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Mathematical Modeling of Biomass and Enzyme Production Kinetics by *Aspergillus niger* in Solid-State Fermentation at Various Temperatures and Moisture Contents

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Abstract

The effect of temperature and substrate moisture content on the growth and production of amylase, protease and phytase by *Aspergillus niger* during solid-state fermentation was investigated. A mathematical model regarding the kinetics of growth and enzyme production was performed to calculate the parameters at different temperatures and substrate moisture contents. The growth kinetics of *A. niger* could be described by the logistic growth model; the mathematical modeling parameters regarding maximum specific growth rate (μ_{max}) and maximum biomass concentration (X_{max}) were obtained by fitting the experimental data to the logistic model. The enzyme production kinetics could be described by the Luedeking-Piret model. The mathematical modeling parameters which included the growth-associated formation constant of the product *i* (α_i) and the non-growth-associated formation constant of the product *i* (β_i) were calculated. The production of amylase, protease and phytase was shown to be exclusively growth-associated. The effect of temperature on μ_{max}, X_{max} and α_i could be described by the cardinal temperature model with inflection (CTMI). Both growth and enzyme formation were clearly influenced by temperature and the optimum culture conditions for growth and enzyme production by *A. niger* were determined to be approximately 34°C with a substrate moisture content ranging from 40 to 60%.

Keywords: *Aspergillus niger*; Solid-state fermentation; Temperature; Moisture content; Mathematical modeling

Introduction

Solid-state fermentation (SSF) is defined as the cultivation of microorganisms on moist solid substrate in the absence of free water and is fundamentally different from submerged liquid fermentation (SLF). The level of water available for microbial growth in SSF is very low which is suitable for the cultivation of fungi [1,2]. SSF processes are used in a number of different industries for the production of enzymes, bioactive products, organic acids and biofuel. Amylase, protease and phytase are enzymes which are widely used in the animal feed industry. These enzymes can be used not only to improve the availability of nutrients in animal feed but also for the reduction of anti-nutritional factors, minimizing environmental pollution and decreasing costs [3,4]. *Aspergillus niger* is one of the most commonly used fungi for the production of enzymes used in the animal feed industry.

Temperature and substrate moisture content are critical factors affecting both growth and enzyme production by *A. niger* in SSF. Earlier publications have shown that the optimum temperature and substrate moisture content for growth of *A. niger* in SSF were 25-38°C [5-7] with a moisture content in the range of 40-70% [8-13]. Both parameters are important for the success of SSF processes. However, they are difficult to control in large scale bioreactors. One of the main problems encountered during large scale fermentations is heat accumulation due to cell metabolism. Heat generation can cause high temperatures in the system and potentially inhibit spore germination, growth and enzyme production [14]. High moisture content can cause substrate agglomeration which can lead to decreased porosity and limited oxygen diffusion within the substrate [15]. Mathematical models can be used as an aid when improving the design and control of SSF processes. Information regarding how temperature and substrate moisture content affect growth and enzyme production is necessary for developing adequate models which can be used for optimizing SSF [16-22]. The research of Hamidi-Esfanhani et al. [9] which focused on the effect of temperature and moisture on the growth of *A. niger* in SSF was further explored and extended in our research. We investigated the effects of temperature and substrate moisture content on the growth of *A. niger*, studied the production of amylase, protease and phytase and developed kinetic models regarding both growth and enzyme production based on the logistic and the Luedeking-Piret model. Additional models describing the effect of temperature on both fungal growth and enzyme production were also developed based on the cardinal temperature model with inflection (CTMI) [23].

This research aimed to investigate the effect of temperature and substrate moisture content on the growth and enzyme production by *A. niger* and more importantly to develop kinetic models for the growth and enzyme production in SSF depending on these two variables. The knowledge gained from our research may contribute to the understanding and control of SSF processes in large-scale bioreactors.

Materials and Methods

Microorganism and preparation of spore suspension

Aspergillus niger ATCC 11414 was obtained from the American Type Culture Collection, Rockville, MD, USA. The culture was

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maintained by inoculating potato dextrose agar (PDA) plates with 1 ml spore suspension (2×10^7 spores/ml), cultivated at 30°C for 5 days and then store at 4°C until use.

