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Article Keywords: Banana waste fermentation: De-lignification: *S. cerevisiae* kinetics

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Highlights

- Growth kinetics of *S. cerevisiae* during solid state fermentation
- Alkaline delignification of waste bananas to solubilize lignin
- Using Chapman model to describe kinetic growth of yeast
- Comparison of Chapman model with Bergter and Andrew kinetic derived parameters
- Chemical characterization of de-lignified and non-delignified waste bananas

Abstract: *S. cerevisiae* (NCYC 431) growth kinetics was studied using mathematical models in order to ascertain the optimum operational parameters for banana waste fermentation. Chapman-Richards model was used to describe yeast growth kinetics under varying pH and temperatures and the results were compared to Bergter and Andrew models. Alkaline-delignification of the wastes was done to solubilize lignin prior fermentation. This is because lignin is a complex organic plant compound that has been reported not to be degraded easily by many microorganisms. From the results temperatures 22-28°C and pH 4.5-5.6 were noted as optimum for yeast growth on delignified waste bananas (DWB). Chapman model results were close to Bergter and Andrew models with very low RMSE. Delignification was noted to aid yeast growth with higher microbial populations (log₁₀ cfu/g) registered with DWB samples as compared to non-delignified waste bananas (NDWB). Also, chemical characterization of the DWB and NDWB indicated higher proteins and lipids in the former than the latter by 3 and 4% respectively. This suggested the possible use of the upgraded wastes as chicken feed supplements. Higher minerals in DWB of 8.6% also suggested the possible use of the waste as a nutrient-rich fertilizer.

Keywords: Banana waste fermentation: De-lignification: *S. cerevisiae* kinetics

1.0 Introduction

Solid-state fermentation (SSF) has been employed greatly to address waste management by upgrading wastes into value added products like biofuels, animal feeds, single cell protein and industrial enzymes among others [1-2]. Several organic wastes i.e. plants and fruit pomace (wheat, cassava, rice, orange peels, banana, etc.) and animal wastes like chicken wastes, cow dung, dairy wastes among others have been upgraded through SSF using yeasts, fungi, algae and bacteria [3-4]. While SSF is viewed as an economic approach to upgrading organic wastes, it suffers a set-back of poor efficiency in terms of incomplete biodegradation which result in higher energy use and costs of production [5-6]. Scientific efforts to increase the efficiency of the bioprocess have resulted in studies like to ascertain optimum operational parameters (pH, water activity, temperature) [7-9]; optimize the substrate loading versus inoculum sizes [10-12]; enhance the fermentation cycle retention times [13-14] among others. In all these endeavors the primary role is to achieve conditions optimum for microbial growth and high population sustainability during fermentation [15-16]. This has also been addressed more accurately through the use of mathematical models to describe the microflora growth kinetics under these optimum conditions [17-18]. They predict the growth dynamics of the microorganisms with changes in the chemical and physical composition in the growth media [19]. *S. cerevisiae* laboratory strain (NCYC 431) is a commonly used microorganism in the wine or bread industries and home-made fermentation processes [20]. This microorganism has not been reported to biodegrade delignified waste bananas (DWB) through solid state fermentation. In this study, a fresh-cultivated pure population of *S. cerevisiae* was used for waste banana fermentation without nutrient supplementation. Nutrients are usually added to industrial wastes or fruit pomace that cannot sustain microbial growth during solid-state fermentation [21].

Several other models have also been successfully employed in microbial modelling like the Baranyi model which is used to describe isothermal and non-isothermal growth curves of microbial populations [22]; Gompertz model is commonly used to describe the sigmoid growth curves of microbial populations in infinite growth media [23]; the GinaFit model highlights the non-log-linear survivor behavior of microorganisms [24]. The Gompertz's equation has also been commonly employed to describe microbial dynamics in bio-processes like solid-state fermentation [23]. The effects of temperature and pH on specific growth rates have been described by the square root model [25]. For this study Chapman-Richard model, Bergter and Andrew model derived parameters were used to determine the effect of pH and temperature on yeast growth during waste banana fermentation.

Further efforts to improve the biodegradability of organic wastes are the pre-treatment of the biomass before solid-state fermentation [26]. This is done using various chemical, physical, biological and mechanical methods [27-28]. Of interest to this study was chemical alkaline pre-treatment method which was employed to solubilize the lignin; a plant complex organic compound reported to be degraded by a few organisms like *Aspergillus Niger* [29-30]. This was done because banana wastes containing lignin compound were the focus for fermentation using a pure microbial population. Chemical characterization of the upgraded delignified waste banana (DWB) and non-delignified waste bananas (NDWB) was also done. The aims of the study, therefore, were to ascertain the growth kinetics of *S. cerevisiae* during DWB and NDWB fermentation. Secondly, to ascertain the use of Chapman model to describe accurately *S. cerevisiae* growth during fermentation as compared to Bergter and Andrew models. And finally to characterize the upgraded DWB and NDWB as a measure to determine the effect of alkaline

delignification on waste banana fermentation and then ascertain the possible use of the upgraded wastes as chicken feed supplements or fertilizer. It should be noted that the world banana producing countries like Brazil, Ecuador, Uganda, etc. are third world countries and have been documented to experience huge wastages during peaks seasons [31-33]. Therefore, bioprocessing would be an alternative pathway for utilization of the un-used or waste bananas into value added products like animal/chicken feeds or fertilizers.

