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Comparative analysis of banana waste bioprocessing into animal feeds and fertilizers

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Abstract:

The efficiency of a bioprocess in terms of cost of production versus product output determines its pragmatism in the field of Bioengineering. Solid state fermentation (SSF) and anaerobic digestion (AD) were compared for banana waste bioprocessing using *Saccharomyces cerevisiae*. The wastes were pretreated by alkaline-delignification and thermal pre-treatment prior to SSF and AD respectively. Optimization of the operational parameters for yeast growth kinetics was done using Chapman, Bergter and Andrew models. From the results generally, pH 3.5 to 5.8 and temperature 22 to 30°C were optimum for both bioprocesses. Comparatively, SSF was a more economic and efficient banana waste bioprocess than AD. The protein content increased by approximately 7.9% after SSF and by 6.7% after AD. And the lipid contents increased by 5.9 and 5.4%, while the mineral contents increased by 6.3 and 7.5% both after SSF and AD respectively. This suggested the possible use of the upgraded wastes as animal feed supplements and nutrient rich fertilizers.

Key words: banana wastes: nutrient rich fertilizers: animal feed supplements

1. Introduction

Over 102 million tons of bananas are produced annually, where 35% of each fruit is a peel, which produces approximately 36 million tons of waste (Vu et al., 2018). Also in various countries like Uganda, India, Brazil and Nigeria where bananas are consumed as food, approximately 70 million tons have also been documented as wasted after ripening in peak

seasons (Fonsah and Amin 2017; Albertson 2016; Sabiiti et al., 2016). Several other agricultural wastes like fruit pomace, wheat bran, coffee husks, banana wastes and sugarcane bagasse among others are also generated in million tons annually (Miller, 2017; Ros et al., 2017). The release of greenhouse gases to the atmosphere from waste incineration and open field dumping sites, and the depletion of non-renewable energy resources has prompted the advancement into environmental friendly bio-processing of agricultural wastes (Yang et al., 2017; Meng et al., 2017). The increase in the world population which prompts more industrial and agricultural waste production boosted the bioprocessing of wastes through solid state fermentation and anaerobic digestion (Akobi et al, 2016; Tao et al., 2017). Solid state fermentation (SSF) and anaerobic digestion (AD) have been successfully used to upgrade wastes into industrial enzymes, adsorbents, biofuels, fertilizers, animal feeds among others (Dennehy et al. 2018 ; Ahmad and Danish 2018). However, also these bio-processing advancements have remained more of 'research-findings' than 'field applications' as they fail to justify the feasibility of the processes in terms of efficiency and costs of production (Colón et al. 2017). These hiccups have been addressed in several other scientific studies of process optimization and mathematical modelling (Wang et al., 2014). Optimization of solid state fermentation has been approached through using mixed culture versus pure populations, optimization of operational parameters like water activity, pH and temperature, optimization of solid waste selection used among others (Canabarro et al., 2017; Rodríguez et al., 2017). While for anaerobic digestion the optimization of hydraulic retention time, effect of inhibitory substances like volatile fatty acids, batch system versus continuous digestion, packing of the beds among others have been documented (Ward et al., 2014; Miller, 2017; Ros et al., 2017). However a few studies have

attempted to compare different bioprocesses while using similar operational conditions and waste substrates as a pathway to optimize the degree of biodegradability. For example Park et al., 2011 reported solid state anaerobic digestion of organic wastes for methane production and Liew et al., 2012 reported solid state anaerobic digestion of lignocellulosic biomass for production of methane. These studies merged solid state fermentation and anaerobic digestion processes and termed it 'solid-state anaerobic digestion'. In this study these processes were studied independently but under similar operational conditions. Secondly, mathematical models were employed to describe the microbial growth kinetics of *S. cerevisiae* and also to ascertain the optimum biodegradation conditions of waste bananas. *S. cerevisiae* has been extensively documented in fermentations for ethanol production (Margeot et al., 2009; Matias et al., 2015; Canabarro et al., 2017), without focusing on the value of the fermented solid residue.

Mathematical models have been employed during SSF and AD to describe microbial growth. For example the Gompertz model has been used in SSF to describe the sigmoid growth of microorganisms in infinite resources (Gompertz 1825); Baranyi model has been used predictively to describe non-isothermal and isothermal microbial growth curves (Baranyi et al., 1996); the GinaFit model has been used successfully to describe the survivor behavior of microbial populations (Versyck et al., 1999). The square root model has also been employed to highlight the effect of temperature and pH on the microbial growth rates during fermentation (Smith-Simpson et al., 2005). For this study Bergter Andrew and Chapman models were used to describe yeast growth during SSF and AD. Andrew and Bergter models have been used in kinetic studies for anaerobic digestion of lignocellulosic wastes and other organic complex

substrates (Yang et al. 2017) but have not been documented for yeast growth during SSF and AD. Also the Chapman model has been used to describe microbial growth in infinite resource environments but not during waste bioprocessing.

