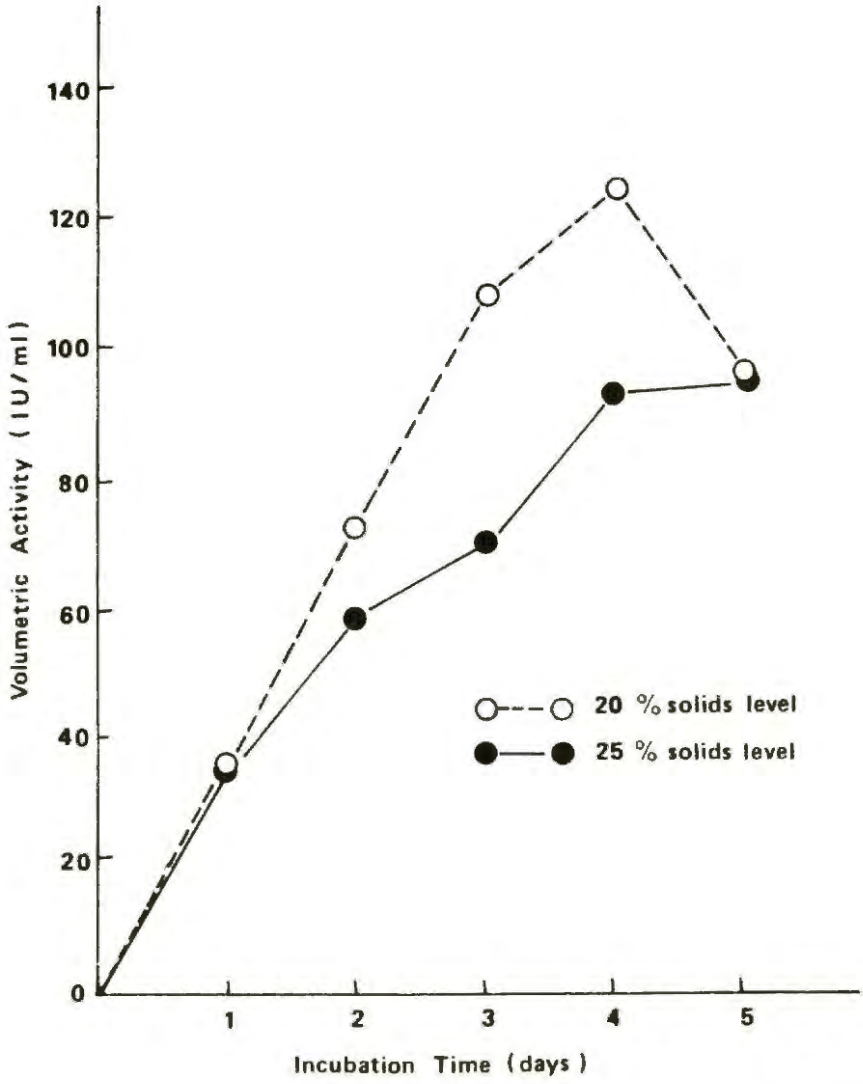


Figure 11. Volumetric activity of glucoamylase produced by *A. awamori* NRRL 3112 at 30°C and pH 5.5 as influenced by variation of solids levels of substrate using 10% inoculum size.

