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Ascorbic Acid Production by *Aspergillus flavus* and *Aspergillus tamarii*; Kinetic and Thermodynamic Study

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Abstract
This study investigated the fermentation kinetics and thermodynamics of ascorbic acid production from Brewery Spent Grain (BSG) using *Aspergillus flavus* and *Aspergillus tamarii*. Ascorbic acid fermentation of *A. flavus* and *A. tamarii* was performed at a temperature of 30 °C, agitation speed of 100 rpm and pH 5.0 at 96 h of fermentation. The thermodynamics, kinetics of the growth parameters and ascorbic acid production were studied using Monod, Contois and Teisser models. Teisser model gave the best fit as it obtained the highest maximum specific growth rate (μ_{max}) and correlation coefficient of 0.184 h^{-1} and 0.997, respectively, at 40 °C, pH 5.0 and 0.6 g of BSG. The result showed that Teisser model gave a better description of each growth parameter. Hence, the production of ascorbic acid by *A. flavus* and *A. tamarii* is growth-associated.

Introduction

Ascorbic acid is a water-soluble vitamin which aids the normal functioning of the human body. This vitamin helps in the stimulation of certain enzymes, collagen biosynthesis, hormonal activation, anti-oxidant, histamine detoxification, formation of nitrosamine, proline hydroxylation prevention of scurvy [1]. Ascorbic acid occurs naturally in a wide variety of plants and animals [2]. It is an organic acid with anti-oxidant properties in chemical and biological systems [3]. The L-enantiomer of ascorbic acid is commonly known as vitamin C in nutritional context and it also encompasses the oxidation product of dehydroascorbic acid with different oxidizing agents [4].

The kinetics of cell growth and product formation using a mathematical model has been in use for elucidation of the complex and multicomponent fungal fermentation system with different approaches to modeling microbial kinetics [5]. The unstructured kinetic model considers cell as a uniform quantity without internal dynamic [6] since cell growth involves various biochemical networks and chemical reaction [7]. Unlike the unstructured kinetic models, the structured kinetic models are dependent on the biomass components, especially the concentration of nucleic acids, protein, metabolism and enzymes [8].

The importance of thermodynamics study cannot be overemphasized as it provides information on the feasibility of a chemical reaction. It is also instrumental in defining the physico-chemical conditions under which reactions can occur. Summarily, thermodynamics plays an important role in many chemical reactions and process development [9]. Thermodynamics has been used in fermentation process to estimate key parameters of the reaction to predict the economic viability of the process, although, it is hardly ever applied in biotechnology [10].

The importance and applications of ascorbic acid to other disciplines including: medicine, agriculture, food industry, flour industry and metallurgy had been reported by different researchers [11]. However, reports on the kinetics and thermodynamics of the chemical reactions involving this important organic acid are scanty [12]. Hence, the objective of this study is to investigate the kinetics and thermodynamics of ascorbic acid production from BSG using *Aspergillus flavus* and *Aspergillus tamarii*.

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Materials and Methods

Fungal Source

Pure cultures of ascorbic acid producing strains of *A. flavus* and *A. tamarii* were obtained from the Microbiology laboratory of the Federal University of Agriculture, Abeokuta, Nigeria. The pure cultures of these fungi were maintained on Sabouraud Dextrose Agar.

Sources of Substrates

Brewery waste was obtained from Sona Breweries, Ota, Ogun State, Nigeria. Cassava starch flour was obtained from a market in Abeokuta, Ogun State, Nigeria.

Production and Quantification of Ascorbic Acid Under Optimum Conditions by *A. flavus* and *A. tamarii*

Spores of *A. flavus* and *A. tamarii* were cultured on the brewery spent grain (BSG) medium according to the methods of Banjo et al. [13]. The fermentation process was monitored for 7 days and the ascorbic acid produced quantified by using the titrimetric method of the Association of Vitamin Chemist [14] and High performance liquid chromatography (HPLC).

Kinetic Studies of Ascorbic Acid Production by *Aspergillus spp*

Kinetic studies were carried out using the different parameters of temperature, substrate concentration and pH. Three kinetic models (Contois, Monod and Teisser) were used in the estimation of the specific growth rate.

Kinetics of Ascorbic Acid Production by *Aspergillus* spp at Different Substrate Concentrations

The effect of different concentrations of the substrate (BSG) in the range 0.2–1.0 g on kinetic parameters, μ, P_{max}, P_0 and R^2 was studied. This was carried out by inoculating the spores of *A. flavus* and *A. tamarii* (2×10^9 spore/ml) on brewery waste medium (0.6% brewery waste, 2% glucose, 0.3% galactose, 0.3% yeast extract, 0.5% peptone, 0.2% monosodium glutamate) at 30 °C and pH 5 in a 250 ml Erlenmeyer flask. The ascorbic acid produced was quantified at 96 h of fermentation as previously described.