Pre-treatment of organo-cellulosic compounds is usually done to dissociate the complex carbon-hydrogen bonds and increase the substrate-microorganism contact ratio (Cai et al. 2016). Thermal pretreatment is a common method employed in SSF which also achieves a sterile-state of the solid substrate (Ghanem et al., 2000). Alkaline delignification involves use of alkaline solutions like sodium or potassium hydroxide at varying concentrations to solubilize lignin. Lignin has been reported to be digested by a few microorganisms like *Aspergillus Niger* (Hinojosa et al. 2017). For this study these pre-treatment methods were employed to enhance the digestibility of banana wastes using *saccharomyces cerevisiae*. Several studies have been documented on the successful use of *saccharomyces cerevisiae* during bioprocessing (Wang and Witarsa, 2016; Lu et al., 2017). Therefore the aim of the study was to compare alkaline delignified SSF and thermal pre-treated AD of banana wastes for possible use as animal feeds or nutrient-rich fertilizer.

2. Materials and Methods

Musa acuminata 'Grand Nain', AAA Group bananas were purchased green from Birmingham UK fruit stores e.g. Aldi and Tesco. The samples were sorted and cleaned to remove the damaged fingers. They were stored in an incubator at 22°C for 9 days up to the waste critical stage (Nannyonga et al., 2016). Whole fruits (peel and pulp) samples were homogenized by

mechanical chopping and blending into smaller particles of approximately 1.5-2.0 mm for AD and SSF.

2.1 Alkaline delignification and solid state fermentation experimental setup

SSF experimental setup was carried out according to (Mantzouridou et al., 2015) with some modification as detailed below. 30g of homogenized wastes (wet weight) were fermented in 300ml flat bottomed flasks for all the experiments (1:10 w/v). Individual samples were adjusted to initial pH levels of 4.5, 5.6 and 6.2 using a citrate-phosphate buffer containing 0.2M dibasic sodium phosphate and 0.1M citric acid and the final pH adjusted using a sensitive meter (± 0.01).

Alkaline delignification pretreatment of banana wastes was done according to (Dahunsi et al., 2016); Wet state sodium hydroxide at varying concentrations of 4, 8, 12 and 16% (w/w) was used. The temperature was controlled in a water bath during the pre-treatment. Sodium hydroxide (NaOH) concentration was calculated as below;

$$NaOH_{conc} (\%) = \frac{m_{NaOH}}{dry\ weight\ of\ WBF} \times 100 \quad [1]$$

Where m = number of moles and BW= whole banana fruit.

The samples were inoculated with 1.0×10^5 to 1×10^7 cfu/ml before fermentation and incubated at different temperatures (22, 28 and 34°C) for a period of 7 days. Sampling was done every 24hrs for further analysis. SSF experiments were carried out in triplicates with blank uninoculated samples run along assays. Results were expressed in log₁₀ cfu/g of the substrate or cell concentrations.

2.2 Thermal pre-treatment and Batch-system anaerobic digestion setup

Batch-system setup according to (Borowski 2015) was employed for banana waste anaerobic digestion with some modifications as detailed below. The loading volumes were scaled down to laboratory scale with the ratio of inoculum; banana waste (w/w) of 2.5; 30, 5; 30 and 7.5; 30. 100ml serum bottles were used for digestion of 30g waste bananas under varying initial pH of 4.6, 5.8 and 6.6 respectively. The adjustment of pH was done as detailed in section 2.1. The samples were thermal pre-treated for 15 minutes at 120°C prior inoculation (Mantzouridou et al. 2015). After cooling the feedstock was inoculated once with 1×10^5 - 1×10^7 cfu/ml at the commencement of the digestion process. Nitrogen gas was flushed into the serum bottles in order to acquire the anaerobic conditions. Biomerieux, France anaerobic atmosphere indicators and a litmus test were used to ascertain the total absence of oxygen. Anaerobic digestion was carried out at three mesophilic temperatures which were 25, 30 and 37°C and for a retention time of 10 days. The experiments were carried out in triplicates with un-inoculated blank samples run along assays. Sampling for further analysis was done in a pattern of 1 day, 2 days and 10 days respectively. Serum bottles that were opened for sampling were subjected to similar oxygen depleting procedures before continuation with further anaerobic digestion. All kinetic results are expressed as log₁₀ cfu/g of the cell concentration or substrate.