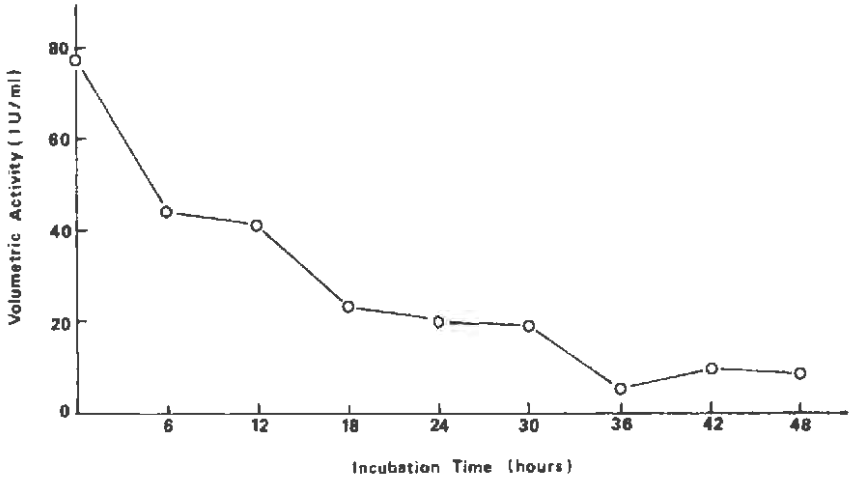


Figure 12. Volumetric activity of glucoamylase during continuous production by *A. awamori* NRRL 3112 at 30°C and pH 5.5 using 20% solids level of substrate.

agreement with those obtained by Smiley (1974) and Osorio (1981) using the same strain of mold. Furthermore, lower glucoamylase activities were obtained at 15% and 25% solids level (Smiley, 1974). Unfortunately, our studies on the effect of 10% and 20% inoculum sizes, corresponding to initial values of 7.20×10^5 and 1.5×10^6 spores per ml of the medium were not conclusive. Continuous-flow glucoamylase production at 30°C and pH 5.5 using 20% solids level of substrate showed a decreasing volumetric activity of the culture filtrate with time, as shown in Figure 12. The results show that the mold exhibits metabolic instability during continuous-flow culture; hence, batch enzyme production is preferable.

Partial purification data for glucoamylase using ethanol precipitation are presented in Figure 13 and Table 5. A four-fold purification of the enzyme was obtained after precipitation of the 50-80% ethanol fraction. The solid enzyme preparation had high gravimetric and specific activities which are comparable with commercial liquid glucoamylase preparations.

Alpha-Amylase Production

The enzyme alpha-amylase is required for the initial dextrinization of starch prior to saccharification by glucoamylase as shown in Figure 2. This enzyme has been produced in our laboratory in an airlift fermenter using Bacillus subtilis NRRL 3411. A simplified process of producing the enzyme was developed using as substrate a mixture of cassava root flour, rice bran, fish meal and soybean meal. Optimal fermentation conditions of pH, temperature, aeration rate and fermentation time were determined in order to obtain maximal yields of the enzyme. The enzyme