Spores were prepared by cultivating *A. niger* for 5 days on PDA plates at 30°C. The spores were then harvested from the surface by adding sterile 0.10% Tween 80 (v/v) solution and scraping the surface with a sterile spatula. The spore suspension obtained was counted by a haemacytometer and spore concentration adjusted to 2×10^7 spores/ml.

Culture on membrane

The glucosamine content at various stages during the growth was studied by cultivating *A. niger* on membrane filters. Whatman No.1 filter papers, previously weighed and sterilized, were placed on PDA plates and one milliliter of spore suspension (2×10^7 spores/ml) was applied to the surface of each filter paper. The cultures were incubated at 30°C for 5 days. The biomass dry weights were determined by peeling off the filter papers from the agar and drying them in a hot air oven at 80°C. Glucosamine content in the filter papers was determined with a colorimetric assay as described by Aidoo et al. [24]; data regarding biomass versus glucosamine were plotted to establish a standard curve for biomass depending on measured glucosamine content (Figure 1). All experiments were performed in triplicate.

Culture on mixed substrate

The experiments were performed at four different temperatures (20, 30, 40 and 50°C) and substrate moisture contents (30, 40, 50 and 60%). Tapioca pulp (residue from the production of tapioca starch) and soybean pulp (residue from the production of soybean milk) at a weight ratio of 6:4 were used as a substrate in all experiments. Moisture content of the mixed substrate was adjusted to 30, 40, 50 and 60% ± 3% with distilled water. Five grams of the mixed substrate were placed on a plate and autoclaved. The substrate was cooled and mixed with 0.50 ml spore suspension (2×10^7 spores/ml) per plate. The culture plates were placed in airtight plastic boxes and incubated at 20, 30, 40 and 50°C for 108 h. Samples were taken every 12 h during the cultivation for measuring moisture content [25], biomass, amylase activity, protease activity and phytase activity. All experiments were performed in triplicate.

Analytical methods

Determination of biomass: Concentration of biomass was determined by measuring the glucosamine and then comparing the determined glucosamine content with the previously developed glucosamine content standard curve (Figure 1).

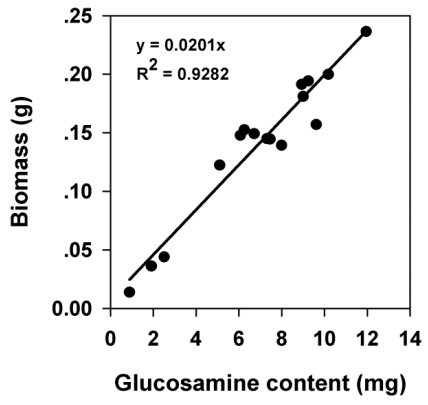

Figure 1: Glucosamine content of *A. niger* during fermentation at 30°C using membrane filter cultures.

Amylase assay: Amylase activity was assayed by measuring the amount of glucose released from the hydrolysis reaction of starch. The glucose concentrations were measured by the dinitrosalicylic acid (DNS) method according to Nelson [26]. One unit of amylase activity was defined as the amount of amylase required to release 1 µg of glucose in 1 minute at 60°C, pH 4.0.

Protease assay: Protease activity was measured according to the modified method of Anson [27] using bovine serum albumine (BSA) as substrate. One unit of protease activity was defined as the amount of protease required to release 1 µg of tyrosine in 1 minute at 60°C, pH 4.0.

Phytase assay: Phytase activity was assayed by measuring the amount of inorganic phosphorus released from sodium phytate using the method of Engelen et al. [28]. One unit of phytase activity was defined as the amount of phytase required to release 1 µg of inorganic phosphorus in 1 minute at 37°C, pH 5.50.

Mathematical models

Microbial growth kinetics: The fungal growth kinetics in solid-state fermentation was described empirically by the logistic equation [19]:

$$\frac{dX}{dt} = \mu_{max}\left(1 - \frac{X}{X_{max}}\right)X \quad (1)$$

Where μ_{max} is the maximum specific growth rate (1/h), X_{max} is the maximum biomass concentration (gram per gram substrate dry weight, g/gsdw), X is the biomass concentration (g/gsdw) and t is time (h). The logistic equation was used to describe the growth profile of *A. niger* during the exponential growth and stationary phase.