2.3 Inoculum stock preparation

Saccharomyces cerevisiae commercial strain (Saf-instant) was collected from the University of Birmingham Biochemical Engineering stock bank. The cultures were revived in potato dextrose broth (PDB) with shaking overnight at 120 rpm at 24°C. For long term storage, the stock cultures were aliquoted in yeast extract broth (YEB) with a composition of (glucose, 10.0g;

peptone, 5.0g; yeast extract, 3.0 g; and malt agar, 3.0 g) supplemented with 20% glycerol and stored in an ultralow-temperature freezer at -80°C (Cerdeja et al., 2017). The inoculum culture was prepared by adding 2ml of the cell suspension into 100ml of YMB (Yeast Malt Broth) from Sigma Aldrich consisting (g/l); Dextrose, 10g; Yeast Extract, 3g; Malt agar, 3g and Peptic Digest of Animal Tissue, 5g. The final pH of the solution was 6.2±0.01 at 25°C. The culture was then incubated at 30°C with shaking at 200rpm for 24hrs. Yeast cells were centrifuged in 50ml BD Falcon, Franklin Lakes, NJ, conical tubes at 8000xg for 10min. They were washed and re-suspended in PBS saline solution. The PBS composition (g/l) was sodium chloride, 8.0; potassium chloride, 0.2; disodium hydrogen phosphate, 1.15; and potassium di-hydrogen phosphate, 0.2. The cell concentration was adjusted to 1×10^6 cfu/ml with sterile YM broth as determined turbid-metrically using a spectrophotometer at 600 nm (Cavaleiro et al. 2017).

2.4 Analytical methods

Total suspended solids and volatile solids

The volatile solids were determined by incubating the sample in a furnace at 550°C for two hours (Aggelopoulos et al. 2014). And total solids were determined by heating the sample at 105°C until a constant weight (Kalia et al., 2000).

Soluble sugars and COD

The soluble sugars were extracted using hot ethanol and analyzed using UV-spectrophotometry at 490nm against a blank and glucose standard (Michel Dubois et al., 1956). The COD analysis were done according to APHA standard method (APHA 2005) A digital Hatch reactor (DRB200,

15x16 mm vial well and 115vac) and a COD cell test kit were used to measure COD of the digestate with a detection range of 100 to 1500mg/l (De-Castro and Sato, 2014).

Crude proteins and lipids

Soxhlet extraction was employed for lipids analysis using petroleum ether and Kjeldahl digestion was used for determination of the protein content (Cordova et al. 1998).

Ash/Minerals, moisture content and total dietary fibre (TDF)

The ash, mineral contents were determined by heating the sample in 525°C and the moisture content was determined by heating at 105°C respectively until a constant weight (Deswal et al., 2011). Total dietary fibers were determined by Happi Emaga et al., 2007 method using Megazyme kits.

Resistant and non-resistant starch

Total starch (resistant and non-resistant) was determined by (Yaro, 2016) method. Banana samples were lyophilised and grounded into powder before the analysis using Megazyme (Megazyme International Ireland A98 YV29) kits (Karthikeyan A, 2010).

Microbial kinetics and modelling

Mathematical models have been employed in SSF and AD to describe the mechanism of growth for microbial populations under varying operational conditions. Chapman and Richard's model is an old model used to describe the growth of microorganisms in infinite resources (Versyck et

al. 1999) and has not been documented in use during SSF and AD. Other models used in this study to describe yeast kinetics were Andrew and Bergter models (Wang and Witarasa, 2016). Chapman-Richards model (1973) is represented as;

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

Where n_0 = the initial population size and n_t = the population size at time, t , μ is the growth rate $[(\beta_0 \beta_2 \beta_3^{\frac{\beta_3}{1-\beta_3}})]$, which is also the gradient of the tangent line, $X_a = \beta_0$ are asymptote functions on the logarithm scale for maximum growth, and λ_z is the lag phase which is the x-axis intercept of the tangent line.

The lag phase is mathematically represented as;

$$\lambda = \frac{\beta_0(1-\beta_1)^{\frac{1}{1-\beta_3}} - \beta_0\beta_3^{\frac{1}{1-\beta_3}} + \mu \frac{\log\left(\frac{\beta_1}{1-\beta_3}\right)}{\beta_2}}{\mu} \quad [2]$$

The Andrew's model is represented as;

$$\mu = \mu_{max} * \frac{S}{K_s + S + \frac{S^2}{K_i}} \quad [3]$$

And Bergter model as;

$$\mu = \mu_{max} * \frac{S}{K_s + S} * \left[1 - \exp \left[\frac{-t}{\lambda} \right] \right] \quad [4]$$

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

3. Results and Discussions

Delignified SSF and thermal pre-treated AD of banana wastes using *saccharomyces cerevisiae* was observed under varying inoculum sizes, pH levels and temperatures to ascertain optimum operational conditions. Under these optimum conditions chemical characterization was done and compared to ascertain the degree of digestability of the waste and the possible use of the upgraded waste. Triplicate runs were carried out under optimum conditions. Mathematical models were applied to describe and compare the growth kinetics of yeast.

