PREFERENTIAL DEGRADATION OF LIGNIN IN WATER HYACINTH USING WHITE ROT FUNGI FOR ANIMAL FEED PRODUCTION

FINAL REPORT

PSTC GRANT: 10.027

Executive Summary:

The above project was undertaken with the goals of finding a pretreatment method for lignocellulosics which will preferentially degrade lignin. This research was carried out in collaboration with scientists from Bangladesh. The following were the main accomplishments of the research:

1) *Pleurotus ostreatus* was the "best" fungi for the preferential degradation of lignin.
2) The optimum moisture content for the solid state fermentation were: 70-80% initial moisture content, initial pH of 6.5, and air flow rate through the solid state reactor of 0.32-0.44 ml/min/g-dry wt of the substrate.
3) The scale up (250 L) required longer fermentation time (2 months).
4) Solid state fermentation was analyzed by developing a novel moethod using instantaneous RQ (respiratory quotient) measurements and staedy state elemental balances. The instantaneous heat-interaction profile was predicted from steady state balances. The solid state fermentation data were checked for consistency by using entropy balance inequality.
5) Growth of *P. ostreatus* on lignocellulosics such as water hyacinth and barley straw was found to be tri-auxic for 30 days fermentation.

One journal paper, two conference papers, and one MS thesis were published from this research. Two scientists from Bangladesh received training in biotechnology, one completed a Master of Science degree on the project. Equipment such as an incubator shaker, a fermentor with appropriate instrumentation, a spectrophotometer, and an HPLC system were donated to the Bangladesh University of Engineering and Technology in order for them to develop the expertise in-house in the Chemical Engineering Department. Overall, the project was a success from the technical point of view.

PERSONNEL INVOLVED:

1. Professor M. Nazmul Karim (Colorado State University), Principal Investigator
2. Professor Jim Linden (Colorado State University), Co-Principal Investigator
3. Ms Linda Henk (Colorado State University), Research Associate
4. Ms Mynette Minyard (Colorado State University), student (undergraduate)
5. Ms Shelly Knupp (Colorado State University), student (undergraduate)
6. Dr. Jasimuzzaman (Bangladesh University of Engineering and Technology)
7. Mr. Pranob Das (Bangladesh University of Engineering and Technology)

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RESEARCH ACCOMPLISHMENTS:

In this final report research accomplishments from October 14, 1992 - September, 1994 will be mainly highlighted. The report will be appended with previous reports and publications.

Abstract:

This study was primarily focused on the degradation of lignin in water hyacinth and barley straw for animal-feed production. The experiment was performed in a 1.5-L Applikon fermenter for 30 days, varying the air flow rate from 0.022 VVM/0.047 VMM to 0.048 VVM/0.102 VMM. A novel approach was introduced for the prediction of a kinetic model by using instantaneous respiratory quotient (RQ) measurements and steady state elemental balances. Growth kinetics were determined for the fungus in a 30-day fermentation with a mixture of barley straw and water hyacinth as the substrate. The instantaneous heat-interaction profile was predicted from steady state balances. Fermentation data were checked for consistency using the entropy balance inequality, and thermodynamic efficiency was calculated to show that degradation of lignocellulosics by *Pleurotus ostreatus* followed more than one metabolic pathway during the course of the fermentation. Growth of *P. ostreatus* on lignocellulosics, such as water hyacinth and barley straw, was di-auxic or possibly tri-auxic during the 30 days of fermentation. From the scale up study it was found that *in-vitro* digestibility increased by 10.4% after 60 days of pretreatment with the fungi. Protein to lignin ratio increased in the case of water hyacinth as the substrate to about 20%.

Materials and Methods:

Organism and inoculum

Pleurotus ostreatus, ATCC 32783, was cultured in slants containing 10 ml of yeast malt agar (3.8 g/100 ml water) at 30°C. After 10 days of incubation, the fungus was transferred to petri plates containing 10 ml yeast malt agar. Barley grain (10% of the wet weight of the substrate) was sterilized in 10 1-L canning jars (each jar containing equal amounts of grain), and the sample was autoclaved for 30 min at 120°C. Each jar was inoculated with 6-8 plugs (No. 9 cork-borer) of the fungus cultured in the petri plates and incubated for 2 weeks at ambient temperature (28–29°C).

Substrate preparation

Water hyacinth plants, excluding roots, were separated into leaves and bulbs. The plants, originally from Florida, were obtained from Raymond E. Smith, Fort Collins, Colorado. The leaves, bulbs and barley straw were chopped according to the following

size distribution: leaves (1-1.5 x 0.5-1 inch), bulbs (0.8-1.0 x 0.5-1 inch) and straw (0.5-1.0 inch). The pH was adjusted to 6 using 90% tris hydroxy methyl-amino methane hydrogen chloride. The pH was measured using a pH meter and taking a 10 g sample, diluting it with 90 ml of water and soaking it for 1 hour.

Analytical methods

Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) of the straw were determined according to the procedure of Agricultural Handbook No. 379 [1]. Total protein was estimated by measuring trichloroacetic acid (TCA)-soluble Kjeldahl protein, and *in vitro* dry matter enzyme digestibility (INVDMED) was determined by the procedure reviewed by Rajarathnam and Bano [2]. Analysis of oxygen and carbon dioxide was accomplished by using a gas chromatograph made by Gow-Mac Instrument Company (Madison, NJ, Model No. 69-550), using a thermal conductivity detector.

Experimental:

Experiments were performed in a 1.5-L Applikon fermenter (Model No. Z81305C001, Foster City, CA). The inlet air was humidified by a humidifying column (115-cm length, 5.5-cm outer diameter) containing Rashig rings. The flow rate of water passing through the column was 100 ml/min. The outlet gas was dried by passing it through a silica-gel dryer (22-cm length, 4-cm outer diameter). The fermenter was insulated with fiber glass insulation (0.5-inch thickness), and the temperature in the fermenter was maintained at 28-30°C. Fig. 1 illustrates the experimental set-up. The fermenter was loaded as follows: wet water hyacinth weighing 450 g (36% leaves and 64% bulbs/stems) was mixed with 22.5 g of dry barley straw. Eighty milliliters of 90% buffer solution (tris-hydroxy methyl-amino methane hydrogen chloride in water) and 105 ml of water were added to the mixture. The mixture was then placed in an autoclavable bag and autoclaved for 30 min at 121°C. Thirty-five grams of mycelium and 16.4 g of barley grain with *Pleurotus mycelium* were added to the bag and thoroughly mixed. Pure mycelium was obtained by inoculating the canning jars which contained 14.8 g of barley grain, incubating the jars for 30 days, and scratching the mycelium from the glass surface. The mixture was then transferred to the fermenter. Initially, the moisture content in the substrate was 75.9%, and the air flow rate in the fermenter varied from 33.5-72.5 ml/min (0.022 VVM/0.047 VMM to 0.048 VMM/0.102 VMM). VVM is defined as the volumetric air flow rate (ml/min) divided by the volume of the fermenter (ml). Similarly, VMM is defined as the volumetric air flow rate (ml/min) divided by the total mass of the wet material (g) in the fermenter.