Enzyme production kinetics: The kinetics of enzyme production were based on the Luedeking-Piret equation [29] and describes the relationship between biomass and enzyme production. This model which combines the growth-associated and non-growth-associated contribution to the enzyme production can be written as follows:

$$\frac{dP_i}{dt} = \alpha_i \frac{dX}{dt} + \beta_i X \quad (2)$$

Where α_i is the growth-associated formation constant of the product i (unit per gram, U/g), β_i is the non-growth-associated formation constant of the product i ((U/g)/h), P_i is the concentration of the product i (unit per gram substrate dry weight, U/gsdw); i represents either amylase, protease or phytase.

The effect of temperature on the growth rate and the enzyme formation: The effect of temperature on the maximum specific growth rate (μ_{max}) was described using the cardinal temperature model with inflection (CTMI) as proposed by Rosso et al. [23]. The three cardinal temperatures (T_{min}, T_{opt} and T_{max}) and the maximum specific growth rate at the optimum temperature (μ_{opt}) were estimated by the following equation:

$$\mu_{max} = \frac{\mu_{opt}(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)]} \quad (3)$$

Where T is the temperature (°C), T_{max} is the maximum temperature (°C), T_{min} is the minimum temperature (°C), T_{opt} is the optimum temperature (°C) and μ_{opt} is the maximum specific growth rate at the optimum temperature (1/h).

Moreover, the CTMI can be extended to describe the influence of temperature during fermentation on the maximum biomass concentration (X_{max}) and the growth-associated formation constant of the product *i* (α_i) with the following equations:

$$X_{max} = \frac{X_{opt}(T-T_{max})(T-T_{min})^2}{(T_{opt}-T_{min})[(T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T)]} \quad (4)$$

and

$$\alpha_i = \frac{\alpha_{iopt}(T-T_{max})(T-T_{min})^2}{(T_{opt}-T_{min})[(T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T)]} \quad (5)$$

Where X_{opt} is the maximum biomass concentration at the optimum temperature (g/gsdw) and α_{iopt} is the growth-associated formation constant of the product *i* at the optimum temperature (U/g).

Numerical solution

The dynamic behavior of cell growth and enzyme production could be described by the set of differential equations presented in Equations (1-5). These equations were integrated numerically and the models were fitted to the experimental data using Berkeley Madonna 8.01 [30].

Results and Discussion

Glucosamine content of *A. niger*

Direct measurement of penetrative fungal biomass in SSF is impossible due to the inability to separate fungal biomass from the substrate. To circumvent the problem one can indirectly measure glucosamine which has been shown to be proportional to the fungal biomass [24]. The conversion factors needed for biomass estimation were determined by cultivating *A. niger* on membrane filters which enables the measurement of both biomass and glucosamine contents. The data obtained could then be used to calculate the relationship between the glucosamine content and biomass.

The relationship between glucosamine content and *A. niger* biomass was determined every 6 h for 120 h. The results showed that the ratio of glucosamine content to biomass was constant with a ratio of 49.75 mg glucosamine per gram biomass dry weight (Figure 1). This value is in agreement with the result of Hamidi-Esfahani et al. [9] who noted a constant ratio of 50 mg glucosamine per gram fungal biomass dry weight for *A. niger* when grown under similar conditions.

Effect of temperature and moisture content on the growth

A. niger were cultivated on substrates containing either 30, 40, 50 or 60% moisture and were incubated at a constant temperature of either 20, 30, 40 or 50°C in order to investigate the effect of substrate moisture content and temperature on the growth. Both substrate moisture content and temperature were found to have a pronounced effect on the growth of *A. niger* (Figure 2). At 30°C with moisture content in the range of 40 to 60%, the biomass concentration increased slowly for the first 24 h whereafter the biomass concentration increased more rapidly until 72 h when the concentration leveled out and reached approximately 0.25 g/gsdw (Figure 2B).

The growth curves looked similar when grown at 30°C or 40°C; however, the maximum biomass concentration was slightly lower when