3.1 Comparison of the initial inoculum concentration during alkaline delignified fermentation and thermal pre-treated anaerobic digestion

Inoculum size, pH and temperature were used to study yeast growth kinetics during banana waste bioprocessing. Mathematical models were employed to describe the biological parameters of the kinetics i.e. lag phases, substrate constants and growth rates.

The optimum initial inoculum concentrations required to digest the same amount of banana wastes under varying pH and temperature were determined during SSF and AD. Initial inoculum sizes of 1.0×10^5 and 1.0×10^6 were used for this study with 30g of banana wastes Figure 1. The selection of the inoculum sizes depended on literature and single test experiments (Mantzouridou et al. 2015).

From the SSF results, the initial inoculum size of 1×10^5 cfu/g increased to a maximum microbial population of $\log 10^8$ cfu/g at approximately 70 hours. While the initial inoculum 1×10^6 cfu/g increased to a higher maximum microbial population of $\log 10^9$ cfu/g also at 70 hours, however it can be seen (Figure 1) that the peak population is followed by a tremendous decline (death) phase. This therefore rendered the initial inoculum size of 10^5 more optimum as compared to 10^6 for delignified SSF of banana wastes.

Considering AD results, the initial size of 1×10^6 cfu/g increased to a higher microbial populations' of $\log 10^7$ cfu/g as compared to initial sizes of 1×10^5 cfu/g. The former sustained the maximum microbial population for approximately 70 hours while the latter sustained the maximum microbial population for approximately 50 hours. This also indicates that the initial size of 10^6

cfu/g is more optimum as the maximum population was sustained longer as compared to the initial inoculum size of 10^5 cfu/g for thermal pre-treated anaerobic digestion of banana wastes.

The results highlighted the significance of inoculum optimization, it should be noted that the size of inoculum to effect a bioprocess is necessary to optimize as very low concentrations results in long digestion cycles which require high energy and costs of production. While high initial inoculums also result in high populations during the initialization stage of the digestion process leading to high death rates before commencement of digestion (Batstone et al., 2015). For comparative evaluation, delignified SSF require a lower inoculum size as compared to thermal pre-treated anaerobic digestion for the same amount of banana waste.

3.2 Effect of pH on the growth kinetics of *saccharomyces cerevisiae* during delignified SSF and thermal pre-treated AD of banana wastes

The effect of pH on *S. cerevisiae* growth kinetics was studied to ascertain the optimum conditions during delignified SSF and thermal pre-treated AD. Initial pH levels of 4.5, 4.6, 5.6, 5.8, 6.2 and 6.6 were considered for this study (Figure 2 and 3).

The growth kinetics of yeast during delignified SSF under varying pH levels indicated pH 4.5 as optimum, followed by pH 5.6 and lastly pH 6.2 (Figure 2). The maximum microbial population obtained was $\log 10^8$ cfu/g and was sustained for approximately 90 hours under pH 4.5

fermentations. Low microbial populations with a maximum of \log_{10}^6 and \log_{10}^4 cfu/g were shown by pH 5.6 and 6.2 respectively.

The thermal pre-treated AD (Figure 3) also indicated similar variations as with SSF, with pH 4.6 as optimum for yeast growth as compared to pH 5.8 and 6.6. The maximum microbial population at pH 4.6 was \log_{10}^7 cfu/g, while the maximum for pH 5.8 and 6.6 were \log_{10}^6 and \log_{10}^5 cfu/g respectively.

Comparative evaluation of yeast growth kinetics under varying pH during SSF and AD indicated higher growth during the former than the latter. Also less time was required to reach the maximum microbial population under all pH levels. For example considering the optimum pH (4.5-4.6), it took about 70 hours to reach a maximum microbial population during SSF and about 120 hours for AD.

Longer lag phase (Figure 3) were also shown during AD as compared to SSF (Figure 2) under similar conditions.

3.3 Effect of temperature on the growth kinetics of *saccharomyces cerevisiae* during delignified SSF and thermal-pretreated AD

The effect of temperature on the growth kinetics of yeast during SSF and AD was determined.

Temperatures 22, 25, 28, 30, 34 and 37°C were considered for this study (Figure 4 and 5).

During delignified SSF, temperature 22 and 28°C indicated similar patterns of growth with higher maximum microbial populations in the former with \log_{10}^8 cfu/g than the latter with \log_{10}^7 cfu/g. Low microbial populations were shown by temperature 34°C with a maximum of \log_{10}^4 cfu/g.