Modeling:

Solid state fermentation (SSF) is a very complex system, and it is very difficult to model a solid state fermentation process via the chemical reaction engineering approach due to the heterogeneity of the substrates, the discrepancy in composition because of its agricultural origin, and the very poor mixing environment in the fermenter. A novel approach was proposed that requires only the measurement of gaseous components from the fermenter by using a black-box approach. Thermodynamic analysis of biological systems is an active field due to following reasons [3,4,7]:

- Biological systems are often not understood from the microbial physiology point of view.
- If metabolic pathways are known, the physical and chemical properties must be assumed because the pertinent information is not available in the literature.

Understanding the microbial system is important in developing a generalized tool for the analysis of biochemical reactions without knowing of the complex intracellular processes. The thermodynamic approach is well suited to this type of analysis because the system can be modeled from a macroscopic point of view. All biological systems proceed via non-equilibrium processes; thus, non-equilibrium thermodynamics must be used instead of equilibrium thermodynamics.

For these reasons, researchers use the black-box approach to model metabolic processes [5,6]. Among the various black box parameters cited in the literature [4], degree of reduction was taken into consideration in this study. As mentioned earlier, solid state fermentation is a complex system, and the analysis of the solid samples from the fermenter does not provide precise information of the reaction. Attempts have been made in this study to obtain kinetic information from stoichiometric information incorporated with the instantaneous respiratory quotient (RQ) measurement.

Prediction of growth kinetics from steady state balances

The fermenter was assumed to be a black-box containing an initial amount of substrate. Saturated air is then pumped into it. Fermentation produces biomass and carbon dioxide. A schematic of the fermenter system as well as the black-box model are shown in Fig. 1 and 2, respectively. The fermenter mass balances are determined via elemental analysis, based on one mole of carbon, and assuming the chemical formula of the substrate and the biomass as $CH_aO_bN_c$ and $CH_xO_yN_z$, respectively.

Introducing $\Delta\phi_j = \phi_j - \phi_j^i$, where j is any species other than water and i is any stream in the fermenter (Fig. 2) and defining $\Delta\phi_w = \phi_w + \phi_{wv} - \phi_w^i$ for water, elemental balances for carbon, oxygen, hydrogen and nitrogen can be obtained as follows:

$$Y_{bo} - Y_{so} + RQ = 0 \tag{1}$$

$$y \bullet Y_{bo} + 2 \bullet RQ + Y_{wo} = 2 + b \bullet Y_{so} \tag{2}$$

$$a \bullet Y_{so} = x \bullet Y_{bo} + 2 \bullet Y_{wo} \tag{3}$$

$$c \bullet Y_{so} = z \bullet Y_{bo} \tag{4}$$

The yield coefficients can be defined as follows:

$$Y_{bo} = \frac{\text{Moles biomass produced}}{\text{Moles } O_2 \text{ consumed}}$$

$$Y_{so} = \frac{\text{Moles substrate consumed}}{\text{Moles } O_2 \text{ consumed}}$$

$$Y_{wo} = \frac{\text{Moles moisture produced}}{\text{Moles } O_2 \text{ consumed}}$$

$$RQ = \frac{\text{Moles } CO_2 \text{ produced}}{\text{Moles } O_2 \text{ consumed}}$$

The compositional formula of the substrate (dry water hyacinth and barley straw mixture) and the fungus (biomass) has been obtained from the elemental analysis of the dry substrate and the fungus. A large quantity of substrate (200 g) and fungal mycelium (100 g) was vacuum dried at 40°C and milled before performing the elemental analysis. The total dry matter used for the elemental analysis of the substrate and biomass was 10 g for each case. The chemical composition of the fungus varies depending on the maturity of the fungus; however, there is no way to obtain the instantaneous biomass composition. Similarly, the substrate (lignocellulosic mixture) chemical formula depends on the maturity of the plant and the proportion of leaves and stems used in the substrate. The best approximation is to assume a constant chemical formula for the biomass and the substrate. Coefficients of substrate ($CH_aO_bN_c$) and biomass ($CH_xO_yN_z$), on the basis of 1 mole of carbon, are: a = 1.692, b = 0.72, c = 0.05, x = 0.17, y = 0.64, and z = 0.08. Solving Equations (1-4) simultaneously and substituting the values of a, b, c, x, y and z in Equations 1-4, provides the following relationships:

$$Y_{so} = 2.667 \ (RQ) \tag{5}$$

$$Y_{bo} = 1.667 \ (RQ) \tag{6}$$

$$Y_{wo} = 2.115 \ (RQ) \tag{7}$$

Thus, measuring the oxygen consumed and the carbon dioxide evolution rate, different yield coefficients can be calculated. From these yield coefficients, the concentrations of biomass and substrate can be calculated.

Energy and entropy balances

The generalized degree of reduction is the number of equivalents of oxygen required for the complete oxidation of that quantity of organic compound contained in 1 gram atom of carbon. This parameter can be used advantageously to simplify the fermenter energy and entropy balances [5]. To perform this simplification, assume: (i) CO_2 and N_2 are in the gaseous state, and H_2O is in the liquid state at standard temperature and pressure; (ii) heat capacity remains constant in the experimental temperature range; (iii) the inlet temperature equals the fermenter temperature; and (iv) the heat of combustion is expressed as $\Delta h_{c,i}^{\circ} = -109.04 \bullet \gamma_i$ kJ/mol [**Error! Bookmark not defined.**]. These assumptions give the simplified energy balance of the fermenter.

Equation 8 represents the final expression for energy balance of the fermenter with the above mentioned assumptions.

$$Q + W = 109.04 \cdot (\Delta\phi_s \gamma_N + \Delta\phi_N \gamma_N + \Delta\phi_b \gamma_b) \quad (8)$$

Here, $h_{c,i}^{o}$ is heat of combustion of species *i* at 298 K, γ_i is the degree of reduction of species *i*, Q and W are the fermenter heat input and the shaft work input, respectively. Division by $(-\Delta\phi_{o_2})$, substitution of $\Delta\phi_i$'s in terms of yield coefficients as defined earlier and insertion of the γ_i's ($\gamma_s = 4.25$ and $\gamma_b = 2.89$) into Equation 8 gives:

$$Q / (-\Delta\phi_{o_2}) = 109.04 \cdot (2.89 \bullet Y_{bo} - 4.25 \bullet Y_{so}) \quad (9)$$

Equation 10, which is the entropy balance on the fermenter, can be obtained from the following assumptions: (i) all the assumptions from which Equation 7 were obtained; (ii) the substrate components behave as an ideal mixture, and (iii) the Gibb's free energy of combustion can be described as: $\Delta g_{c,i}^{o} = -110.23 \bullet \gamma_i$ kJ/mol [5].

$$\gamma_s \bullet Y_{so} - \gamma_w \bullet Y_{wo} + \gamma_{o_2} - \gamma_{co_2} \bullet RQ + \gamma_b \bullet Y_{bo} \leq 0 \quad (10)$$

With the insertion of Y_{so}, Y_{bo} and γ_i values into Equation 10, Equation 11 is obtained.

$$T \bullet S_{generation} / (-\Delta\phi_{o_2}) = 6.517 \bullet RQ + 4.0 \geq 0 \quad (11)$$

Here, $S_{generation}$ is entropy generated in the fermentation process. Another important thermodynamic parameter, η, is the thermodynamic efficiency of the system. Roels and Suijdam) [7] proposed a definition of the thermodynamic efficiency (Equation 12).

$$\eta = 1 - \frac{T \cdot S_{generation} / (-\Delta\phi_{o_2})}{\Sigma_j^{products} \gamma_j Y_{jo} - \Sigma_j^{reactants} \gamma_j Y_{jo}} \quad (12)$$

The relationship between the thermodynamic parameters derived in this section with the fermentation experimental results are established later.

