# Kinetic Modelling of Oxalic Acid Production from Cassava Whey by *Aspergillus niger*

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

| *Original Research Article* |

## ABSTRACT

The transition from eco-harmful chemical processes to bio-based production of organics has been challenged by the complex nature of fermentation processes. The growth kinetics and modelling of oxalic acid production from cassava whey by *Aspergillus niger* (MW188538) was studied in a batch fermentation system. The production kinetics of the fermentation study was fitted into the Monod, Leudeking-Piret and Andrews kinetic models. The oxalic acid, reducing sugar, cell dry weights were determined according to the experimental design. The results showed that the production of oxalic acid was associated with *A. niger* with significant $R^2$ value of 0.96 and growth rate of 0.065 biomass/day using the cassava whey as a sole carbon source. The substrate consumption rate of 14.28 and 11.16 mg/g/day with an $R^2$ value of 0.94 and 0.96 suggest there was a healthy utilization of the Cassava whey and yeast extract as described by the Leudeking-Piret and Monod models.

*Keywords: Leudeking-Piret; Aspergillus niger; cassava why; oxalic acid.*

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# 1. INTRODUCTION

In recent years, the need for a sustainable environment has been at the forefront of global debate. The necessity to create industry-critical chemicals in a more sustainable manner has prompted a search for less expensive and environmentally friendly feedstock as well as environmentally friendly manufacturing procedures. Because of the necessity to safeguard the environment, old chemical processes have been replaced by biologically based manufacturing of organic acids, exposing fungus as true striking cell factories. Fungal fermentation of organics has steadily grown and carved out a position in the chemical industry [1,2]. Organic acids have a wide range of industrial applications, including serving as building blocks for synthetics and the development of novel bio-based materials that can replace non-renewable petroleum-based polymers [3]. The organic acid market was valued at over 16 billion dollars globally in 2016, and is predicted to reach 29 billion dollars by 2027. Although bulk commodity organic acids such as citric acid have a large market presence, oxalic, succinic, and other organic acids have made inroads into the additive, pharmaceutical, and preservation industries [4,5,6]. The development of sustainable bioprocesses with a focus on low-cost renewable resources has been a key component of the transition to bio-based industrial production of organic acids. Microbial fermentation is now widely regarded as a reliable, low-cost, environmentally benign, and practical option for commercial organics production with lower carbon footprints [7,8].

Due to its importance in the pharmaceutical and metallurgical industries, oxalic acid, a dicarboxylic acid, has acquired popularity in the organic acid sector. Oxalate can be used as a preservative [9,10,11]; a cleaning agent (Guru et al., 2001); and a kaolin iron remover [9,10,11,12,13]. The chemical industry currently supplies the majority of the world's oxalic acid, which is produced using non-sustainable processes [14]. However, as enumerated by authors in a recent paper, there have been multiple examples of microbial synthesis of oxalate by fermentation by certain microbes (Chioma and Agwa, 2019). Because of its ease of handling, saprophytic nature, and high yield, A. *niger* is the biotechnologist's inoculum of choice for oxalate synthesis during fermentation (Emeko et al., 2015).

Microorganisms' metabolite synthesis is influenced by the inoculum's strains and species, culture conditions, and growth medium [15]. Minor changes in the aforementioned elements might have a significant impact on the quality and quantity of fermentation products. As a result, every fermentation process must be optimized by finding the proper media composition and growth conditions for maximum yield [16]. The creation of kinetic models and experimental designs can be used to evaluate the microbial refinery [17,18]. The use of kinetic models is critical for reducing the number of tests required to evaluate operation conditions for optimization and control [19]. The structured and unstructured mathematical models are commonly employed in fermentation research [20], Gadjil and Venkatesh, 1997; [21,19]. Unstructured models use simply a global parameter like cell mass to explain the biological system, cell development, or product production, whereas structured models consider some basic elements of cell shape, function, and composition. For the explication of metabolic steps and the computation of kinetic parameters, theoretical models are typically created and applied [22]. Recent oxalate research has centered on optimizing oxalate production using RSM, but little or no work has been done on proposing a kinetic model for the creation of the organic acid.

The objective of this study was to kinetically model the fermentation process oxalic acid production by A. *niger* grown on cassava whey medium. In this study, the Monod's model and Leudeking-Piret model is used to describe the oxalate yield from the cassava whey and yeast extract.