Microbial growth studies during AD under varying temperatures indicated that operation under 25 and 30°C could sustain similar maximum microbial populations of up to \log_{10}^7 cfu/g however, under 30°C the population declines just after 20 hours.

Optimization of pH and temperatures during SSF and AD did not only reduce the lag phases but also increased the growth rates (Figure 2-5). It was concluded that the optimum pH for yeast during banana waste bioprocessing was between 4.5-4.6, and temperature 22-30°C.

Optimisation of operational parameters like pH and temperatures have been documented to increase the production of biofuels and industrial enzymes during agro-waste bioprocessing (Lee et al., 2017; Leite et al., 2016; Wang et al., 2018).

From the results it was noted that similar optimum ranges of temperatures i.e. between 22 to 30°C were shown for banana waste AD and SSF using *S. cerevisiae*. Comparatively, higher microbial populations and less time to reach the maximum population were required during delignified SSF than thermal pre-treated AD.

3.4 Mathematical modelling of the growth kinetics of *S. cerevisiae* during delignified SSF and thermal pre-treated AD

Chapman, Bergter and Andrew's models were employed to describe yeast growth kinetics during SSF and AD of banana wastes. Chapman model results were derived from the mathematical functions of the sigmoid growth curve and they were compared to the derived kinetic parameters of Bergter and Andrews model (Table 1). Temperature 22 and 25°C were used as reference for delignified SSF and thermal pre-treated AD respectively under varying pH levels. The kinetic derived parameters from Bergter model were lag phase (λ), maximum growth rate (μ_{\max}) and substrate constant (K_s) while from Andrew's model, the derived parameters were maximum growth rate (μ_{\max}) and substrate constant (K_s) and inhibitor constant (K_i).

Chapman model results for SSF indicated pH 4.5 as optimum with the lowest lag phase of 8.353 ± 0.030 and highest growth rate of 2.391 ± 0.015 as compared to other pH ranges. Also for AD pH 4.5 indicated the optimum conditions with the lowest lag phase of 10.024 ± 0.011 , and high growth rate of 1.024 ± 0.011 . From the selected pH levels for both processes it was noted that pH 4.5 to 4.6 were optimum for yeast growth during banana waste bio-processing. Similar trends were followed for maximum growth rates (μ_{\max}) of yeast under varying pH levels. The maximum rate was observed during delignified SSF as 2.580 ± 0.021 as compared to thermal pre-treated AD with 1.106 ± 0.031 . Lower pH ranges i.e. 3.5 to 5.6 were considered optimum for SSF and AD of waste bananas using *Saccharomyces cerevisiae*.

The comparative results for the mathematical models indicate close values between Chapman and Bergter models as compared to Andrew's model (Table 2). For example lag phases for Chapman model during pH digestions were 10.024 ± 0.015 and 10.024 ± 0.010 for Bergter model. The RMSE for predicted model results and actual experiments were very low with 0.011 for Bergter model.

Also the kinetic derived parameters for delignified SSF and thermal pretreated AD indicate lower lag phases and maximum growth rates. For example the predictive maximum growth rate during SSF was 2.581 ± 0.015 and that of AD was 1.098 ± 0.020 using Bergter and Andrew models respectively. Also Chapman model results indicated delignified SSF as a more efficient process as compared to thermal pre-treated AD, in terms of shorter lag phases and faster growth rates under similar operating conditions.

Mathematical model predictive results highlighted the ability of Chapman and Bergter models to describe yeast growth as compared to Andrew's model (Table 2). The results also indicated higher efficiency in terms of less time, inoculum size, and maximum population registered during SSF as compared to AD. The greatest hiccup of agro waste bioprocessing is higher energy and costs of production as compared to the value of the product generated (Jena et al. 2017; Puyol et al., 2017). Long cycles can be minimized by reducing the lag phases by using mathematical models to determine optimum operational conditions before large scale bioprocessing (Rasmussen et al., 2010; Ruan et al., 2014).

3.5 Comparative chemical characterization of banana wastes during delignified SSF and thermal pre-treated AD

Chemical analysis was done on the upgraded banana waste after delignified SSF and thermal pre-treated AD, Table 3. The purpose of chemical characterization was to compare the degree of digestability of waste bananas during SSF and AD in terms of upgrading the wastes into animal feed supplements and fertilizers. Several parameters were considered and compared with literature, Table 3. The key parameters to be considered for the above purposes are proteins, lipids and mineral compositions.

Comparatively, higher protein levels were registered after delignified SSF as compared to thermal pre-treated AD, the former consisted 10%, while the latter consisted 8.8% from an initial of before bio-processing 2.1%. Also the crude lipid content followed similar trends with higher percentages reported during SSF (7.8%) as compared to AD (7.0%) from an initial of 1.6%.