2.0 Materials and Methods

2.1 Waste banana preparation

Musa acuminata ('Grand Nain', AAA) Group banana samples were purchased from local Birmingham UK stores. The banana clusters were sorted, cleaned and placed on spacious trays to avoid damage. They were stored in incubators at 22°C for 9 days up to the waste critical stage [34]. All samples used for solid-state fermentation were bananas at the waste critical stage. The bananas were homogenized by blending using Phillips HR219/01 Avance (stainless steel) Blender. For standardization, uniform amounts of bananas were blended at the same speed and time i.e. 400g of bananas were blended at 2-level speed for 2.5 minutes to achieve an approximate size of 1.5-2.0mm determined by laboratory sieves. This procedure was done for the first experiments as method development and was not repeated for every experiment. The wastes were pre-treated, then adjusted to varying initial pH levels of 2.7, 3.5, 4.5, 5.6 and 6.2 before inoculation and fermented at temperature ranges of 16, 22, 28 and 34°C for the kinetic studies. The samples were manually agitated once a day during the period of fermentation.

2.2 Alkaline and thermal pretreatment of samples and SSF setup

SSF experiments were set up according to [35] with some modifications. The fermentation was carried out in 300 mL flat-bottomed flasks. 30 g of homogenized waste bananas (wet weight basis) was used in all experiments (3:30 w/v). A citrate buffer i.e. 0.1 M Citric acid and 0.2M Dibasic sodium phosphate were used for pH adjustment and the final pH adjusted with a sensitive pH meter (± 0.01). Two methods of waste banana pre-treatment were used in this study. First, the NDWB were thermally pretreated at 120°C for 15min. After cooling, the substrates were inoculated with 1.0×10^5 cfu/g and incubated at different temperatures i.e. 16, 22, 28 and 34°C respectively for a period of 7 days. The second pre-treatment was according to Chongkhong and Tongurai et al. [36] with a few modifications. 10g of banana wastes (whole fruit) were first chopped to a length of 1.5 to 2.5cm. Wet-state sodium hydroxide at different concentrations was used to pre-treat the waste bananas as shown in Table 1. The sodium hydroxide concentrations used were 0, 4, 8, 12 and 16%, while the pretreatment retention times were 8, 12, 16, 20 and 24 hours. The temperature during pre-treatment was controlled in a water bath and the set temperatures were 16, 22, 28 and 30°C. The pre-treated wastes were washed with water until a pH 7.03 ± 0.01 . The concentration of sodium hydroxide (NaOH) was calculated as below;

$$NaOH_{conc} (\%) = \frac{mNaOH}{dry\ weight\ of\ WB} \times 100 \quad (1)$$

Where m = moles and WB = waste bananas. Blank samples were alkaline pre-treated under similar conditions. Sampling was done at specific time intervals of 0, 24, 48, 72, 96, 120, 144 and

168 hours for further sample analysis. The fermentation experiments were carried out in triplicates, a set of un-inoculated blanks were run along assays. The kinetic results were expressed in \log_{10} cfu/g of the wet weight substrate or as natural logarithms of the biomass concentration or otherwise stated.

2.3 Microorganism culturing

S. cerevisiae laboratory strain (NCYC 431) strain was collected from the University of Birmingham, Biochemical Engineering stock bank. The cultures were revived by shaking at 120 rpm overnight in potato dextrose broth at 24°C. Yeast extract broth was used to aliquot stock cultures in cases of storages for long periods. The composition of the yeast extract broth (10g, glucose; 5g peptone; 3g, yeast extract; and 3g, malt agar) supplemented with glycerol (20%) and stored at -80°C in an ultra-low temperature freezer. The preparation of the inoculum was done by adding 2mL of the cell suspension into 100mL of yeast malt broth (Sigma Aldrich) with the composition (g/l): yeast extract, 3.0; peptic digest of animal tissue, 5.0; dextrose, 10.0; malt extract, 3.0. The final pH of the broth was 6.2 +/- 0.2 at 25°C. The culture was then incubated on a rotating shaker (for sample homogenization) at 200 rpm, 30°C for 24hrs. The yeast cells were centrifuged (8000xg, 10min) washed and then re-suspended in a phosphate buffered saline solution. Phosphate buffered saline composition (g/l) was potassium Chloride, 0.2; sodium chloride, 8.0; potassium dihydrogen phosphate, 0.2; di-sodium hydrogen phosphate, 1.15 [35]. The concentration of the cells was adjusted using sterile yeast malt broth to 10^5 - 10^7 cfu/ml as

determined turbid-metrically using a spectrophotometer at 600 nm [37]. A concentration of 1×10^5 cfu/ml was used for all experiments.