# 2. METHODOLOGY

## 2.1 Sample Collection and *A. niger* Isolation

The cassava whey employed for this research was collected from small cassava processing plants within Choba community of Obio/Akpor Local Government Area, Rivers State, Nigeria. The A. *niger* utilized for this study was isolated from dried banana peels and thereafter, identified using the internal transcribed spacer sequence (ITS) region of the nucleotide sequence and identified with the NCBI gene bank. The inoculum was maintained in Potato dextrose agar slants and stored at 4$^{o}$C for future reference.

## 2.2 Fermentation Medium

The medium for the production of oxalic acid was prepared in a 500 mL glass flask containing 200 mL of the medium. The media formulation described by Emeko et al., (2015) was modified and consisted of 0.05 g/L of yeast extract, 0.5g/L of $MgSO_4.7H_2O$ and 1.0 g/L $KH_2PO_4$ and 20 g/L of cassava whey.

## 2.3 Preparation of Inoculum for Fermentation

The inoculum was prepared by transferring a loopful of cells from a 48 hr slant to the 250-ml Erlenmeyer flasks containing 50 ml of the medium as described by Emeko et al. (2015), which contained 0.025g/L of yeast extract, 0.025g/L of $MgSO_4.7H_2O$ and 0.5g/L $KH_2PO_4$. The medium was adjusted to pH 6.0 using 4M NaOH solution prior to sterilization and 4% glucose. This inoculum was cultivated at 30 °C, for 48 h under agitation (200 rpm).

## 2.4 Submerged Fermentation of Oxalic Acid

A batch of 200ml medium in 500ml Erlenmeyer flask with 4% cassava whey as carbon source were used. The flasks were sterilized by autoclaving at 121°C for 15 minutes, then 2.5 x $10^6$ spores/ml of *A. niger* were inoculated and incubated at 30°C, 200 rpm using a rotary shaker incubated for 14 days. The fermentation medium was maintained at pH of 6.0 throughout the period using 3.0 M NaOH (Betiku et al., 2014).

## 2.5 Kinetic Models

### 2.5.1 Microbial growth associated production of oxalic acid and other process parameters

The use of a variety of logistic and non-parametric indicators, such as the Leudeking-piret, Monods, and Andrews models, has been highlighted as one of the most important techniques to understanding the function of growth-related processes revealed in this study. The rate of growth of microbiological feed stock utilizing Cassava whey and yeast extract has been determined in this study. The growth rate calculation, which supports the role of the exponential phase in batch and continuous fermentation, has been expressed using first order kinetics and pseudo-first order as a

verification and assessment approach, as shown in Equation 1.

$$\mu = \frac{Ln\left(\frac{N1}{Ni}\right)}{T1-T0} \qquad (1)$$

Where the N1 and Ni are the Growth number (Microbial Counts) for the initial and final durations in days while the T0 and T1 are the initial and final durations of the fermentations.

The Leudeking-Pirets and Monod's models fits a number of non-logistic parametric considerations of the fermentations such as ones applied for the present study. The report of Linville et al. (2003); Manikandan et al., 2008 and Song et al. [23] have identified the role of unstructured, nonsegregated and iterative modeling of process parameters in the determination of the synthesis of metabolites by microbes which have been describes by Abu [24] and Agbaji et al. [25] as the factory of enzymes needed for biotechnology and bioprocess

$$\mu x = \frac{Ln\ (dx)}{(dt)} \qquad (2)$$

Given that $\quad \mu = \frac{\mu max}{(Ks+S)} \qquad (3)$

Where $\mu$= specific growth rate (per day); $\mu_{max}$= Maximum specific growth rate; Ks= saturation constant; S= Substrate concentration (mg/ml).

The inability and flaws of the Monod's Model to fit all growth associated conditions is a drawback. The need to apply other non-conventional models such as Andrews models as reported by Olorunnisola et al. (2018) as presented in Equation (4) shows the considerations of the Andrew's Model

$$\mu = \frac{\mu max S}{(Ks+S+s^2/Ki)} \qquad (4)$$

where $K_i$ is inhibition constant and other parameters remain constant as presented in equation 3 above.

### 2.5.2 Considerations and model fit assumptions

i.   A number of non-logistic models, such as Monod's and Leudeking-Piret Models, can forecast the generation of organic acids like oxalic acid. They have been observed to adequately suit secondary metabolite production, and they may be used to determine the specific growth rate using the exponential function of changes in

microbial count and time, as detailed by Abu et al., [24]; Olorunnisola et al., (2001). (2018).

ii. Microbial growth dynamics in regulated solid-state fermentations follow standard growth settings using monocultures, with lag and exponential phases lasting variable amounts of time. This could be due to a number of variables, including the lack of a symbiotic interaction. This could also change depending on the rate of product synthesis and the isolate's unique growth.

iii. The Halden Model, as well as the modified Michalis-Menten and Andrews equation, can be used to determine substrate absorption for both cassava whey and yeast extract.