Results and Discussions:

Solid state fermentation is a very heterogeneous system, and it was pointed out before that it is very difficult to collect precise information by measuring the solid samples from the fermenter. In order to circumvent this problem, only gaseous components were measured. The implication of these measurements will be described in this section.

The fermenter is aerated from the top via a tube with an outer diameter of 0.5 cm (Fig. 1). When the aeration is performed in one section of the fermenter, the oxygen diffusion to other sections is minimal; hence, the sections can be considered independent from one another. Moreover, no stirring is performed because the fungal mycelium of the *P. ostearatus* is extremely sensitive to shear force [8]. The performance of the fermenter is determined by day-to-day measurements of the respiratory quotient (RQ). The RQ is calculated from the ratio of evolved carbon dioxide and consumed oxygen. Fig. 3

illustrates the RQ values *versus* sampling time. Each point is the average of two fermented samples that were analyzed in duplicate via gas chromatography. The error bar is calculated by dividing the standard deviation by the mean of the four measurements. Generally, Fig. 3 shows a relatively large error for a given RQ measurement, which indicates that the biological fermentation varies during the growth due to inhomogeneities in the fermented material. Even if no solid samples are withdrawn and analyzed during the fermentation period of 30 days, one assumes that both anaerobic and aerobic fermentation are operative in some regions of the fermenter. This statement is not unreasonable because thermodynamic efficiencies vary with time (Fig. 8), and as mentioned by Dawes [9], this can only be explained by the presence of an anaerobic metabolic process. Three maxima can be observed from the RQ profile in Fig. 3. These peaks in fermentation activity are in the vicinity of 4, 10, and 25 days. The presence of these three humps indicates that more than one component is present in the growth substrate. Inhomogeneities in the substrate can also be invoked to rationalize this behavior. As mentioned earlier, due to the heterogeneity of the fermentation environment, anaerobic processes will be dominant in some regions of the fermenter, and because the fermentation proceeds readily, this implies that in the oxygen poor regions, the fungus adapt and survive, and carry the reaction via a secondary metabolic pathway.

At the end of the fermentation period (30 days), the total fermented matter was dried overnight at 100°C. The determination of the residual components (lignin, cellulose, hemicellulose, and soluble sugars) are summarized in Table I. After fermentation, reduction in all the residual components is observed (Table I). Evidently, this decrease indicates an effective fermentation process. Fermentation leads to a consumption of 63% of the lignin, 85% of the cellulose, 65% of the hemicellulose, and 43% of the soluble sugars on the basis of the original amount of each component (Table I). One of the objectives of this study was to find a fungus that would specifically degrade lignin. The results presented in this table are not satisfying because the *P. ostreatus* is not lignin-specific. Nevertheless, these results show that the fungus degrades twice the amount of lignin relative to the total amount of cellulose and hemicellulose. The reduction of the protein content relative to the control is a bit surprising, however; this may be due to the loss of soluble protein during analysis and the production of volatile nitrogenous fermentation by-products.

In view of the objectives, the fermented matter also should exhibit a protein enrichment. It is clear that this objective is not fully achieved (Table I), but if the protein to lignin ratio is calculated (Table II), it is shown that this ratio increases by a factor of 2 relative to the control. Although after fermentation the absolute content in proteins decreases, the second objective is met because the *P. ostreatus* leads to an effective increase of the proteins relative to the lignin. The *in vitro* enzyme digestibility test, INVDMED, is a convenient way to characterize how ruminants will digest the feed. However, these determinations are fraught with experimental uncertainties. The determination of the INVDMED from the fungus-treated feed is usually lower relative to the control (Table II).

Modeling

Using Equations 5 and 6, the biomass and substrate concentrations can be calculated, respectively, from the measured RQ values illustrated in Fig. 3. The biomass and the substrate profiles obtained from the application of both equations are illustrated in Fig. 4. Fig. 4 emphasizes that a di-auxic (or possibly a tri-auxic) growth is an operative mechanism. The filled circles (λ) represent the biomass, while the filled squares (ν) describe the substrate. The di-auxic growth or tri-auxic growth imply that more than one component is degraded by the fungus. Preliminary in-house results on a similar system exhibited the same type of behavior [10]. Typical fermentation usually leads to the utilization of soluble sugars in the first stage, while less favorable components such as hemicellulose, cellulose and lignin are degraded in the second stage. In Fig. 4, it is shown that after 30 days, the model evaluates the final substrate content to be approximately 1.8 mol. This compares relatively well with the determined experimental substrate content (2 mol), and suggests the model is insensitive to the heterogeneity of the substrate.

Fig. 5 illustrates the instantaneous heat-interaction profile generated via Equation 9. The instantaneous heat interaction profile can be used in the on-line control of the fermenter. Rearrangement of the energy balance (Equation 8) yields the ratio of the generated heat per produced biomass:

$$\frac{Q}{\Delta\phi_b} = 436.2 \bullet Y_{ob} \tag{13}$$

By use of the above equation, the biomass production rate can be regulated by controlling the heat-exchange through the fermenter. The process control of the fermenter using Equation 14 is beyond the scope of the current study; however, the heat profile clearly indicates that the fermentation process is exothermic. Moreover, it was observed that the heat generation maxima corresponded relatively well to those previously observed (Fig. 3). This correspondence between the peaks provides further evidence that during those days, one specific substrate component gets processed by the fungus which in turn generates the calculated heat excess.

If the fermentation process is operative, the Second Law of thermodynamics dictates that the entropy generation must be positive. As discussed by Schrödinger, microorganisms feed on negative entropy [11]. This is ascertained by the application of Equation 11 to the experimental RQ values. Results from Equation 11 give rise to the entropy generation profile illustrated in Fig. 6. All the generated entropy values are positive indicating that the measured values are consistent, and the fermentation process is viable.

Data presented in Fig. 4 can be expressed in terms of the specific growth rate. Fig. 7 illustrates the specific growth profile for the fermentation. As expected, higher growth rates are observed at the beginning of the fermentation, i.e., between 0.8 and 1 day^{-1}. The low growth rate values centered around 6.2 mol are due to the initial instability of the fermenter. At approximately 5 mol of substrate, the growth rate decreases dramatically, and the growth exhibits a plateau behavior. The average value of the plateau oscillates at 0.1 day^{-1}. In view of previous discussions and Fig. 3 and 5, Fig. 7 shows that the soluble sugars are degraded at a higher rate relative to the other substrate components (hemicellulose, cellulose or lignin). Moreover, since the growth rate levels off, this implies that the remaining components are degraded at similar rates.