2.4 Delignification design of experiment (DoE) and Data Analysis

Sodium alkaline pretreatment was optimized using the central composite design (CCD) response surface methodology. Three parameters were considered in this alkaline pre-treatment DoE i.e. temperature, time and alkaline concentration as shown in Table 1. The levels were selected depending on literature results [34], industrial-scale real values [30], and single factor tests [38]. The regression coefficients for the experimental data were calculated using the Design-Expert software version 8.0.

2.5 Chemical Characterization of DWB and NDWB

Chemical analysis of DWB and NDWB samples was done after solid state fermentation as detailed below;

The total starch (resistant and non-resistant) was determined using APHA methods [39]. The digested waste samples were lyophilized and grounded into powder for subsequent analysis. Megazyme (Megazyme International Ireland A98 YV29) kits were used for starch analysis. The absorbance of the samples against a reagent was determined using a UV-vis spectrophotometer at 510nm [37]. The moisture content and total mineral content were determined by i.e. 2g of lyophilized samples were incubated at 105 and 525°C until a constant weight was attained respectively [40]. The analysis of total sugars was done using 80% hot ethanol extraction and the crude fiber analysis was done using Megazyme kit (Megazyme International Ireland A98 YV29).

Using a UV-spectrophotometer the absorption was measured at 490nm for simple sugars and 520nm for crude fibers [37] against a reagent blank. Carbohydrate composition was done by correction from 100%. Finally, the analysis of lipids and proteins was done using Total Kjeldahl nitrogen and Soxhlet thimble extraction methods respectively [41].

2.6 Microbial kinetics and mathematical modelling

S. cerevisiae growth kinetics was described using Chapman-Richards, Eq. 2, Bergter and Contois (Eq. 11 and 12) growth models as described by [42];

$$p_t = \log|n_t/n_0| = X_a \left| 1 - (1 - \beta_3) \exp \frac{\beta_3 \beta_3^{-1} \mu}{X_a} (\lambda_z - t) + \beta_3 \right|^{\frac{1}{1-\beta_3}} \quad (2)$$

Where p_t = population size at time t , n_0 = initial inoculum concentration and n_t = inoculum concentration at time t , $X_a = \beta_0$ are asymptote functions on a logarithm curve for maximum growth, $\mu = (\beta_0 \beta_2 \beta_3^{\frac{\beta_3}{1-\beta_3}})$ = the gradient of the tangent line of the logarithmic curve, λ_z = the x-axis intercept of the tangent line during lag phase of the logarithmic curve, t -time.

For an enclosed system with limited nutrients, the colony increase is a constant with time i.e. P organisms will multiply into 2P, then 2²P populations in time, (t_d);

$2^0P = 2^1P = 2^2P = 2^nP$, where $n = \frac{t}{t_d}$ (is the number of populations at time, t)

Therefore at t_0 ;

$$P_t = P_0 2^n \rightarrow \frac{P_t}{P_0} = 2^{\frac{t}{t_d}} \quad (3)$$

Taking natural logarithms

$$\left(\ln \frac{P_t}{P_0}\right) / t = 0.693 / t_d \quad (4)$$

The relative rate of microbial growth, μ ; is the gradient of the logarithmic curve tangent line, at the point of the inflection point. The time to reach the inflection point, t_1 was calculated by differentiating with respect to t, the second derivative of the equation below,

$$y_t = \beta_0 \left[1 - \beta_1 \exp^{-\beta_2 t}\right]^{\frac{1}{1-\beta_3}} \quad (5)$$

$$\frac{dy_t}{dt} = \frac{\beta_0 \beta_1 \beta_2 e^{\beta_2 t} (1 - \beta_1 e^{-\beta_2 t})^{\frac{1}{1-\beta_3} - 1}}{1 - \beta_3} \quad (6)$$

$$\frac{d^2 y_t}{dt^2} = \frac{\beta_0 \beta_1^2 \beta_2^2 \left(\frac{1}{1-\beta_3} - 1\right) e^{-2\beta_2 t} (1 - \beta_1 e^{-\beta_2 t})^{\frac{1}{1-\beta_3} - 2}}{1 - \beta_3} \quad (7)$$

$$- \frac{\beta_0 \beta_1^2 \beta_2^2 e^{-\beta_2 t} (1 - \beta_1 e^{-\beta_2 t})^{\frac{1}{1-\beta_3} - 2}}{1 - \beta_3} \quad (8)$$

Therefore the growth rate is;

$$\mu = \beta_0 \beta_2 \beta_3^{\frac{\beta_3}{1-\beta_3}} \quad (9)$$

Lag phase:

The logarithmic line passing through the point of inflection or the intercept of the tangent is the lag phase;