## 2.6 Analytical Techniques

The oxalic acid concentration in the fermentation medium was determined through catalytic kinetic spectrophotometric method described by Jiang et al. (1996). The reducing sugar and cell dry weight were determined using the methods reported by Saqib and Whitney (2011) and (Abd-aziz et al., 2008) respectively.

## 3. RESULTS AND DISCUSSION

The result presented in Fig.1 shows the linear regression plot for the estimation of the growth kinetics. The plot shows the linear regression of Log CFU/g against time duration. The values were fitted into an exponential model of $y=3.1514e^{0.065x}$ with a regression coefficient of 0.9617. Similarly, the result presented in Fig 2 shows the linear regression plot of growth rate against time using the growth indices for utilization of the cassava whey as a source of carbon and energy. The linear plot in Fig. 2 was observed to have a fit model regression coefficient $R^2$ of 0.91 with an equation $y = 0.0041x +0.0691$. The plot presented in Fig 3 and Fig 4 represents the Leudeking – Piret's and Monod's model equation using the product formation rate against specific growth rate and the data was fit into a model $y=0.3322x + 0.0944$ with regression coefficient $R^2$ of 0.43 while the Monod regression using the specific growth rate against the inverse of substrate concentration [1/S]. Andrew's modelling was presented in Fig. 5 using the specific growth rate and substrate concentration. The logarithm modelling had a Regression coefficient $R^2$ was 0.94 with a mathematical equation $y= 0.029ln(x)+0.082$. The result presented in Fig. 6 shows the regression model for yeast extract as nitrogen source. Regression coefficient $R^2$ of 0.9373 with an equation of $y=4.4047 + 0.051$ was obtained for the utilization of the yeast extract as presented below.

The stages of development phase, ranging from lag phase to death phase, have been found for solid-state fermentation [26]. A variety of drawbacks have been observed with monoculture and solid-state fermentation methods. Between the 2nd and 10th days of the fermentation investigation, this study observed a sharp and sustained exponential growth phase. This demonstrates that, independent of the culture circumstances, the performance of the inoculum utilized in the study can progress through the fermentation process, despite the feed stock conditions. The deduction of the specific growth rate and doubling time, as shown in Table 1 reveals that the inoculum response between the exponential and linear growth rates is similar.
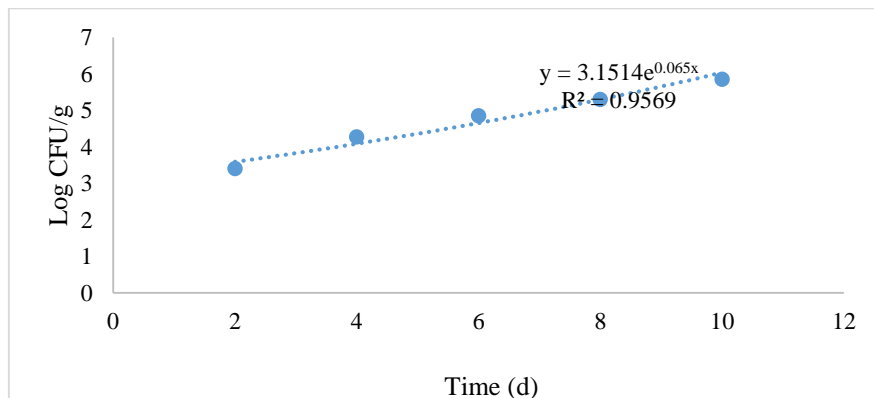