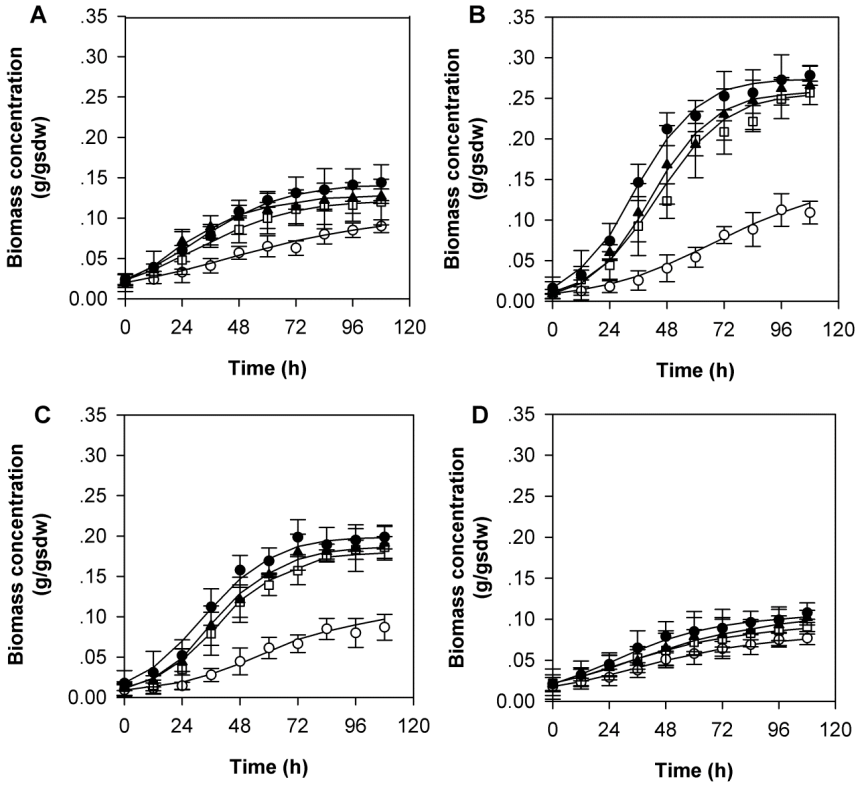

Figure 2: Comparison of the model predictions (solid lines) and experimental data (symbols) for the biomass concentration of *A. niger* in fermentations over time at temperatures of (A) 20°C, (B) 30°C, (C) 40°C and (D) 50°C with substrate moisture contents of 30% (○), 40% (□), 50% (▲) and 60% (●).

incubated at 40°C. Cultivation of *A. niger* at 20°C or 50°C was clearly less favorable for the growth regardless of substrate moisture content. Low concentrations of biomass were also found when the substrate had a moisture content of 30% at all temperatures investigated. Similar studies have shown that *A. niger* does not grow well at temperatures exceeding 45°C [8-9,11,20]. The conditions in our investigation which gave the best growth of *A. niger* were a temperature of 30°C and a substrate moisture content in the range of 40-60%. Under these conditions, the biomass reached a concentration of approximately 0.25 g/gsdw. The biomass concentration reached under these conditions agrees with the result of Hamidi-Esfahani et al. [9] who studied the influence of temperature and substrate moisture content on the growth of *A. niger* when using wheat bran as substrate. The highest concentration of biomass they obtained was identical to ours and reached 0.25 g/gsdw when fermenting at 35°C and with a substrate moisture content of 55%.

During SSF of *A. niger,* pH of the substrate decreased from an initial pH of 5.7 to approximately pH 3.8 after 72 h (data not shown). This decrease agrees with the results of Nagel et al. [31] who also found that the substrate's pH decreased during fermentation from an initial pH 6 to pH 4. Similar studies have been conducted by Jecu [7], Donnell et al. [32] and Silveira et al. [33] who in addition observed that growth and enzyme production by *A. niger* did not differ significantly when cultivated at pH values between 3.0-6.0.

Effect of temperature and moisture content on enzyme production

Production of amylase, protease and phytase by *A. niger* at various temperatures and substrate moisture contents are shown in Figures 3-5. The activity over time for the different enzymes had approximately the same profiles as the one obtained for growth and accumulation of biomass (Figure 2). The production of all three enzymes increased slowly for the first 24 h followed by a more rapid increase during the exponential growth phase and slowed down in the stationary phase when the fermentation temperature was kept at 30°C and the substrate moisture content between 40 and 60%. The lowest production rate was observed at a temperature of 50°C for all enzymes, regardless of substrate moisture content. The decrease in enzyme production at higher temperatures agrees with the results of Jecu [7] who also noted a decrease in endoglucanase production by *A. niger* at temperatures above 34°C. Publications regarding the effect of temperature and substrate moisture content on the enzyme production by *A. niger* by SSF suggest an optimal temperature in the range of 28-34°C and a substrate moisture content between 40 and 70% [5-7,34]. Those data are in agreement with our results.