Mathematical representation;

$$\lambda = \frac{\beta_0(1-\beta_1)^{\frac{1}{1-\beta_3}} - \beta_0\beta_3^{\frac{1}{1-\beta_3}} + \mu \frac{\log\left(\frac{\beta_1}{1-\beta_3}\right)}{\beta_2}}{\mu} \quad (10)$$

The Bergter model, 1983 is mathematically represented as;

$$\mu = \mu_{max} * \frac{S}{K_s+S} * \left| 1 - \exp\left[\frac{-t}{\lambda}\right] \right| \quad (11)$$

And Andrew's model is also represented as

$$\mu = \mu_{max} * \frac{S}{K_s+S+\frac{S^2}{K_i}} \quad (12)$$

Where μ_{\max} = maximum growth rate (h^{-1}), μ = growth rate (h^{-1}), λ = lag phase (h^{-1}), S = substrate concentration, t = acceleration time, K_i = Inhibition coefficient (mg/l^{-1}) and K_s = substrate concentration constant (mg/l^{-1}).

3.0 Results and discussion

3.1 Effect of pH and temperature on the growth kinetics of *S. cerevisiae* (NCYC 431) during DWB fermentations

The growth kinetics of *S. cerevisiae* was observed during DWB fermentations under varying pH levels and operational temperatures. As already described in methods, the DWB samples were adjusted to initial pH levels of 2.7, 3.5, 4.5, 5.6 and 6.2 before inoculation and fermented at temperature ranges of 16, 22, 28 and 34°C for the kinetic studies. First, preliminary studies to optimize the alkaline-delignification process using RSM, central composite design (DoE), Table 1 was done by chemical analysis of the delignified samples. Total sugars, moisture and ash contents were considered among the important nutrients required to sustain microbial growth during fermentation. Several studies reported the recovery of sugars as the key criteria to approve a biomass pre-treatment method before SSF [43-44]. For this study, the highest percentage recoveries of these chemical components were considered the optimum for waste banana delignification. The initial *S. cerevisiae* concentration of 1×10^5 cfu/ml was used for DWB fermentations. Fig. 1 and 2 highlights the kinetics of *S. cerevisiae* under varying pH and temperatures. All graphic data points are mean values of 17 individual experiments.

From the results pH and temperature as expected affected the growth of *S. cerevisiae* on DWB during SSF. pH 4.5 fermentations indicated the maximum *S. cerevisiae* population of $\log_{10}^8\text{cfu/g}$, with this maximum population being sustained for approximately 90 hours as shown in Fig. 1. The operational conditions that can sustain high populations for long periods during fermentation have been documented to reduce costs of production [28, 41] since the efficiency of the fermentation process largely depends on the microbial populations [16, 29]. pH 3.5 fermentations sustained higher *S. cerevisiae* populations up to $\log_{10}^8\text{cfu/g}$ for only 20 hours as compared to pH 5.6 fermentations, with a maximum microbial population of $\log_{10}^6\text{cfu/g}$ sustained for approximately 45 hours. It could be noted that *S. cerevisiae* (NCYC 431) preferred moderately acidic conditions but could not survive in very acidic fermentations of pH 2.7 where the maximum populations were only $\log_{10}^5\text{cfu/g}$. However, this was more preferred as compared to pH 6.2 fermentation with the highest growth as $\log_{10}^4\text{cfu/g}$.

As already indicated in literature *S. cerevisiae* generally flourishes in moderately acidic conditions [45], however, several studies have indicated the specificity of the optimum pH range depending on the solid growth media [10, 46]. All these studies among others indicated the optimum pH range for *S. cerevisiae* growth to occur between pH 4.3 and 5.4. For this study pH, 4.6 was considered optimum for *S. cerevisiae* growth during DWB fermentations.

The growth kinetics under varying temperatures also quickly highlighted temperatures 22°C as the most preferred for *S. cerevisiae* on DWB wastes as shown in Fig. 2. The maximum microbial population was $\log_{10}^8\text{cfu/g}$ for temperature 22°C and $\log_{10}^7\text{cfu/g}$ for fermentations under 28°C .

It is probable that temperature 28°C could sustain higher population if more regular shaking to release the internal heat was done during fermentation. For this study, the flasks were manually shaken once a day. It has been noted in the literature that fermentations operated at higher temperatures could sustain higher microbial populations if constant shaking or aeration is enabled [40, 43].

Higher temperature like 34°C also indicated low growth rates of *S. cerevisiae* as well as the low temperature of 16°C on the DBW. It should be noted that the purpose of delignification was to degrade lignin that has not been documented to be degraded by *S. cerevisiae* before fermentation. However, the common hiccup of alkaline delignification process is the degradation of other organic compounds, that the delignified material cannot sustain growth without nutrient supplementation [33, 45]. It was concluded that alkaline delignification by Chongkhong and Tongurai, [36] with some modification was able to degrade lignin and the delignified material was able to sustain growth without nutrient supplementation. Nutrient supplementation may be done but it increases the cost of production compared to the value of products attained [28]. Also, thermal pre-treatment of the NDWB was considered as an economically feasible banana waste pre-treatment method as compared to alkaline pre-treatment.