**Fig. 1. Growth rate determination of *A. niger* using the Log of microbial concentration against Time**
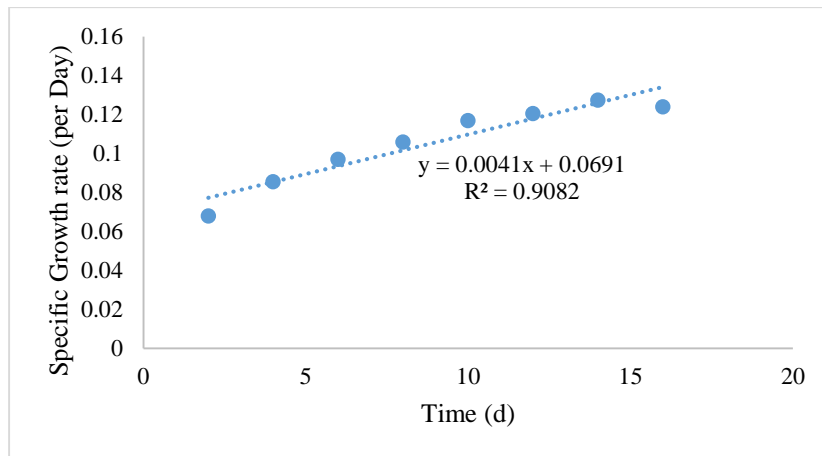
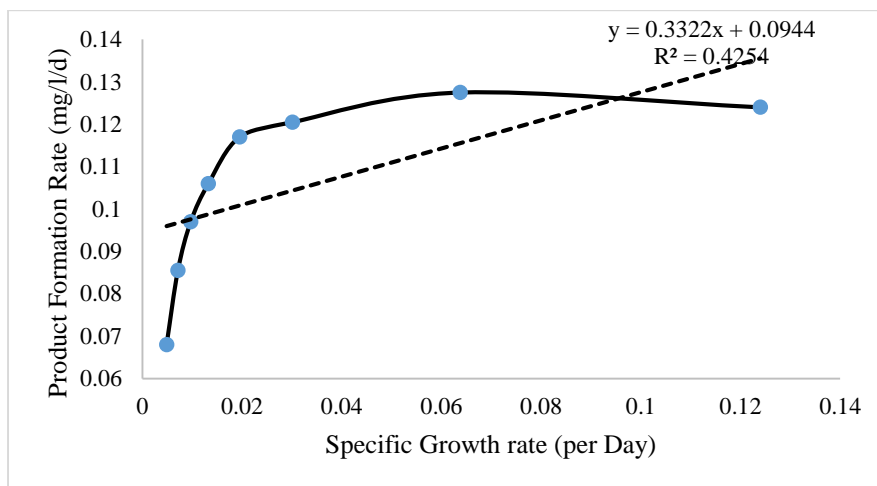**Fig. 2. Plot of the specific growth rate against time for the Cassava whey utilization by *A. niger***