The other chemical compositions for banana wastes after SSF and AD indicate a uniform trend of higher digestability during the former than the latter. For example the recovered soluble sugars after SSF were 12.6% and 16% after AD. Higher mineral compositions were registered after AD as compared to SSF with percentages of 9.8 and 8.6 respectively.

Chemical characterization of the feedstock, highlighted higher proteins and lipids in the delignified SSF wastes as compared to thermal pre-treated AD wastes. This concluded the former as nutrient rich animal supplement as compared to the latter. However higher mineral composition in anaerobic digested wastes as compared to the fermented wastes indicated the

former as a nutrient rich fertilizer than the latter. This highlighted how comparative evaluation of bioprocess can aid large scale applications with a more specified purpose.

3.6 Conclusions

Comparative evaluation of delignified SSF and thermal pretreated AD clearly highlighted the necessity to compare different bioprocesses in order to determine the more efficient for large scale and commercial applications. Delignified SSF was considered more economical and feasible as compared to thermal pretreated AD in terms of initial inoculum size, retention time for bioprocessing, larger spectrum of pH and temperature tolerance for the microbial population to survive among others. Also higher microbial populations were registered during SSF which resulted in a more upgraded waste with higher protein, lipids and mineral compositions. Optimization of operational parameters (inoculum sizes, pH and temperature) also indicated higher bioprocessing rates under the optimum conditions, with pH 3.5 to 5.8 and temperature 22 to 30°C being optimum for yeast growth. Chemical characterization of the upgraded wastes for both bioprocesses indicated increase in proteins by 7.9% after SSF and by 6.7% after AD. The lipids increased by 5.9 and 5.4%, while the mineral contents increased by 6.3 and 7.5% both after SSF and AD respectively. This suggested possible use of the upgraded banana wastes as animal feed supplements or fertilizer. These bioprocessing methods can be employed to address banana wastage in peak seasons from third world countries like Uganda and Brazil.

'E-Supplementary data for this research study can be found in e-version of this paper online'

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Figure 1: Growth kinetics of *S. cerevisiae* during de-lignified SSF and thermal pre-treated AD at varying initial inoculum sizes

Figure 2: Growth kinetics of *S. cerevisiae* during de-lignified SSF under varying pH levels

Figure 3: Variation of *S. cerevisiae* growth kinetics during thermal pre-treated AD under varying pH levels

Figure 4: Variation of *S. cerevisiae* growth kinetics during de-lignified SSF under varying temperatures

Figure 5: Variation of *S. cerevisiae* growth kinetics during thermal pre-treated AD under varying temperatures

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Table 1: Chapman Richards model results of *S. cerevisiae* growth kinetics during de-lignified SSF and thermal pre-treated AD

Delignified SSF (ref, 22°C)

Thermal pre-treated AD (ref, 25°C)

pH	λ (h)	μ (h)	μ_{\max} (h)	pH	λ (h)	μ (h)	μ_{\max} (h)
3.	8.92±0.125 ^b	1.8 ^c ±0.073 ^b	2.16±0.082 ^a	4.	10.02±0.011 ^a	1.02±0.012 ^a	1.11±0.031 ^a
5				6			
4.	8.35±0.032 ^a	2.39±0.015 ^c	2.58±0.021 ^b	5.	12.71±0.104 ^c	0.94±0.026 ^b	1.02±0.012 ^c
5				8			
5.	9.85±0.082 ^d	1.17±0.030 ^a	1.39±0.017 ^c	6.	13.91±0.217 ^b	0.62±0.031 ^a	0.98±0.043 ^b
6				6			
6.	10.91±0.264 ^c	1.01±0.105 ^c	1.25±0.019 ^a	7.	14.11±0.328 ^a	0.59±0.006 ^c	0.88±0.084 ^b
2				0			

Where λ is the lag phase, μ = growth rate, μ_{\max} = maximum growth rates, values are presented as means \pm SEM of 17 individual experiments. Also means with different letters are $p > 0.05$ significantly different

Table 2: Comparative evaluation of *S. cerevisiae* growth kinetics using the derived parameters of Bergter and Andrew versus Chapman model during SSF and AD