Kinetics of Ascorbic Acid Production by *Aspergillus* spp at Different Temperatures

The effect of *temperature* on ascorbic acid production was studied by inoculating the spores of *A. flavus* and *A. tamarii* (2×10^9 spore/ml) on brewery waste medium (0.6% brewery waste, 2% glucose, 0.3% galactose, 0.3% yeast extract, 0.5% peptone, 0.2% monosodium glutamate) at optimum BSG concentration. The effect of temperature on kinetic parameters, μ, P_{max}, P_0 and R^2 was evaluated at 30, 35, 40 and 45 °C. The ascorbic acid produced was determined by titration as previously described.

Kinetics of Ascorbic Acid Production by *Aspergillus spp* at Different pH

Spores of *A. flavus* and *A. tamarii* (2×10^9 spore/ml) on brewery waste medium (0.6% brewery waste, 2% glucose, 0.3% galactose, 0.3% yeast extract, 0.5% peptone, 0.2% monosodium glutamate) at optimum BSG concentration and temperature. Effect of pH on kinetic parameters, μ, P_{max}, P_0 and R^2 was evaluated at pH range 4.0–8.0 (pH 4.0, 5.0, 6.0, 7.0 and 8.0) and the quantity of ascorbic acid produced was determined at 96 h of fermentation as previously described.

Product Formation

The ascorbic acid concentration profile was obtained by direct computing as shown in the equation [15]

$$P = \frac{P_0 e^{p_r t}}{1 - \left(\frac{p_0}{p_m}\right)\left(1 - P_0\right)}$$

where P_r is specific growth rate (h^{-1}), P_0 is initial cell concentration (g/L) and P_m is maximum cell concentration (g/L).

Thermodynamics of Ascorbic Production

Thermodynamics studies of ascorbic acid production by *A. flavus* and *A. tamarii* was carried out to determine the activation energy (E_a) required for the reaction using the Arrhenius equation;

$$K = Ae^{-E_a/(RT)}$$

where A is pre exponential factor, T is absolute temperature and K is reaction rate constant.

Mathematical Models

The kinetic parameters of the ascorbic acid fermentation were studied by direct computing using three different kinetic models namely Monod, Contois and Teisser using the *SCIENTIST* micromath software.

Monod Model

The most widely utilized unstructured kinetic model is Monod model given by [16];

$$\mu - \frac{\mu_{\max} S}{K_s + S}$$

where μ is the specific growth rate in h^{-1}, S is substrate concentration in g/L, K_s is the Monod constant and $\mu_{\max}$ is the growth rate.

Contois Model

Contois kinetic is a modified Monod model [17]

$$\mu = \frac{\mu_{\max} S}{K_x X + S}$$

where K_X is the Contois kinetic constant.

Teisser Model

Teisser model expresses the growth kinetic by relating μ to S exponentially [18]. This model is being adapted from Monod model given by the following equation [19]

$$\mu = \mu_{\max}\left(1 - \exp^{\frac{S}{ks}}\right)$$

Results and Discussion

Production and Quantification of Ascorbic Acid by *A. flavus* and *A. tamarii* using High Performance Liquid Chromatography (HPLC)

Studies on the fermentation of the BSG medium with *A. flavus* and *A. tamarii* showed that ascorbic acid yield peaked at 96 h of fermentation. At this optimum fermentation time of 96 h, ascorbic acid produced by *A. tamarii* and *A. flavus* as quantified by HPLC are 7.25 g/L and 6.25 g/L, respectively (Figs. 1, 2). Thus, 96 h was adopted as the optimum fermentation time for further studies. The yield of ascorbic acid by *A. tamarii* and *A. flavus* are inversely proportional to the fermentation time (Table 1). However, ascorbic acid yield was completely degraded in the fermentation broth containing *A. flavus* at 120 h of fermentation. The loss of ascorbic acid may be a result of the increase in the activity of Ascorbate oxidase produced by the microorganisms in the fermentation medium. This enzyme is implicated in the