In view of the cost of production required by alkaline pre-treatment, the NDWB were thermal pre-treated and chemically characterized after SSF (section 3.4). It was noted that while alkaline pre-treatment resulted in higher value wastes also thermal pre-treatment could be used for waste sterilisation prior bioprocessing.

3.2 Growth kinetics of *S. cerevisiae* on DWB and NDWB wastes using the Chapman-Richard model

The Chapman model, Eq. (2) has been documented to describe microbial growth in infinite resource media and has not been used in growth kinetics during solid state fermentation [42]. This model derives the biological parameters (lag phase (Eq. 10), growth rates (Eq. 9)) of the microbial population from the mathematical functions of the sigmoid growth curve (section 2.6). The results of this model were compared with the Bergter, Eq. (11) and Andrew, Eq. (12) model derived parameters to describe the *S. cerevisiae* kinetics during DWB and NDWB fermentations. The growth kinetics were studied under varying pH at a reference temperature of 22°C. From the results, the DWB wastes indicated better growth conditions of *S. cerevisiae* as compared to the NDWB during fermentation under varying pH levels as shown in Table 2 and 3.

Lower lag phases were obtained during fermentations with the lignified wastes (DWB) as compared to the non-delignified wastes (NDWB) under similar operational conditions. For example, under pH 2.7 the *S. cerevisiae* growth lag phase was approximately 11 hours on DWB while it was approximately 13 hours on the NDWB wastes. This trend was uniform for all the other pH levels on the DWB and NDWB wastes. Long lag phases have been documented to increase the costs of production and waste a lot of time and energy [16, 18]. Also, the growth rates and maximum growth rates as highlighted by the Chapman model results indicated higher growth rates of *S. cerevisiae* on DWB as compared to the NDWB.

The maximum growth rate (μ) on the DWB was 2.391 ± 0.015 and 1.704 ± 0.013 both obtained under pH 4.5 fermentations. While the relative maximum growth rates (μ_{\max}) on the DWB and

NDWB were 2.580 ± 0.021 and 1.907 ± 0.636 respectively. From the model results pH 4.5 was considered optimum for *S. cerevisiae* growth on DWB and also concluded that higher growth and lower lag phases were registered on the DWB as compared to the NDWB samples. The Chapman model also revealed the ability to describe effectively the growth of *S. cerevisiae* during DWB fermentations.

3.3 Comparison of Chapman-Richards versus Bergter and Andrew models for *S. cerevisiae* kinetic growth on DWB during solid state fermentation

The Bergter and Andrew derived model parameters as shown in Table 4 were compared with the Chapman Richards model because this model is in many research studies referred to as a basic microbial growth model. The derived parameters from Bergter model were lag phase (λ), substrate concentration constant (K_s) and maximum growth (μ_{max}). While the derived parameters from Andrew's model were, maximum growth (μ_{max}), substrate constants (K_s); and inhibitor constant (K_i) respectively. The optimum pH of 4.5 and 3.5 (from previous studies) at 22°C were used as reference conditions for the comparative studies.

Bergter and Andrew models have been employed to describe microbial kinetics in packed-bed fermentation systems [25, 46] to digest complex organic wastes that produce inhibitory substances like volatile fatty acids [47-48]. These models have not been documented as used in solid state fermentation and considering the kinetic derived parameters, they described with a high level of precision the growth kinetics of *S. cerevisiae* during DWB fermentation.

The Chapman model was used for comparison of the kinetic derived parameters. From the results, Bergter model described closely the lag phases of *S. cerevisiae* with mean lag phases as 8.750 ± 0.025 as compared to 8.748 ± 0.016 from Chapman under pH 3.5 fermentations, as shown in Table 4. Also, the maximum growth rates 2.060 ± 0.080 and 2.059 ± 0.074 for Bergter and Chapman models respectively. The RMSE (root mean square errors) were very low which highlighted the closeness of the predicted model results and actual experimental data.

This trend (for lag phase and maximum growth rates) were similar for pH 4.5 fermentations. Baranyi et al. [22] highlighted the need to use RSME to compare kinetic derived parameters and actual experimental values. Andrew's model results of the inhibitory and substrate constants indicated also a high level of accuracy determined by low RSME. From these results, it was concluded the Chapman model could successfully describe *S. cerevisiae* growth on DWB wastes during solid state fermentation and indicated the lowest RSME.

3.4 Chemical characterization of DWB and NDWB during solid state fermentation

Chemical analysis of the DWB and NDWB samples after *S. cerevisiae* fermentation was done to ascertain the effect of alkaline delignification and the possible use of the upgraded wastes. The main chemical components of interest were proteins, lipids and total mineral compositions.