Fig. 8 shows the thermodynamic efficiency profile obtained using Equation 12. In the early stages of the fermentation (up to 10 days), the thermodynamic efficiency profile shows a variation in the efficiency (0.93 to 0.99). After this period, the efficiency levels off at 0.985. This behavior is almost identical to that observed in Fig. 7 and can be rationalized using the same arguments mentioned above.

Table I Fiber components on a wet basis after 30 days of fermentation

Sample	Lignin	Cellulose	Hemicellulose	Soluble sugar	Proteins
	(%)	(%)	(%)	(%)	(%)
Control	0.60	3.4	2.8	13	4.4
After 30 days	0.22	0.50	0.96	7.2	3.4

Table II Relevant parameters determined after 30 days of fermentation

Sample	INVDMED [1]	Proteins to lignin ratio
	(%)	(%)
Control	88	7.4
After 30 days	83	15

1) The INVDMED abbreviation represents *in vitro* dry matter enzyme digestibility.

Scaleup studies:

A large scale fermenter was built with the rationale of technology transfer. The fermenter was of box type (250 liter working volume). It was a rectangular box of 85 cm x 85 cm x 30 cm made from wood (2 cm thick). The top was made from plexi glass (0.5 cm thick), and it was sealed using silicone rubber. Two pieces of copper tubings (0.5 cm o.d) were inserted from the top, one was for aeration (37 cm long) and the other (11 cm long) for measuring the produced gas due to biochemical reactions. In the aeration tube, five 3 mm holes were made at 12 cm lengths. On one side of the fermenter two copper tube rakes with comb like nails (1.5 cm long) were interested. These were used for intermittent mixing of the substrate. There were four windows (8 cm square) built for talking samples from the fermenter. The inside surface was covered with plastic so that the ferementer could be reused. The schematic diagrams of the fermenter are shown in Figures 9 and 10.

Three kilograms of barley straw chopped to 0.5 to 1.0 inches were mixed with 7800 ml of water to provide an initial moisture content of 70%. The initial pH was adjusted to 6.5 adding 5333 ml of 90% tris-hydroxy methyl-amino methane hydrogen chloride buffer. The inlet air flow was at 7l/min (0.028 VVM). The substrate was "composted" for 11 days after which it was inoculated with 7.2 gms of pure mycelia planted at 2.54 cm deep and at 9 cm apart in 8 rows. The fermentation was carried out for 60 days when samples were taken from four different parts of the fermenter for analysis. The fermenter was run under non-sterile condition. Table 3 and 4 shows results of the analysis. After 60 days, the protein to lignin ratio (PL) increased by 21%. There was a decrease in overall protein content (20%) which might have been due to protease activities. The ***in vitro*** dry matter enzyme digestibility (INVDMED) increased by 10% after 60m days of fermentation which indicates that this digested product could ben used as animal-feed.

Table 3: Fiber components for a 60 day period fermentation; results from the scaleup studies (250 liters fermenter)

Days	Lignin %	Cellulose %	Hemicellulose %	Soluble Sugars %
Day 0	11.45	33.50 ± 0.21	27.50 ± 0.02	12.14 ± 0.02
Day 60	7.56 ± 0.01	37.99 ± 0.03	20.20 ± 0.04	22.71 ± 0.01

Table 4: Protein and INVDMED changes during 60-day fermentation

Days	Protein %	INVDMED %	Protein/Lignin Ratio
Day 0	11.21 ± 0.10	59.86 ± 0.0.5	0.98 ± 0.10
Day 60	8.99 ± 0.03	66.11 ± 0.01	1.19 ± 0.04

International Collaboration:

This project was undertaken with the goal to develop collaboration with scientists from Bangladesh. The personnel from the Chemical Engineering Department at Bangladesh University of Engineering and Technology (BUET) were involved in the project.

Dr. Jasimuzzaman spent two months at Colorado State University to gain hands on experience in the PI's laboratories. He was trained the analytical and as well as microbiological techniques needed to conduct research in biotechnology in Bangladesh. Equipment were donated to BUET to start a biotechnology laboratory. The main equipment that were donated are: incubator shaker, a fermenter with ancillary instrumentation, a spectrophotometer, an HPLC with appropriate columns and data acquisition modules for analysis of data. Another scientist, Pronab Das visited Colorado State University (CSU) for long term collaborative research. He completed a Master of Science degree in Chemical Engineering at CSU while conducting his research in this field. A journal paper has been published from his efforts. Professor Karim made yearly trips to Dhaka, Bangladesh to discuss the progress of the project with his counterparts in Bangladesh. He gave two seminars during his trips. One of the main problems of this type of international collaboration is the lack of local funding (Bangladesh) which is needed to continue to work on the project. Adequate funding from the local government is necessary for personnel and for supplies to make a project such as this successful and rewarding for both the parties. It is the opinion of the PI that more could have been done from the Bangladesh side with respect to more substantial research performed at BUET. In the future, funding agencies should pay attention to the local financial support for the collaborative work.

Publications:

1. P. Das and M. N. Karim. Mass balance and thermodynamic description of solid state fermentation of lignocellulosics by *Pleurotus ostreatus* for animal feed production. J. of Industrial Micribiol., **15**, pp 25-31 (1995).

2. L. L. Henk, M. N. Karim and P. Das. Solid state fermentation of water hyacinth and barley straw by *Pleurotus ostreatus*. Poster presentation at the 5th Annual Colorado Biotehnology Symposium, September 22, 1992, Fort Collins, CO.

3. L. L. Henk and M. N. Karim. Comparative studies of white rot fungi for the delignification of forage materials. Poster presentation at the 4th Annual Colorado Biotehnology Symposium, September 24, 1991, Boulder, CO.

4. P. Das. Degradation of lignin in water hyacinth by *Pleurotus ostreatus* for animal feed production. MS thesis (advisor: M. N. Karim), Colorado State University (1993).

Conclusions:

The technical goals of the project were met. One journal paper, two conference papers, and one MS thesis were published from this research. International collaboration was established. Two scientists from Bangladesh received training in biotechnology, one completed a Master of Science degree on the project. Equipment such as an incubator shaker, a fermentor with appropriate instrumentation, a spectrophotometer, and an HPLC system were donated to the Bangladesh University of Engineering and Technology in order for them to develop the expertise in-house in the Chemical Engineering Department. It is up to the scientists of Bangladesh to take the results of this collaboration for further technology transfer research. Overall, the project was a success from the technical point of view.

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10. P. K. Das. 1993. Degradation of Lignin in Water Hyacinth by *Pleurotus ostreatus* for Animal Feed Production. pp. 30-37, M.S. Thesis, Colorado State University, Fort Collins.