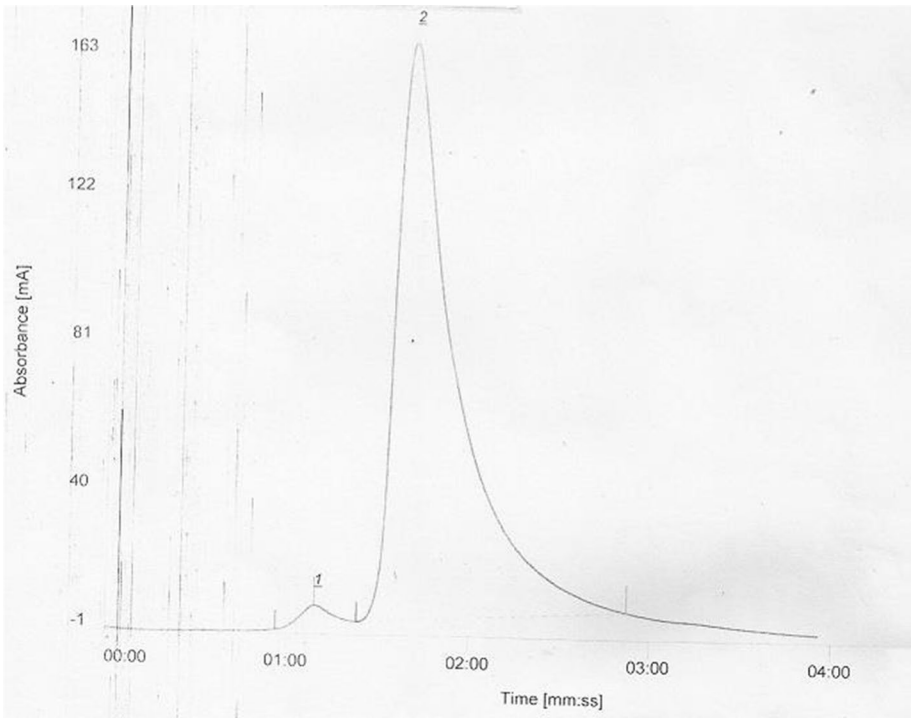

Fig. 1 High performance liquid chromatography of ascorbic acid produced by *A. flavus*

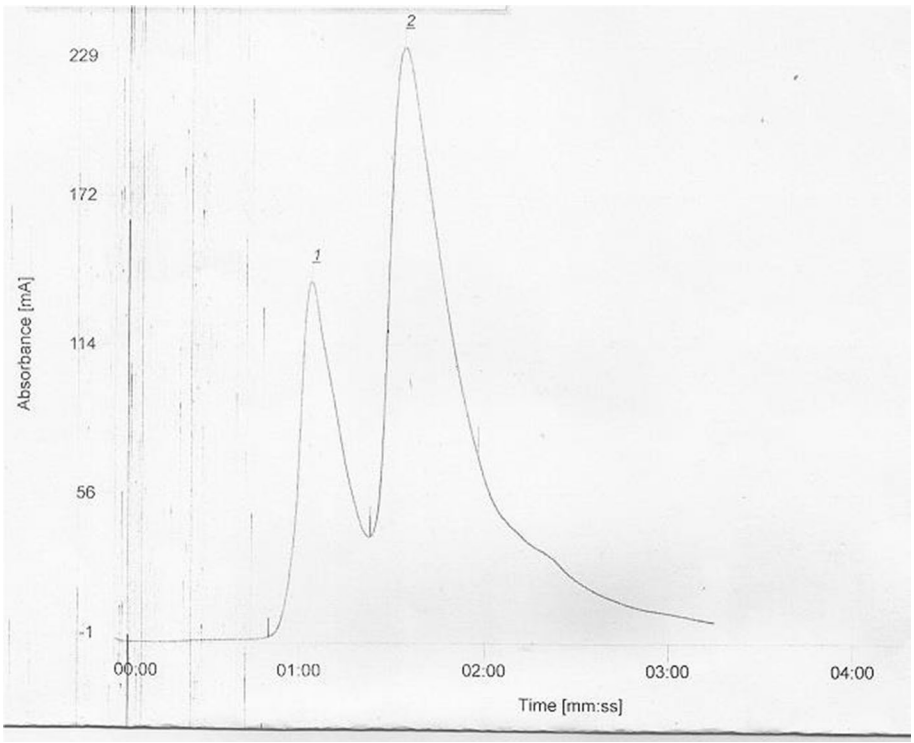

Fig. 2 High performance liquid chromatography of ascorbic acid produced by *A. tamarii*

Table 1 Production of ascorbic acid from brewery spent grain by *A. flavus* and *A. tamarii*

Isolates	Fermentation time (Hours)										
	12	24	36	48	60	72	84	96	120	144	168
Aspergillus flavus	0	0	1.6	2	3.2	4	5.6	6.25	0	0	0
Aspergillus tamarii	0	0	2	2.5	3.6	4.25	6.4	7.25	5	2.25	0.25

conversion of the ascorbic acid produced in the fermentation medium to other analogues of ascorbic acid [20].