From the results (Table 5), the protein content of upgraded DWB and NDWB was 10.089 ± 0.121^{ac} and 8.131 ± 0.089^{ac} (%) respectively from both an initial composition of 2.131 ± 0.086^{bc} . The protein content differed by approximately 2% in the DWB and the NDWB. The lipid content in the DWB and NDWB increased from an initial composition of 1.614 ± 0.072^b to 7.539 ± 0.071^{bd} and

6.649±0.072^c (%) respectively. The lipid content also differed by approximately 1% for the former and latter respectively. This indicated a better value of upgraded DWB for use as chicken or animal as compared to the NDWB. It should be noted that these were laboratory scale experiments that can be scaled-up using the above mathematical models which may result in higher nutrient percentages [30]. Also, the NDWB wastes were thermal pre-treated and could considerably be used as chicken feed with protein and lipid contents of 8.1 and 6.6% respectively. Many studies use thermal pretreatment during solid state fermentation [44, 47] and could still be considered however comparing the delignification effect on the waste bananas, it indicated higher protein and lipid levels which was one the purposes of the study.

The total mineral compositions for the DWB and NDWB samples also indicated higher percentages of 8.685±0.033 and 6.246±0.035 respectively from an initial percentage of 2.365±0.015^a. This suggested the possible use of both upgraded wastes as nutrient-rich fertilizer. It was noted that the delignification process resulted in a significant increase of total mineral content by approximately 6.3 and 3.9% for the DWB and NDWB respectively. These results were compared to the existing literature of the chemical composition of banana wastes (peel and pulp) [49-50] and it was noted that the *S. cerevisiae* fermentation of the DWB had significantly added value to the waste bananas. The bio-process is economical and could also be adapted for use in third world countries. It is also liable to large-scale production through the use of the mathematical models described above.

Lower levels of total sugars were detected in the NDWB as compared to DWB with percentage compositions of 16 and 12 respectively. This suggested higher utilization of sugars by *S. cerevisiae* in the delignified waste fermentations as compared to the non-delignified fermentations. This

suggested the enhancement of the biodegradation of organic compounds after removal of lignin during fermentation. The total dietary fibers in the NDWB and DWB were 40 and 43% which is vital for use of the upgraded wastes as feed supplements.

Conclusions

Several conclusions were derived from this study among which includes; the Chapman model could accurately describe *S. cerevisiae* growth during solid state fermentation even when it is usually described as a basic model. This was confirmed by application of other advanced models (Bergter and Andrew) to compare the kinetic derived parameters. Also, the RSME aided to determine the accuracy of the actual data to the predicted model parameters.

Secondly, was that *S. cerevisiae* could bio-degrade NDWB and DWB without nutrient supplementation as indicated by a significant increase in the proteins, lipids, and total mineral content. Thirdly, alkaline delignification aided the efficiency of *S. cerevisiae* fermentation as revealed in protein and lipid contents in the DWB which were higher than for NDWB by approximately 2 and 1% respectively. And the DWB and NDWB may be used as chicken feed supplements or nutrient-rich fertilizer. Thermal pretreatment also indicated the potential of enabling sustenance of *S. cerevisiae* during banana waste fermentation. This could be used as a lower cost of production pre-treatment method as compared to alkaline pre-treatment.

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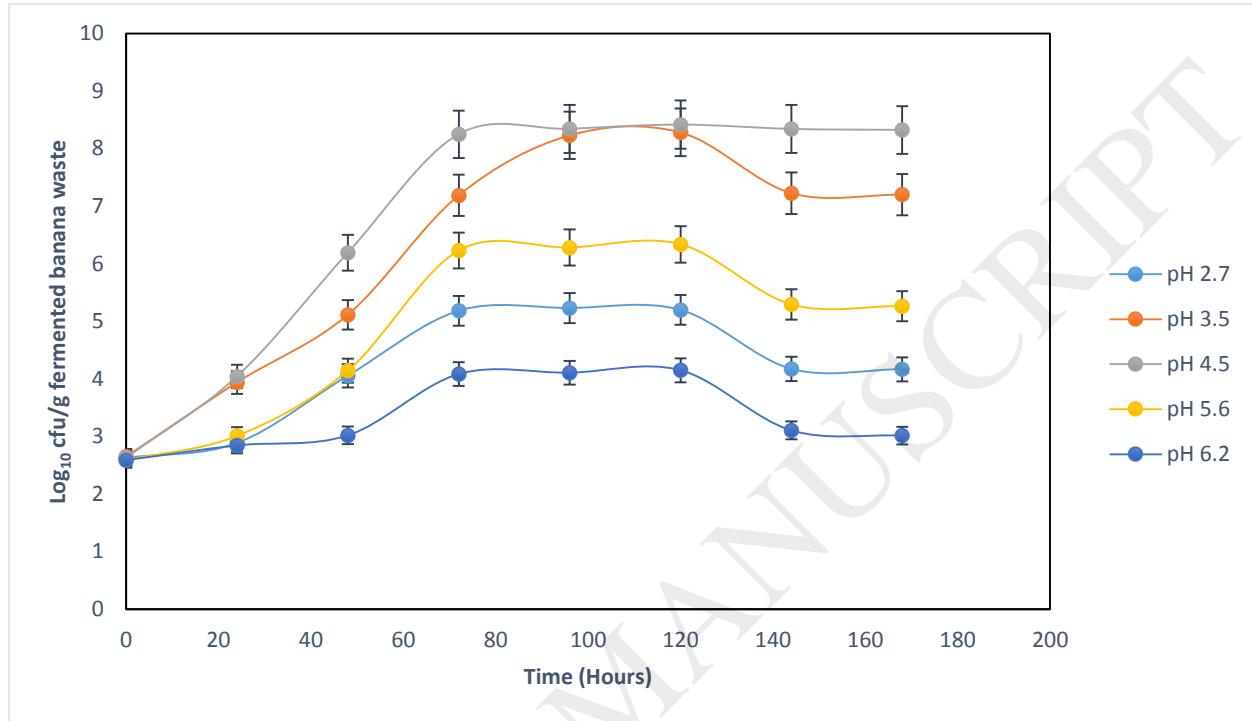
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Figure 1: Growth kinetics of *S. cerevisiae* during DWB fermentation under varying pH ranges at 25°C