11. R. Hasse. 1990. Thermodynamics of Irreversible Processes. pp. 493-495, Dover Publications, New York, NY.

FIGURES

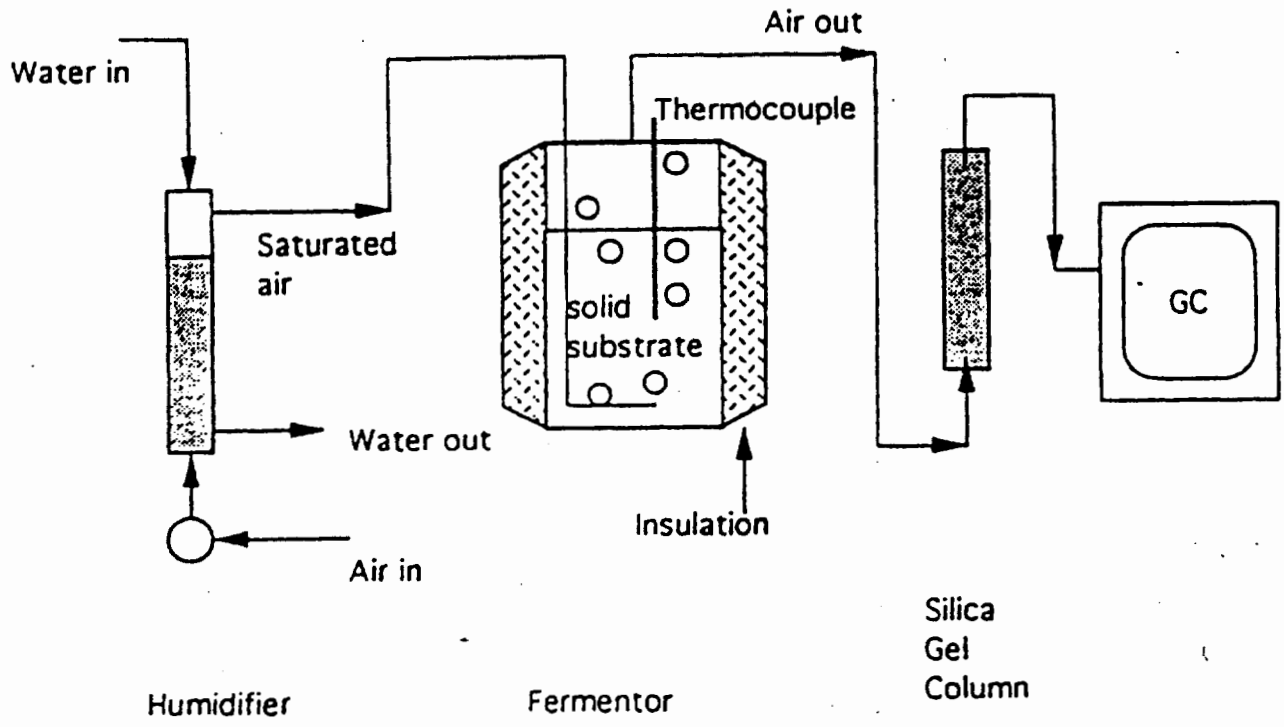

Figure 1 Experimental set-up for 30-day fermentation

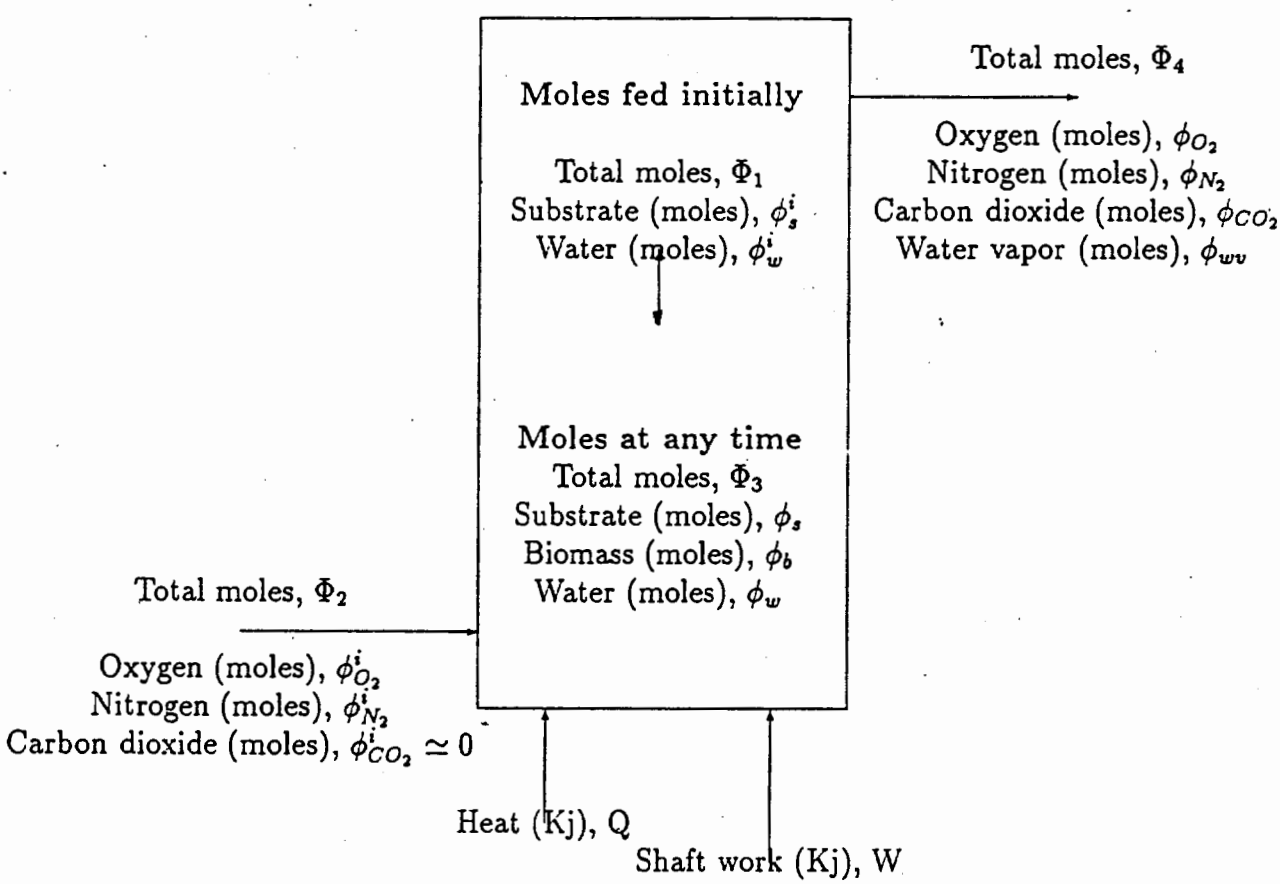