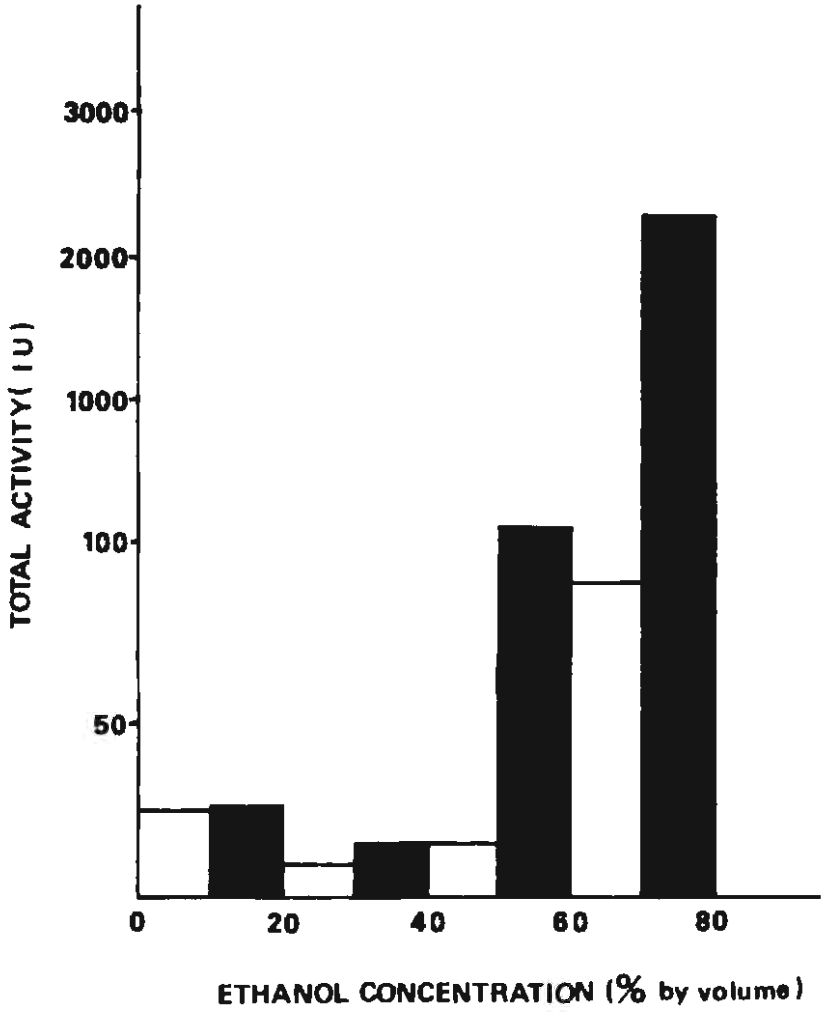


Figure 13. Total activity of glucoamylase precipitated at varying ethanol concentrations.

was then partially purified and concentrated and suitable stabilizing agents were added in order to produce stable liquid or solid enzyme preparations (E.J. del Rosario and T.V. Den, Phil. Patent Pending).

Acknowledgements

The author is grateful for having been awarded one of the Outstanding Young Scientist Awards in 1981. The technical data presented in this paper were based

Table 5. Comparison of laboratory and commercial preparations of glucoamylase

Glucoamylase Preparation	*Volumetric Activity (IU/ml) or Gravimetric Activity (IU/g)*	*Protein Concentration (mg/ml or mg/g)*	*Specific Activity (IU/mg protein)*
Crude enzyme (culture filtrate)	130	9.3	14.1
Precipitate (50-80% ethanol fraction)	3,992	69.2	57.7
SIGMA Amyloglucosidase No. A-9268	1,443	57.7	27.9
NOVO Amyloglucosidase 200L	6,805	171.7	39.6

primarily on the masteral theses of L. Bondoc, Jr. and A. Acabal, which were done under the author's supervision, and on collaborative research with Dr. Truong Van Den. Research support provided by BIOTECH-UPLB and F.E. Marcos Foundation, as well as the grant of VISCA-PCARRD and SEARCA Scholarships to L. Bondoc, Jr. and A. Acabal, respectively are gratefully acknowledged.

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Lydia Joson, Discussant

The development of technology for the production of cellulolytic and amylolytic enzymes is indeed of a vital importance if the utilization of agricultural produce and wastes is to be maximized for conversion into energy, food, and chemical feedstuff. The technology for the enzymatic hydrolysis or saccharification of starches and cellulosics has long been known but its industrial application has been hindered by the high cost of enzyme production. To reduce the cost, cheaper and more efficient production methods should be developed to make the process economically viable. The UPLB group of researchers under the able leadership of Dr. del Rosario has been contributing greatly towards the development of enzyme and microbial technology in the country.

In the development of microbial process or product for industrialization, many factors come into play which should be considered:

1) *Raw Materials*

The cost of raw materials usually constitutes about one-half to three-fourths of the cost of the product. The Philippines being a tropical agricultural country has an almost inexhaustible source of cheap raw materials. Dr. Swaminathan this morning mentioned the different methods of how agricultural residues could be processed into products of more economic value. Dr. del Rosario and his co-workers have developed a laboratory process for the production of enzymes using these same materials, which could be the basis for an industrial plant. These enzymes are necessary for the processing of large bulk of cellulosics and starchy materials into liquid biofuel, food, and chemicals.

2) *Microorganisms*

Of course, the most important in the development of a microbiological process is the microorganism. In the studies of Dr. del Rosario *et al.*, cellulase was produced by several cellulolytic fungi. The most active, however, was a local isolate of *Penicillium* using untreated bagasse as substrate. This is very advantageous for no pre-treatment is necessary which otherwise can add to the cost of production. The same result, that is cellulase production by *Penicillium*, has been found by the group of researchers of the International Center of Cooperative Research and Development in Microbial Engineering in Japan. They found that *Penicillium purpurogenum* (Sasaki *et al*, 1983) could also produce cellulase more active than *Trichoderma reesei*. *Trichoderma reesei*, the well-known cellulolytic fungus, is noted for its high production of a well-balanced cellulase complex required for the hydrolysis of crystalline cellulose. The enzyme system, however, is not sufficient to degrade native cellulose which the *Penicillium* of Dr. del Rosario could degrade. Other limitations of *T. reesei* enzymes are the weak beta-glucosidase activity; the need for inducers for enzyme production; the feed back inhibition of enzyme production by glucose; and its low yield. To remedy these deficiencies, strain improvement programs have been developed by two groups of researchers from the Quartermaster Laboratory of the U.S. Army Natick Research and Development and