Figure 2: Growth kinetics of *S. cerevisiae* during DWB fermentation under varying temperatures at pH 4.5



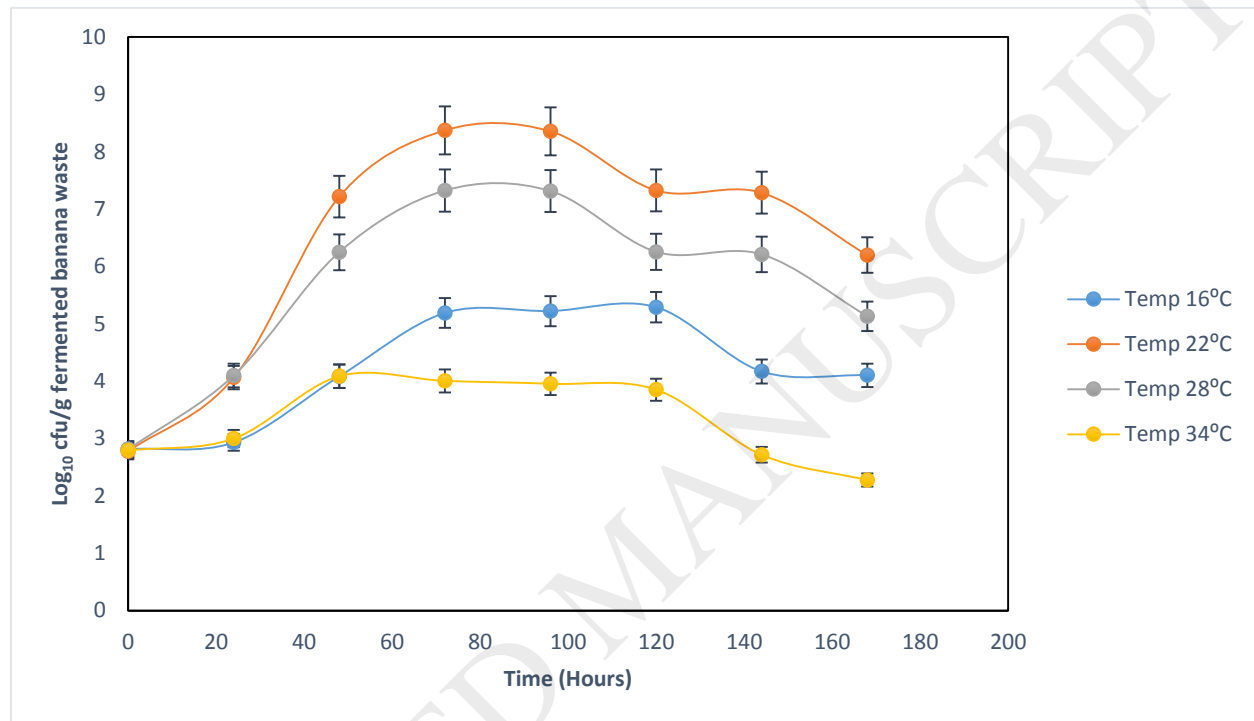


Table 1: Actual delignification pre-treatment conditions and encoding levels of CCD

| Parameter | Levels | | | | |
|-----------------------------------|--------|----|----|----|----|
| | -2 | -1 | 0 | 1 | 2 |
| X ₁ -NaOH (w/w, %) | 0 | 4 | 8 | 12 | 16 |
| X ₂ -Time (hours) | 8 | 12 | 16 | 20 | 24 |
| X ₃ - Temperature (°C) | 16 | 22 | 28 | 30 | 34 |