Figure 2 : Black–box representation of a fermenter

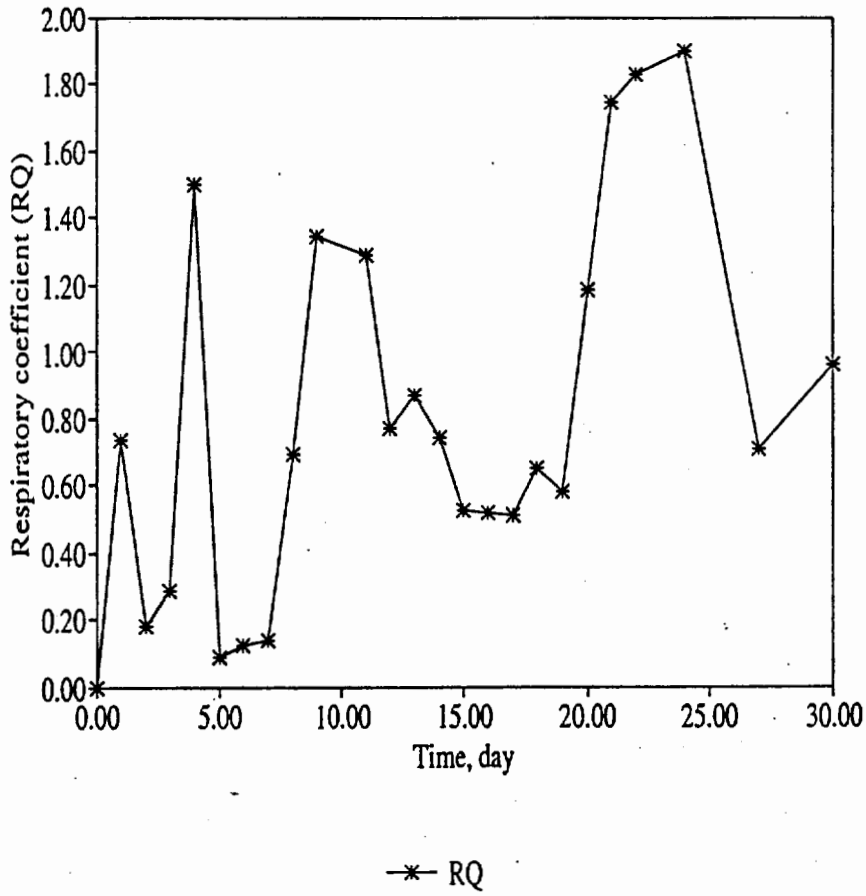

Figure 3 : Respiratory coefficient profile in the Applikon fermenter.

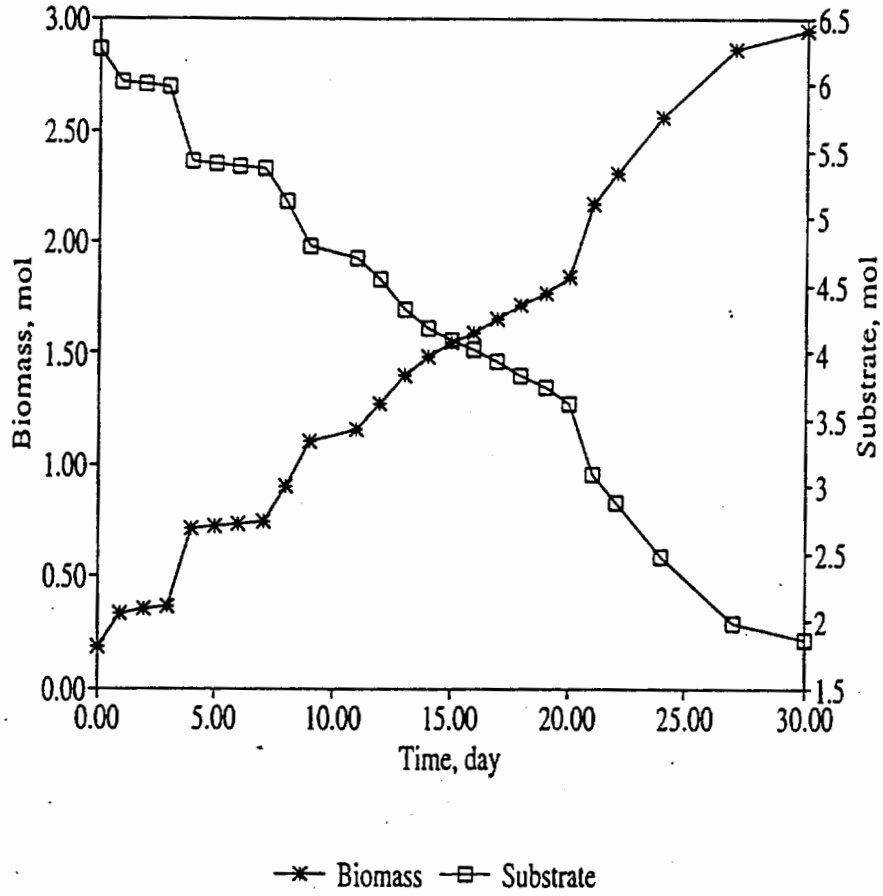

Figure 4 : Calculated biomass and substrate concentration profile for 30 days of fermentation