Effect of Substrate Concentration on Kinetic Parameters

Kinetic studies on ascorbic acid production by Strains of *A. flavus* and *A. tamarii* were investigated. Different concentrations of substrate (BSG) resulted in varying growth rates with optimum specific growth rate values of 0.084 h^{-1} and 0.067 h^{-1} by *A. flavus* and *A. tamarii*, respectively, at a BSG concentration of 0.6 g. Also, the optimum value of P_{max} (maximum cell concentration) was 9.885 mg/L by *A. tamarii*. The results showed that there is a strong correlation between ascorbic acid production and microbial growth or cell concentration. The growth of the two isolates is optimum at BSG concentration of 0.6 g, promoting maximum cell concentration and this contributes to high ascorbic acid production. At high initial substrate concentrations, exponential growth is supported and the specific growth rate remains at its maximum value. However, increase in the substrate concentration resulted in a reduced specific

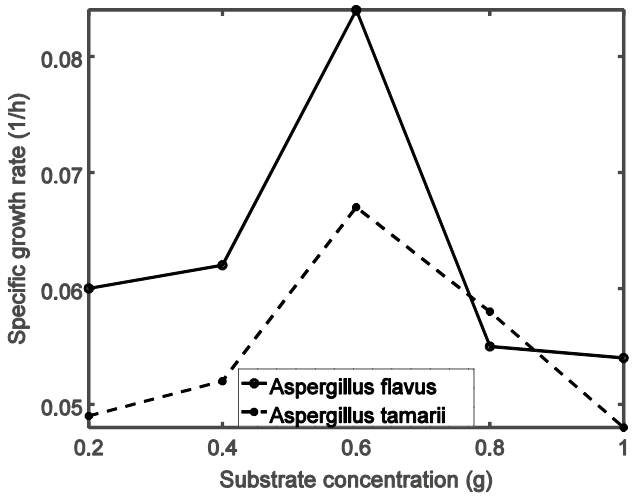

Fig. 3 Effect of substrate concentration on specific growth rate of *A. flavus* and *A. tamarii*

growth rate of the cells (Fig. 3). The high substrate concentrations can result in substrate inhibition, which significantly lowers the hydrolysis rate [21]. Furthermore, high substrate

concentration affects the primary rate and yield of enzymatic hydrolysis which might be responsible for the reduced ascorbic ac id production [22].

Effect of Temperature on Kinetic Parameters

The effects of the different temperature employed in this study on the kinetics of ascorbic acid production revealed that *A. tamarii* gave a higher specific growth rate value of 0.083 h^{-1} compared to 0.065 h^{-1} by *A. flavus* at an optimum temperature of 40 °C. Also, the optimum value of P_{max} (maximum cell concentration) was 7.887 mg/L by *A. tamarii*. The growth of microorganisms is influenced by reactions brought about by enzymes within the cell. Increase in temperature resulted in decrease in microbial growth or cell concentration. The maximum concentration of cell decreased with increase in temperature. This decrease in the specific growth rate of the microorganisms might be as a result of the elevated temperature above the optimum (40 °C), resulting in loss of activity by the enzymes (Fig. 4). The results revealed that there is a direct relationship between ascorbic acid production and microbial growth or cell concentration. The growth of all the isolates were optimum at 40 °C, promoting maximum cell concentration and this contributes to high ascorbic acid production. This agrees with the findings of Rahid [23] who reported that microbial growth or cell growth influenced the rate of production of organic acid.

Effect of pH on Kinetic Parameters

The different pH values adopted in the production of ascorbic acid resulted in different growth rates of the microorganism under study. At an optimum pH of 5, *A. flavus* and *A. tamarii* gave specific growth rate values of 0.104 h^{-1} and 0.126 h^{-1}, respectively. Increase in pH up to 8 resulted in a decreased specific growth rate value by the two isolates. Thus the highest specific growth value was at pH 5 for the two isolates (Fig. 5). There is a direct correlation between the specific growth rate and the biomass production. The higher the Specific growth rate, the higher the rate of biomass production. Also, at pH 5 the maximum cell concentration optimum value (P_{max}) of 8.253 mg/L was given by *A. tamarii*. Therefore an environment, which is too acidic, neutral or alkaline, is not conducive for ascorbic acid. Hence, the pH of the culture medium directly influences the growth of microorganisms and the biochemical processes they perform [24, 25]. The finding of the present study is also in line with that of Chaurasia et al. [26]who reported an optimum pH of 5.0 in their work on organic acid production by a fungus (*Sclerotium rolfsii*).