Microbial growth kinetics

The logistic equation was used to describe the growth kinetics of *A. niger* at different temperatures and substrate moisture contents. Figure 2 shows both the experimental data and the predicted curves obtained after fitting the experimental data to the logistic equation. The estimated values of the logistic model parameters; maximum specific growth rate (μ_{max}) and maximum biomass concentration (X_{max}) are shown in Table 1. Good correlations between the experimental data and model prediction data were obtained with correlation coefficients (R^2) exceeding 0.99. In all experiments the values of μ_{max} and X_{max} obtained from the model were within the range of 0.03-0.08 1/h and 0.10-0.27 g/gsdw, respectively. Incubation at 30°C with a substrate moisture content of 60% during the growth gave the highest μ_{max} and X_{max} reaching 0.08 1/h for μ_{max} and 0.27 g/gsdw for X_{max}. Our estimated

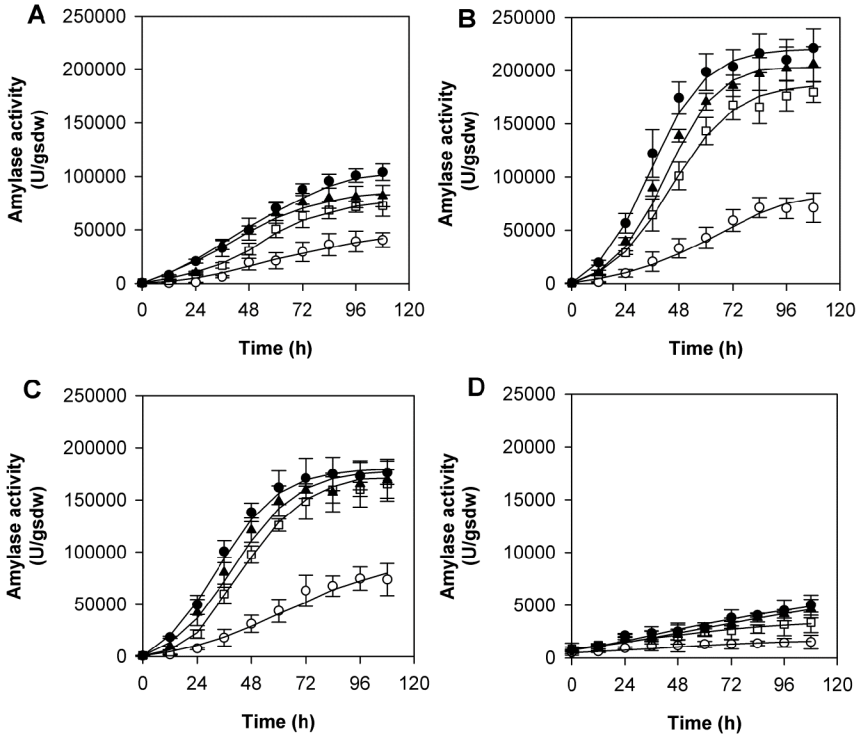

Figure 3: Comparison of the model predictions (solid lines) and experimental data (symbols) for the amylase formation of *A. niger* in fermentations over time at temperatures of (A) 20°C, (B) 30°C, (C) 40°C and (D) 50°C with substrate moisture contents of 30% (○), 40% (□), 50% (▲) and 60% (●).