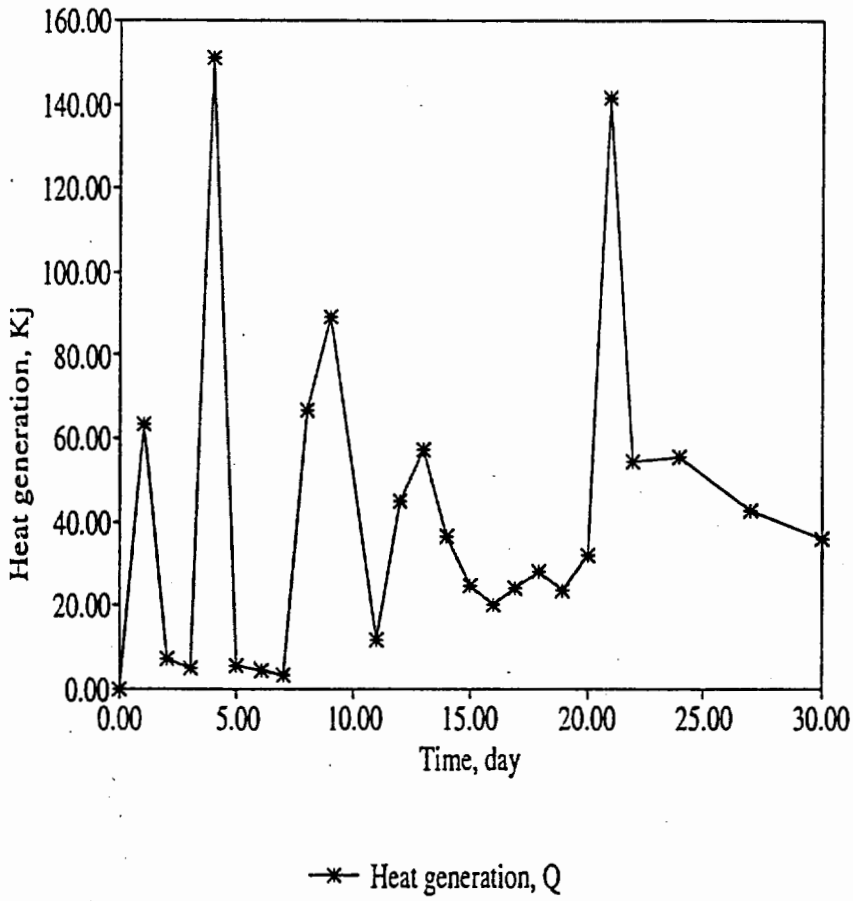

Figure 5 . Calculated instantaneous heat–interaction profile

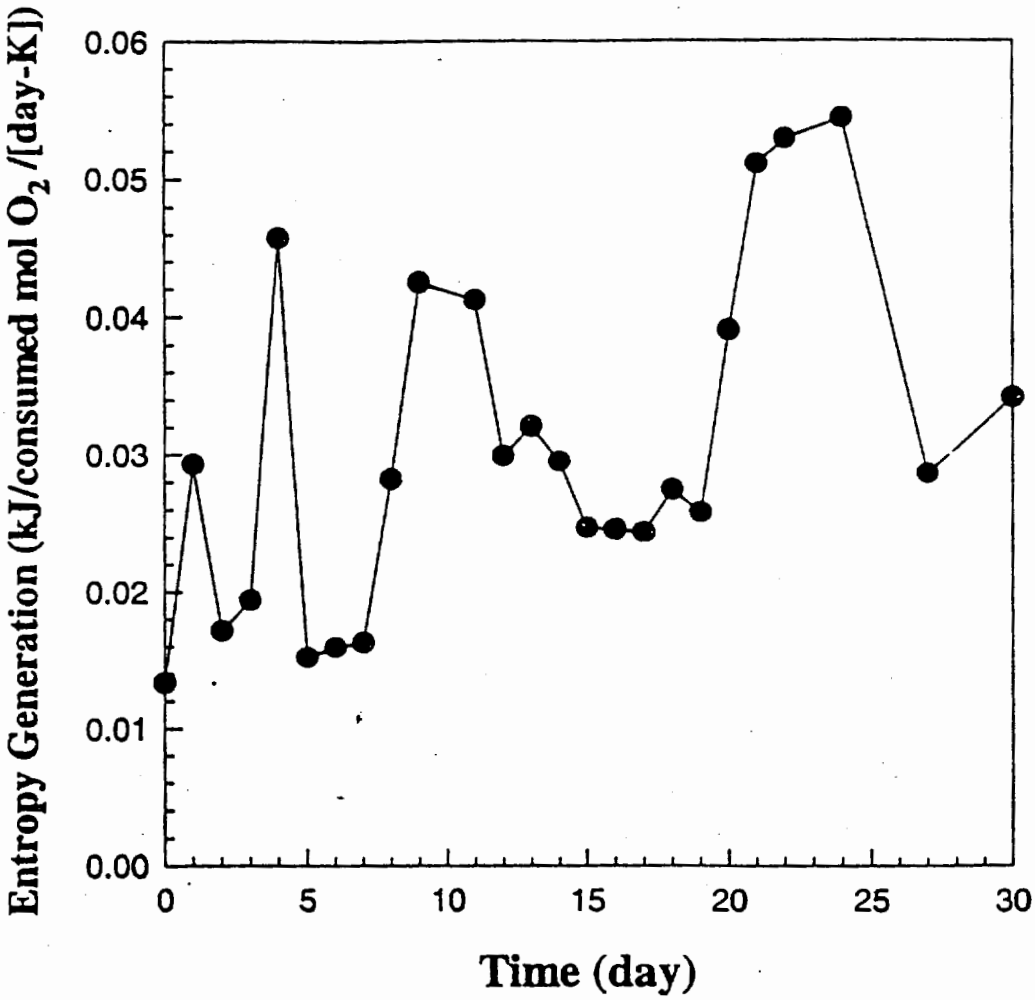

Figure 6 Entropy generation profile for 30-day fermentation

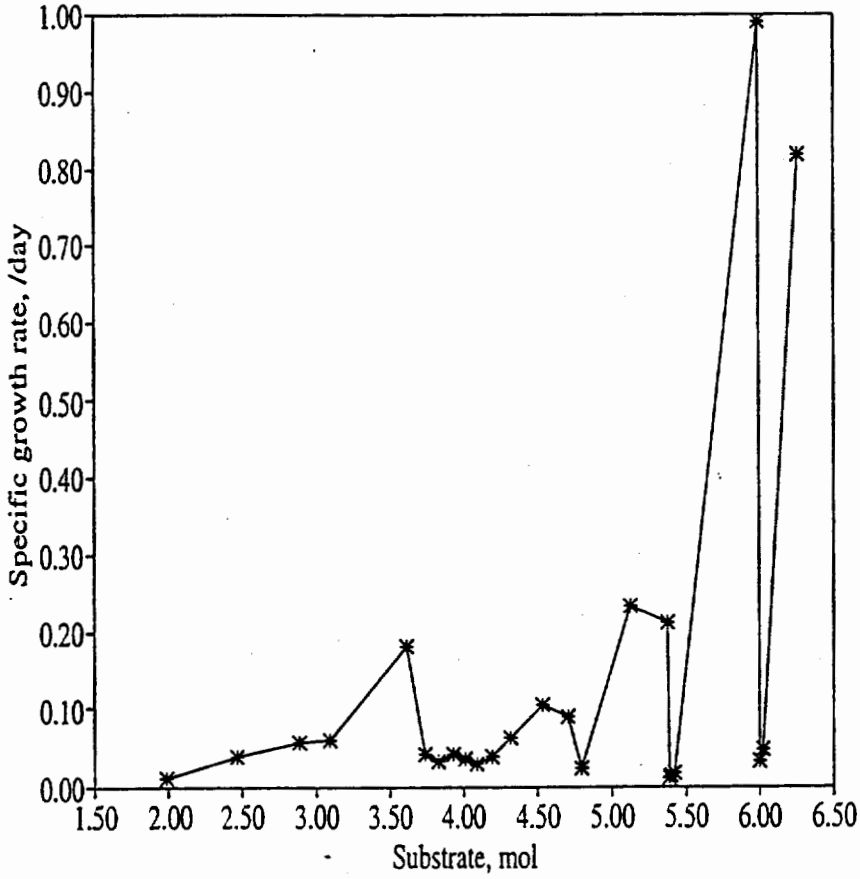

Figure 7.: Calculated specific growth rate profile with substrate utilization

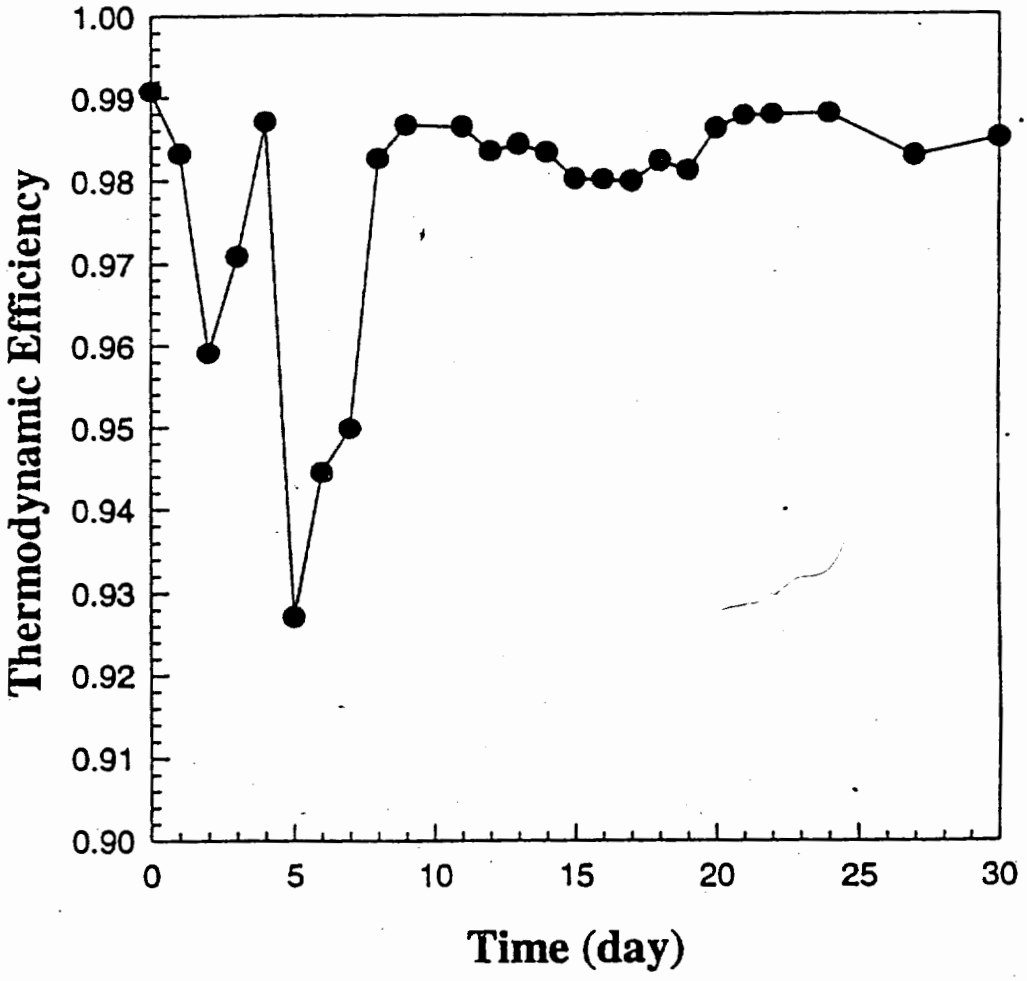

Figure 8 Thermodynamic efficiency profile

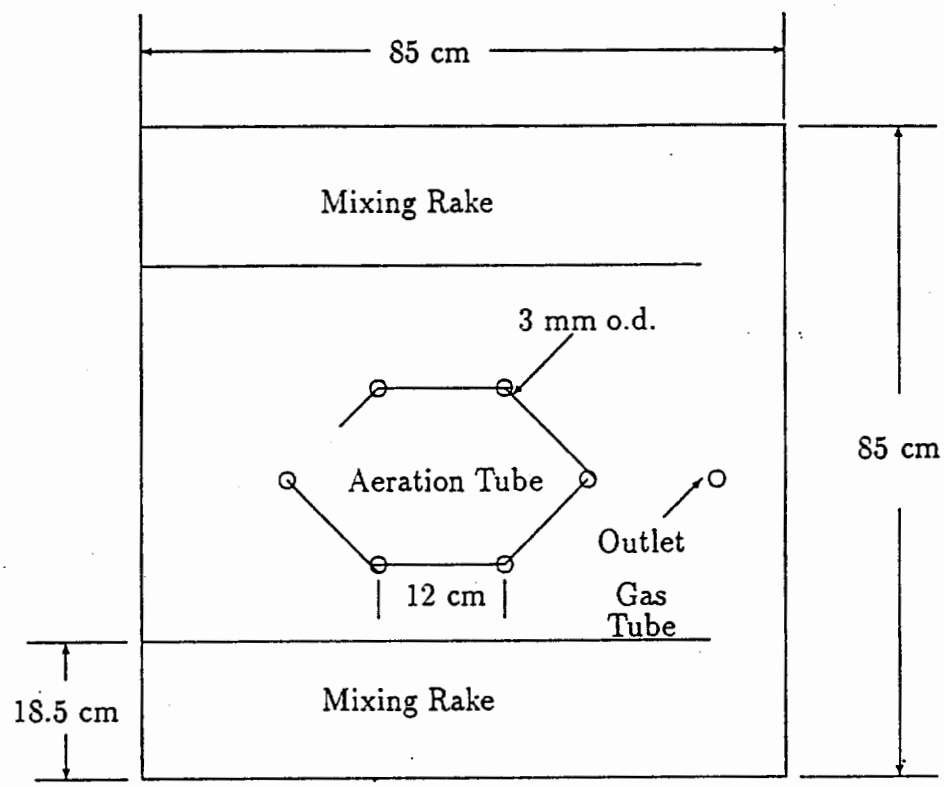