Thermodynamics of Ascorbic Production

Thermodynamics studies of ascorbic acid production by *A. flavus* and *A. tamarii* were investigated. The result showed that the pre exponential factor (A) value of 360.911 h^{-1} was obtained for *A. flavus* while 4.639 h^{-1} was obtained for *A. tamarii.* Also, the activation energy (E_a) of 22,060.92 and 11,311.57 kJ/mol were obtained for *A. flavus* and *A. tamarii*, respectively. This shows that *A. tamarii* requires low energy, which means it is the most cost-efficient in terms of energy.

Comparison of Teisser, Contois and Monod Models in Ascorbic Acid Production

A comparative study on Teisser, Contois and Monod models was carried out on the fitting of the growth kinetic data obtained. Among the three models, Teisser model with the highest maximum specific growth rate (μ_{max}) of 0.066 h^{-1}

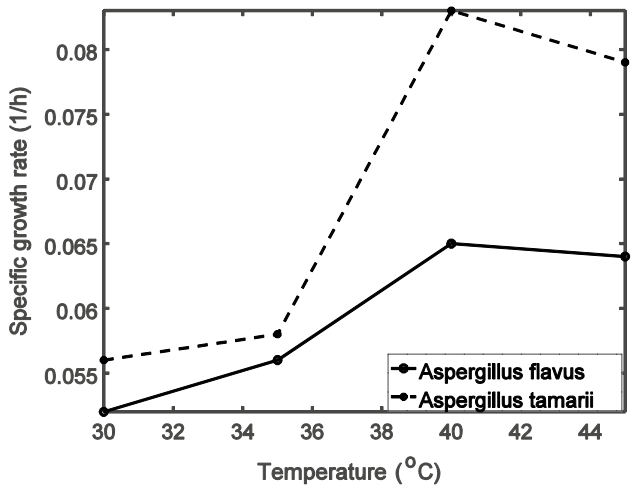

Fig. 4 Effect of temperature on specific growth rate of *A. flavus* and *A. tamarii*

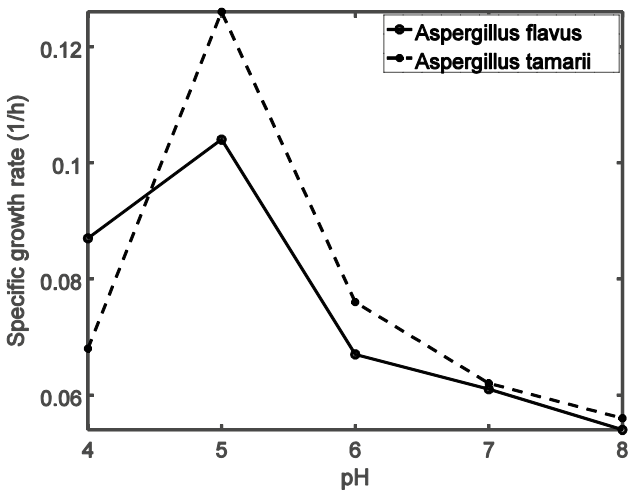

Fig. 5 Effect of pH on specific growth rate of *A. flavus* and *A. tamarii*

Table 2 Kinetic parameters for Teisser, Contois and Monod models

Models	Parameters	*A. flavus*	*A. tamarii*
Teisser	μ_{max}	0.066	0.060
	K_s	0.121	0.179
	R^2	0.997	0.975
Contois	μ_{max}	0.063	0.058
	K_x	0.147	0.158
	R^2	0.902	0.984
	X	1.000	0.158
Monod	μ_{max}	0.063	0.058
	K_s	0.003	0.025
	R^2	0.969	0.984

by *A. flavus* with a correlation coefficient (R^2) value of 0.997 gave the best fit for ascorbic acid production (Table 2). This is in agreement with the reports of Ahmad et al. [27] in which Teisser model fit better for ethanol production than other models investigated.

Conclusion

In conclusion, optimum ascorbic acid production was achieved at substrate concentration of 0.6 g, pH 5.0 and temperature of 40 °C. Among the three models, Teisser model with the highest maximum specific growth rate (μ_{max}) of 0.066 h^{-1} by *A. flavus* with correlation coefficient (R^2) value of 0.997 gave the best fit for the production of ascorbic acid. The teisser model was able to describe the kinetic parameters as it affects the growth of microorganisms during the fermentation process. Hence, ascorbic acid production via fermentation by *A. flavus* and *A. tamarii* is growth-associated.

Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflicts of interest.

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