Model	λ (h)	μ_{\max} (h)	K_s (mg/l ⁻¹)	K_i (mg/l ⁻¹)	RMSE	SSE	R ²
Delignified SSF (ref, 4.5)							
Chapman-Richards	8.353±0.031 ^{ab}	2.580±0.022 ^b			0.001	0.010	0.99
Bergter	8.357±0.021 ^{bc}	2.581±0.015 ^{cd}	173		0.011	0.112	0.99
Andrew		2.551±0.032 ^{ab}	125	268	0.025	0.143	0.97
Thermal pre-treated AD (ref, 4.6)							
Chapman-Richards	10.024±0.015 ^{ab}	1.106±0.012 ^{ac}			0.002	0.101	0.99
Bergter	10.024±0.010 ^{bc}	1.108±0.020 ^{ab}	159.6		0.020	0.109	0.98
Andrew		1.098±0.020 ^c	52.6	396	0.031	0.138	0.97

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 3: Chemical characterization of de-lignified SSF and thermal pre-treated AD banana wastes

Parameter (g/100g)	Non-bioprocessed banana wastes (%)	Delignified SSF banana wastes (%)	Thermal-pretreated AD banana wastes (%)
pH	4.60±0.017 ^c		4.48±0.011 ^a
Minerals (Ash)	2.37±0.015 ^a	8.685±0.069 ^{ab}	9.88±0.037 ^b
Total Volatile solids (DW %)	90.02±0.031 ^b		86.26±0.012 ^b
Moisture (FW %)	90.02±0.085 ^b	93.07±0.014 ^{ac}	
Soluble Sugars (DW %)	72.57±0.044 ^{ab}	12.60±0.211 ^{ad}	16.03±0.151 ^{ab}
Protein (DW %)	2.13±0.086 ^{bc}	10.09±0.121 ^a	8.85±0.035 ^{cd}
Total solids (FW %)	92.04±0.106 ^{cd}		88.25±0.021 ^b
Total dietary fiber (DW %)	11.05±0.221 ^c	43.06±0.405 ^b	
COD (g O ₂ /litre)	64.72±0.016 ^a		38.73±0.067 ^b
Crude Lipids (DM %)	1.61±0.072 ^b	7.54±0.079 ^c	7.04±0.111 ^{bc}

Where FW = fresh weight; DW = dry weight; COD = chemical oxygen demand; means (±) SEM for 3 individual experiments; means with different letters are $p > 0.05$ significantly different

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Highlights

- Banana waste bioprocessing and mathematical modelling
- Biomass pre-treatment prior solid state fermentation and anaerobic digestion
- Chemical characterisation of upgraded wastes as animal feeds and fertilizers

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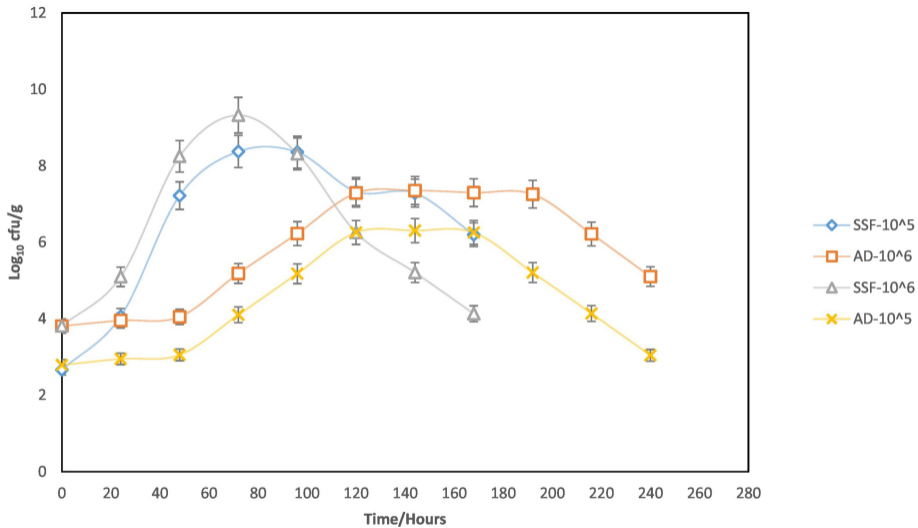