Figure 9 : Top view: Cross-section of the box–fermenter

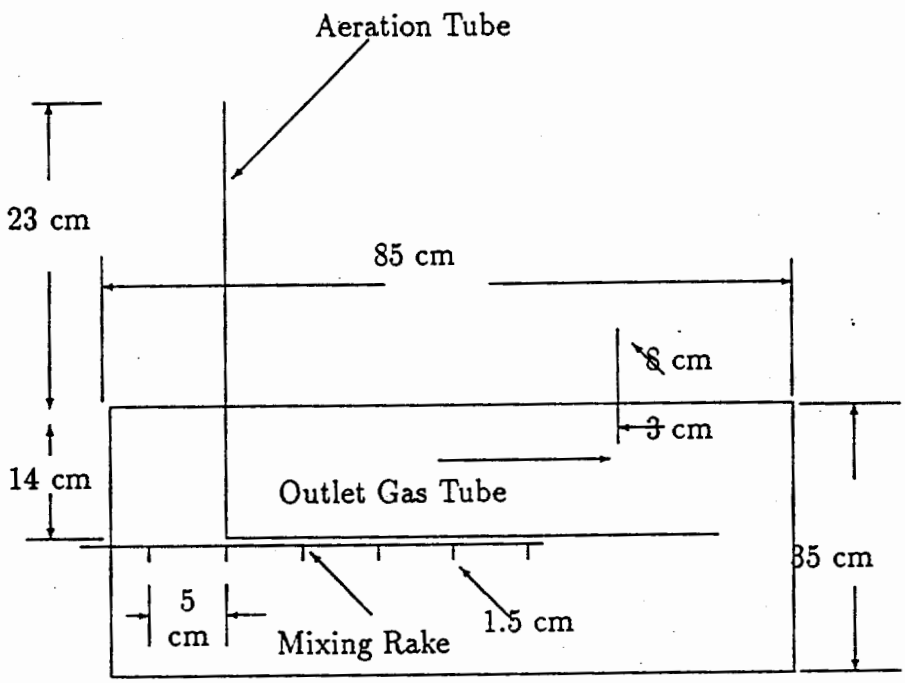

Figure 10 : Side view: Cross-section of the box–fermenter

ATTACHMENTS

1. Copy of the paper, "Mass balance and thermodynamic description of solid state fermentation of lignocellulosics by *Pleurotus ostreatus* for animal feed production"

2 . Copy of the thesis: "Degradation of lignin in water hyacinth by *Pleurotus ostreatus* for animal feed production"