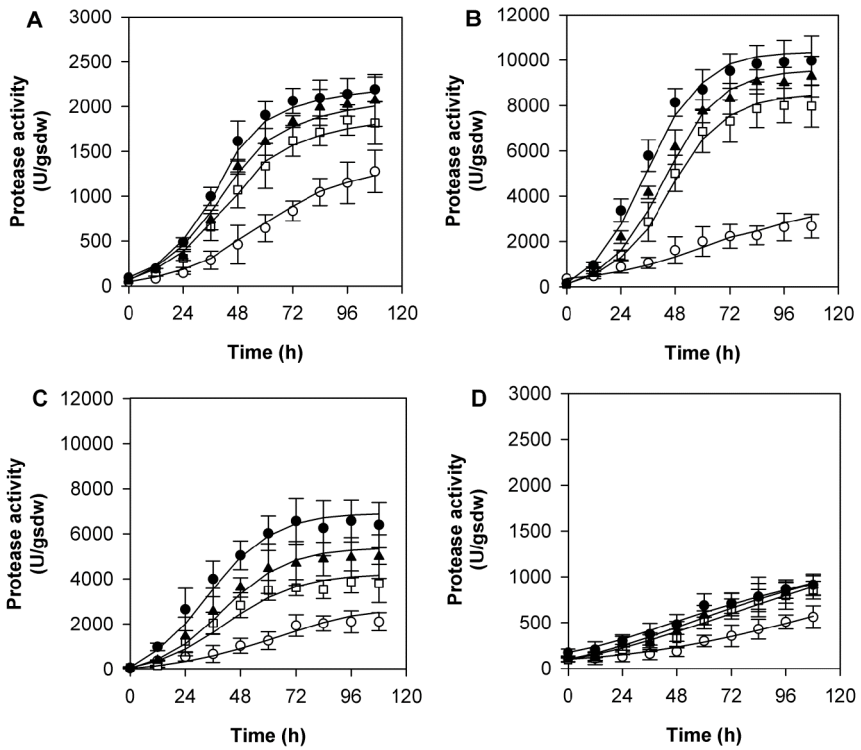

Figure 4: Comparison of the model predictions (solid lines) and experimental data (symbols) for the protease formation of *A. niger* in fermentations over time at temperatures of (A) 20°C, (B) 30°C, (C) 40°C and (D) 50°C with substrate moisture contents of 30% (○), 40% (□), 50% (▲) and 60% (●).

values of μ_{max} and X_{max} agree well with the result of Hamidi-Esfahani et al. [9] who reported that the highest values of μ_{max} and X_{max} were 0.29 1/h and 0.22 g/gsdw, respectively, when grown on wheat bran at 35°C and a substrate moisture content of 55%. In experiments performed by Szewczyk and Myszka [13], the optimum temperature for growth of *A. niger* on wheat bran and beet pulp was achieved at 35°C with a substrate moisture content of 60% which gave a μ_{max} of 0.25 1/h.

Enzyme production kinetics

Enzyme production kinetics was investigated by experiments where the SSF conditions, namely temperature and substrate moisture content, varied. The values of α_i and β_i can be estimated from the enzyme activity (U/gsdw) during fermentation by the Leudeking-Piret equation (Equation 2). The enzyme activity profiles for amylase, protease and phytase obtained from the experiments were numerically fitted to the equation and the model was shown to accurately describe the enzyme production under all these conditions. There was an excellent correlation between the experimental data and the model prediction, with R^2 values greater than 0.90. Comparisons between the experimental data and the predicted data are shown in Figures 3-5.

The estimated parameters regarding production of the three different enzymes which were derived from the model are shown in Table 2. In all experiments, the production of amylase, protease and phytase was closely correlated to cell growth. There was no significant difference regarding α_i values for the enzymes when the fermentations were performed at either 30°C or 40°C and a substrate moisture concentration in the range of 40-60%.

The expression profiles for the three different enzymes were shown to closely resemble the ones obtained for growth. This correlation was reinforced by the estimated β_i values which were close to zero. It affirmed the assumption that the enzymes are associated with growth and that enzyme activity is almost exclusively correlated to biomass accumulation.

Development of models for the effects of temperature on the growth rate and enzyme production

The influence of temperature on μ_{max} and the relationship between them can be described by the cardinal temperature model with inflection (CTMI) [23]. It can be used to estimate the three cardinal temperatures (T_{min}, T_{opt} and T_{max}) and the maximum specific growth rate at the optimum temperature (μ_{opt}) (Equation (3)). The CTMI can also be extended to correlate X_{max} and α_i with the three cardinal temperatures and X_{opt} or α_{iopt} (Equations (4)-(5)).

The μ_{max} parameters derived from the logistic model at different temperatures and substrate moisture concentrations were numerically fitted to the CTMI. The cardinal temperatures obtained for *A. niger* at various substrate moisture concentrations varied between 34-35°C for T_{opt}, 10-12°C for T_{min} and 54-56°C for T_{max} as listed in Table 3. The μ_{opt} and X_{opt} obtained varied more depending on substrate moisture content but were close to the estimated values of μ_{max} and X_{max} when cultivated at 30°C. An excellent correlation between the experimental data and the model prediction was obtained giving R^2 values greater than 0.90. Similar studies where *A. niger* was grown on malt extract agar have given T_{opt} values within the range of 31-34°C [35-37].