Table 2: Chapman-Richards model results for *S. cerevisiae* growth during DWB fermentations under varying pH levels at 22°C

| Temp (°C) | λ (h) | μ (h ⁻¹) | μ_{\max} (h ⁻¹) | R ² |
|-----------|----------------------------|---------------------------|---------------------------------|----------------|
| 2.7 | 10.850±0.092 ^{ac} | 1.178±0.092 ^{ac} | 1.199±0.003 ^{ac} | 0.98 |
| 3.5 | 8.952±0.073 ^{bc} | 1.800±0.073 ^b | 2.167±0.082 ^{bc} | 0.99 |
| 4.5 | 8.351±0.110 ^{cd} | 2.391±0.015 ^{bc} | 2.580±0.021 ^b | 0.99 |
| 5.6 | 9.860±0.080 ^c | 1.169±0.030 ^{ab} | 1.390±0.016 ^{ab} | 0.99 |
| 6.2 | 10.941±0.111 ^{bd} | 1.009±0.105 ^{cd} | 1.250±0.009 ^b | 0.98 |

Where λ = lag phase, μ = growth rate and μ_{\max} = maximum growth rate; h = hours; means (\pm) SEM for 17 individual runs; means with different letters are $p > 0.05$ significantly

Table 3: Chapman-Richards model results for *S. cerevisiae* during NDWB fermentations under varying pH values at 22°C

| pH | λ (h) | μ (h ⁻¹) | μ_{\max} (h ⁻¹) | R ² |
|-----|----------------------------|---------------------------|---------------------------------|----------------|
| 2.7 | 13.362±0.114 ^a | 0.886±0.024 ^{ac} | 1.082±0.012 ^{ac} | 0.99 |
| 3.5 | 8.816±0.053 ^{bc} | 1.401±0.061 ^b | 1.692±0.081 ^b | 0.98 |
| 4.5 | 6.326±0.003 ^{ab} | 1.704±0.013 ^{ac} | 1.907±0.636 ^{cd} | 0.99 |
| 5.6 | 11.509±0.082 ^{bc} | 0.974±0.051 ^{bd} | 1.128±0.401 ^{cd} | 0.99 |
| 6.2 | 13.721±0.003 ^{cd} | 0.879±0.081 ^{cd} | 0.903±0.205 ^d | 0.99 |

Where λ = lag phase, μ = growth rate and μ_{\max} = maximum growth rate; means (\pm) SEM for 17 individual runs; means with different letters are $p > 0.05$ significantly

Table 4: Bergter and Andrew models' derived kinetic parameters versus Chapman model results for *S. cerevisiae* growth during DWB fermentation

| Model | λ (h) | μ_{\max} (h ⁻¹) | K _s (mg/l ⁻¹) | K _i (mg/l ⁻¹) | RMSE |
|---------------|---------------------------|---------------------------------|--------------------------------------|--------------------------------------|-------|
| pH 3.5 | | | | | |
| Chapman | 8.750±0.025 ^{ab} | 2.060±0.080 ^{bc} | | | 0.021 |
| Bergter | 8.748±0.016 ^b | 2.059±0.074 ^c | 166 | | 0.011 |
| Andrew | | 1.851±0.012 ^b | 113 | 253 | 0.024 |
| pH 4.5 | | | | | |
| Chapman | 8.302±0.120 ^{cd} | 2.180±0.021 ^{ab} | | | 0.021 |
| Bergter | 8.298±0.036 ^b | 2.178±0.040 ^d | 151 | | 0.010 |
| Andrew | | 2.098±0.027 ^{bc} | 109 | 207 | 0.031 |

Where λ = lag phase; μ_{\max} = maximum growth rate; RMSE, root mean square error; K_s = substrate constant; K_i = inhibitor constants; means (\pm) SEM for 17 individual runs; means with different letters are $p > 0.05$ significantly different

Table 5: Proximate analysis of DWB and NDWB after solid state fermentation using *S. cerevisiae* (NCYC 431)

| Parameter (g/100g) | UBW samples (%) | NDWB samples (%) | DWB samples (%) |
|----------------------------|----------------------------|----------------------------|----------------------------|
| Total Minerals (% DW) | 2.365±0.013 ^a | 6.246±0.035 ^a | 8.685±0.033 ^c |
| Moisture (% WW) | 76.019±0.085 ^b | 86.019±0.015 ^{bc} | 90.072±0.014 ^{ac} |
| Total dietary fiber (% DW) | 11.053±0.221 ^c | 40.059±0.221 ^c | 43.054±0.104 ^b |
| Protein (% DW) | 2.131±0.086 ^{bc} | 8.131±0.089 ^{ac} | 10.089±0.121 ^{ac} |
| Lipids (% DW) | 1.614±0.072 ^b | 6.649±0.072 ^c | 7.539±0.071 ^{bd} |
| Total sugars | 72.570±0.044 ^{ab} | 16.894±0.211 ^{ad} | 12.604±0.151 ^{ab} |

Triplicate Mean values ± Standard Error, DW = dry weight, WW = wet weight, UBW= unfermented banana wastes and column values with different number differ by p>0.05

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