Figure 1

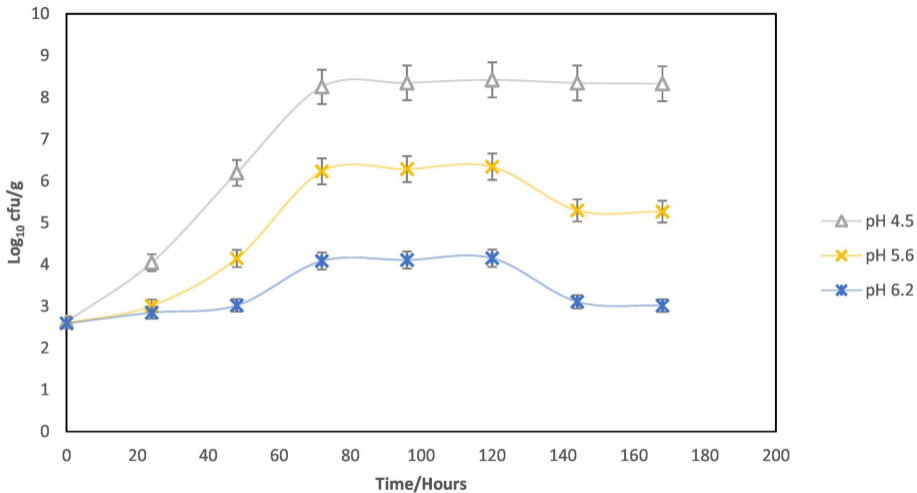


Figure 2

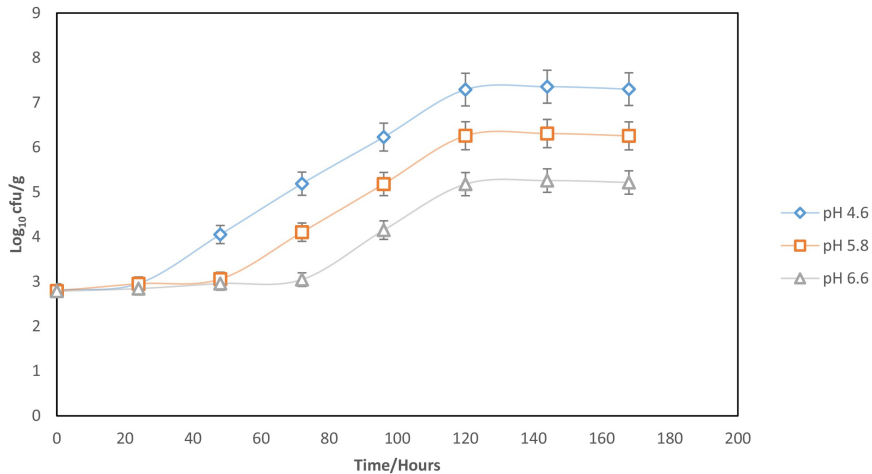


Figure 3

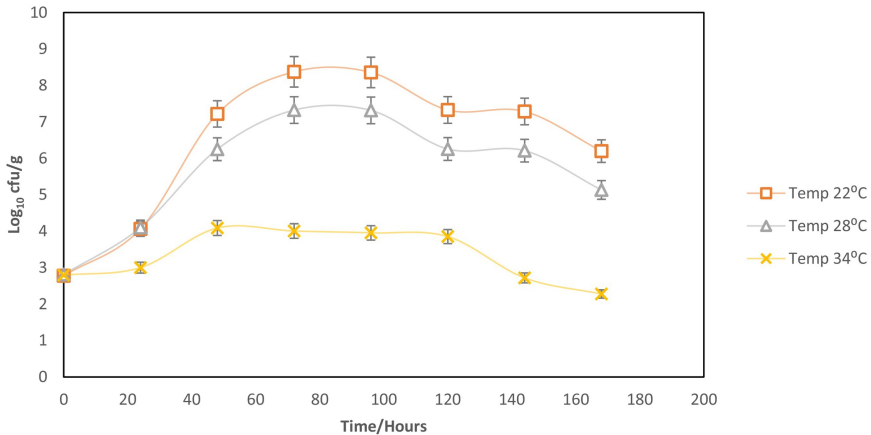


Figure 4

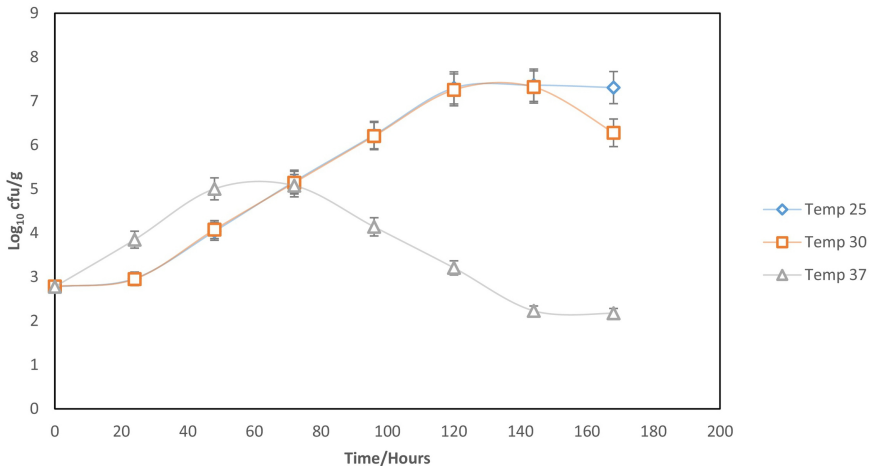


Figure 5