As previously mentioned, the CTMI can be extended to describe the correlation between α_i and temperature. The three cardinal temperatures and the α_{iopt} for enzyme production could be estimated after the experimental data was fitted to equation (5). This was done for

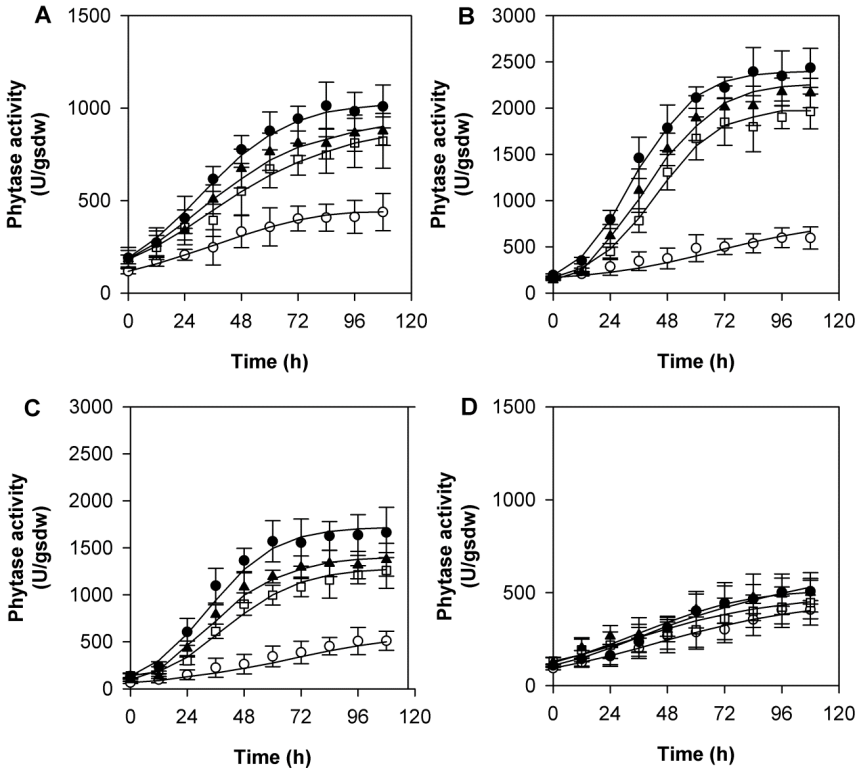

Figure 5: Comparison of the model predictions (solid lines) and experimental data (symbols) for the phytase formation of *A. niger* in fermentations over time at temperatures of (A) 20°C, (B) 30°C, (C) 40°C and (D) 50°C with substrate moisture contents of 30% (○), 40% (□), 50% (▲) and 60% (●).

Temperature (°C)	Moisture content (%)	μ_{max} (1/h)	X_{max} (g/gsdw)	R^2
20	30	0.032	0.102	0.997
	40	0.049	0.123	0.996
	50	0.055	0.130	0.995
	60	0.053	0.146	0.998
30	30	0.040	0.147	0.998
	40	0.070	0.257	0.999
	50	0.078	0.266	0.996
	60	0.081	0.273	0.999
40	30	0.039	0.128	0.996
	40	0.068	0.187	0.987
	50	0.070	0.188	0.998
	60	0.070	0.199	0.996
50	30	0.033	0.086	0.997
	40	0.038	0.094	0.999
	50	0.036	0.106	0.995
	60	0.040	0.104	0.996

Table 1: The maximum specific growth rate (μ_{max}) and the maximum biomass concentration (X_{max}) derived from the logistic growth model at different temperatures and substrate moisture contents.

all three enzymes and the obtained values are summarized in Table 4. Again, the correlation was good and R^2 values were greater than 0.93. The optimum temperature (T_{opt}) for protein expression of all three enzymes was in the range of 34-35°C which is the same as the T_{opt} estimated for growth.

Conclusion

Both temperature and substrate moisture content of the media clearly affected both growth and enzyme production by *A. niger* in solid-state fermentation. We found that the logistic growth model could successfully be used to describe the growth kinetics of *A. niger*. The production of amylase, protease and phytase was found to follow the Luedeking-Piret model and by fitting the model with the experimental data it was shown that the expressions of these enzymes were growth-associated and directly correlated to the biomass.