from Rutgers University in New Jersey, as given by Dr. del Rosario. Through UV and nitrosoguanidine mutagenesis and of resistance to kabicidin, they were able to develop highly active strains. Among these are are RUT NG-14 and RUT-C30 which are 5 times more active than the mutant QM 9414 (Montenecourt *et al.*, 1979); MCG 77 which can grow in glucose and other soluble substrates and produce high cellulase complex after substrate exhaustion (Gallo *et al.*, 1978); and MCG 80 which has lost its feed back inhibition to glucose and can utilize soluble substrates such as lactose and glucose (Allen and Andreotti, 1982). The proper choice of microorganism as the *Penicillium* of Dr. del Rosario is very important for the economic production of the enzyme.

3. *Method of Propagation*

There are two general methods of propagating microorganisms for the production of enzymes; solid substrate fermentation and submerged aerated process. The method of choice depends on the type of microorganism involved. Filamentous fungi because of their nature can be propagated by both methods with success, but not bacteria. As discussed by Dr. del Rosario, the submerged aerated process is easier to control and can be easily scaled up to commercial production. It is, however, more capital intensive but less labor intensive than solid substrate fermentation.

The airlift fermentor developed by Dr. del Rosario which is much cheaper than the commercial ones is indeed a positive step towards the industrial production and utilization of these enzymes in the country. I would like to add, however, that the National Institute of Science and Technology is also involved in the production of these enzymes. A systematic screening of soils for the presence of mesophilic and thermophilic cellulolytic and amylolytic bacterial fungi is being undertaken. A technology has been developed for the production of glucoamylase by solid substrate fermentation using the same organism *Aspergillus niger* NRRL 3112. Yields of about 150 IU/gram of substrate (rice bran and cassava bagasse) have been obtained. Amylase production of *Bacillus licheniformis* IFO 1220 by submerged aerated process is being undertaken.

Priscilla C. Sanchez, Discussant

Developing countries like the Philippines would do well if they could harness nature's gift, that is the abundant renewable raw materials from plant biomass. Microbial conversions of transformations of this important resource into goods, industrial and energy products could help greatly in improving the living conditions in these countries.

The key to the bio-transformations and synthesis of new materials and compounds from plant biomass (the celluloses, starch and sugars) are the microorganisms and enzymes they produce. These biological catalysts (enzymes) hold the key to the full utilization of the abundant plant biomass around us.

Considerable advances have been made in the utilization of starches and sugars but much have to be done with cellulose which commonly abound with lignin in nature to form lignocellulose. The only useful means of utilizing lignocellulose by biological conversion is to use it in growing mushrooms.

Dr. del Rosario's work demonstrated the possibilities of the production of enzymes for industrial use. We have an abundance of raw materials for the purpose and we should proceed in the pilot production of these enzymes to test the economic feasibility. Similarly, local isolates of microorganisms must be screened. These local isolates are more adapted to our conditions hence efficiency will be much higher.

The isolates which Dr. del Rosario used (except for Penicillium) are standard isolates from other countries. Possibly, comparison of the efficiency of these isolates done in other countries and the result of study should have been made. This would at least give us an idea of how much more work must be done to have a full use of the technology.

Now many cellulolytic fungi have been found for commercial cellulose production such as Onozuka (Kinki Yakult Co., Ltd.) from *Trichoderma reesei* (*T. viride*) and cellulosin (Ueda Chemical, Ltd.) from *Aspergillus niger* (CANUZ). But these are not so active in the treatment of cellulose in higher temperature, and so cellulolytic thermophilic fungi could play an active role in the decomposition of cellulose at higher temperature. Application of thermophilic microorganism in degrading the abundant raw materials in the Philippines offer a solution in efficiently utilizing these materials.

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