The cardinal temperature model with inflection (CTMI) was applied

		Amylase			Protease			Phytase		
Temp	MC	α_{amy}	β_{amy}	R^2	α_{pro}	β_{pro}	R^2	α_{phy}	β_{phy}	R^2
20°C	30%	13,488	0.001	0.973	9,270	0.001	0.985	4,192	0.001	0.997
	40%	433,364	0.001	0.993	16,858	0.001	0.970	4,891	0.001	0.995
	50%	405,862	0.001	0.992	16,849	0.001	0.968	5,808	0.001	0.994
	60%	421,204	0.001	0.990	16,255	0.001	0.971	6,894	0.001	0.961
30°C	30%	33,767	0.001	0.958	14,068	0.001	0.957	4,420	0.001	0.961
	40%	789,996	0.005	0.958	31,065	0.005	0.961	8,097	0.005	0.985
	50%	837,629	0.005	0.951	36,732	0.005	0.956	8,271	0.005	0.976
	60%	855,027	0.005	0.972	39,703	0.005	0.975	8,462	0.005	0.996
40°C	30%	254,626	0.001	0.975	16,670	0.001	0.969	4,406	0.001	0.963
	40%	709,612	0.001	0.956	28,856	0.005	0.972	6,603	0.002	0.989
	50%	783,851	0.002	0.955	30,146	0.005	0.959	7,399	0.002	0.977
	60%	793,756	0.002	0.965	37,763	0.005	0.961	8,077	0.002	0.989
50°C	30%	16,855	0.001	0.960	5,831	0.001	0.994	4,282	0.001	0.992
	40%	29,677	0.001	0.979	6,060	0.001	0.991	4,285	0.001	0.990
	50%	24,310	0.001	0.991	6,286	0.001	0.993	4,294	0.001	0.982
	60%	24,320	0.001	0.987	6,904	0.001	0.992	4,396	0.001	0.994

Table 2: Estimation of the growth-associated formation constant (α) and the non-growth-associated formation constant (β) predicted by the Luedeking-Piret model for expression of amylase (α_{amy}, β_{amy}), protease (α_{pro}, β_{pro}) and phytase (α_{phy}, β_{phy}). α is expressed as (U/g), β as ((U/g)/h) and moisture content (MC) in percent.

Moisture content (%)	μ_{max} (1/h)					X_{max} (g/gsdw)				
	μ_{opt} (1/h)	T_{min} (°C)	T_{opt} (°C)	T_{max} (°C)	R^2	X_{opt} (g/gsdw)	T_{min} (°C)	T_{opt} (°C)	T_{max} (°C)	R^2
30	0.040	12	35	56	0.989	0.143	10	34	56	0.981
40	0.070	10	34	54	0.995	0.237	13	34	53	0.947
50	0.078	10	34	54	0.999	0.242	12	34	53	0.925
60	0.079	10	34	55	0.967	0.250	12	34	54	0.948

Table 3: Estimated values of the parameters derived from the CTMI describing the effect of temperature on the growth rate (μ) and accumulation of biomass (X) for *A. niger* and the predicted μ_{opt} and X_{opt} at different substrate moisture contents.

	Moisture content	α_{opt} (unit/g)	T_{min} (°C)	T_{opt} (°C)	T_{max} (°C)	R^2
amylase	30%	337,671	19	35	50	0.976
	40%	829,575	18	34	50	0.999
	50%	890,583	10	34	50	0.996
	60%	914,782	17	34	50	0.992
protease	30%	16,701	10	35	52	0.992
	40%	29,440	10	34	51	0.979
	50%	36,507	15	34	51	0.996
	60%	40,304	14	34	51	0.999
phytase	30%	1,437	10	35	55	0.987
	40%	7,408	10	34	57	0.937
	50%	8,235	9	34	57	0.996
	60%	8,921	11	34	55	0.999

Table 4: Estimated values of the parameters derived from the CTMI describing the effect of temperature on the growth-associated formation constant (α) and the predicted α_{opt} for amylase, protease and phytase at different substrate moisture contents.

to describe the effect of temperature and moisture content on μ_{max}, X_{max} and α_i and used to calculate T_{min}, T_{opt}, T_{max}, μ_{opt}, X_{opt} and α_{iopt}. All the data showed a high level of correlation with the proposed models and could have applications when scaling up fermentations in bioreactors.

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