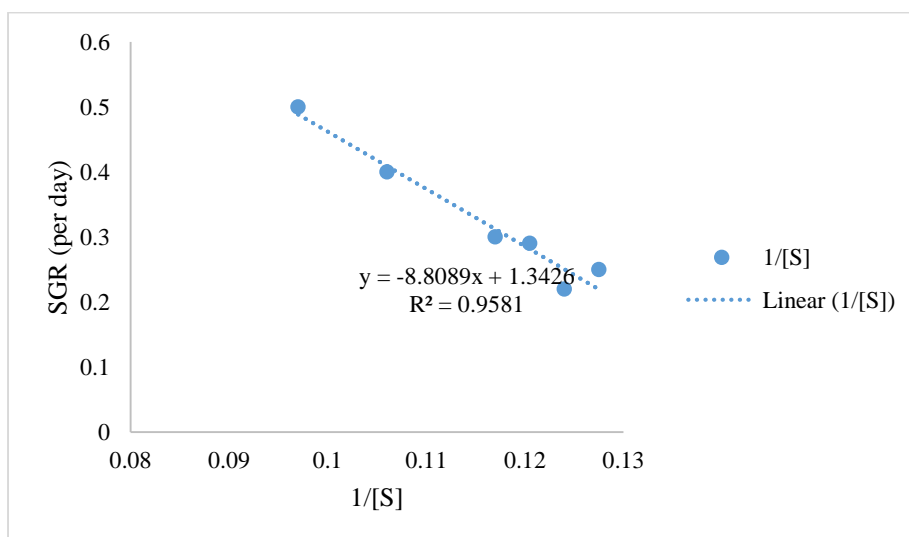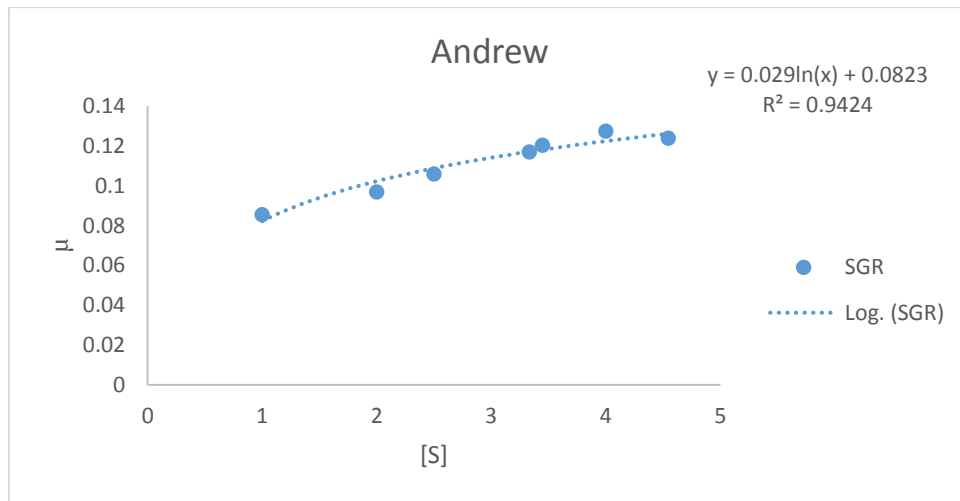**Fig. 3. Leudeking- Piret Plot for deduction of the substrate utilization rate (Cassava Whey)**
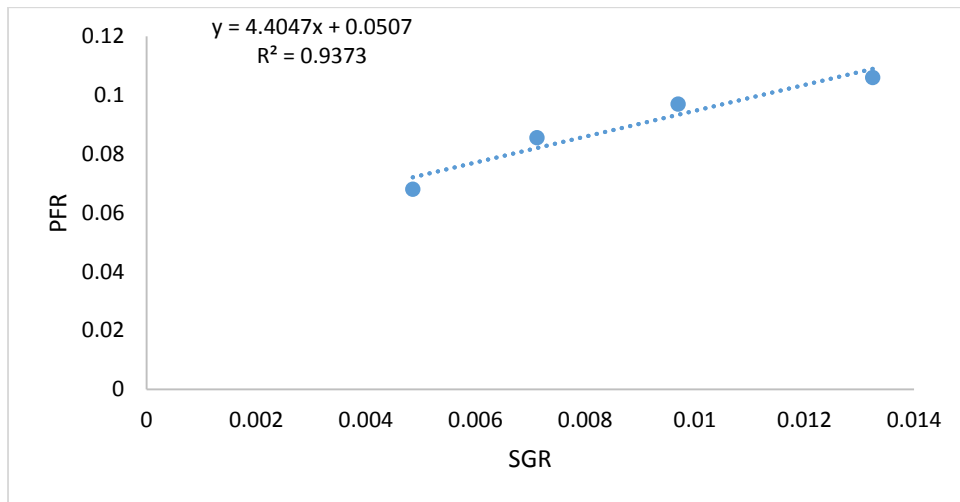


**Fig. 4. Monods Modelling plot of the Specific Growth Rate against inverse of substrate concentration (Cassava Whey)**

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**Fig. 5. Andrew's Modelling of the Cassava whey substrate utilization using the specific growth rate against substrate concentration**



**Fig. 6. Monods modelling plot of the product formation rate against specific growth rate**

**Table 1. Summary of growth kinetics and modeling for cassava whey and yeast extract**

| Sample | Growth Rate (per day) | $R^2$ | Generation Time (Gen/day) | General Growth Specifics | Luedeking-Piret Model | Monods Model | Andrew's Model |
|---|---|---|---|---|---|---|---|
| C: Cassava Whey | 0.065 | 0.96 | 24.39 | $\mu$=0.065 (d$^{-1}$) $R^2$=0.96 | $\mu$=0.065 (d$^{-1}$) $R^2$=0.85 $\mu_{max}$=0.13 $K_s$= 14.29 | $\mu$=0.004 (per Day) $R^2$=0.91 | $\mu$=0.05 (per Day) $R^2$=0.50 |
| N: Yeast Extract | | | | $\mu$=0.0006 (d$^{-1}$) $R^2$=0.98 | $\mu$=0.065 (d$^{-1}$) $R^2$=0.94 $\mu_{max}$=0.13 $K_s$= 11.11 | $\mu$=0.056 (per Day) $R^2$=0.96 | $\mu$=0.024 (per Day) $R^2$=0.94 |
| Consideration | dx/dt | Regression coefficient | t.d= 0.693/ $\mu$ | d$\mu$ /dt | [dp/dt] /d[$\mu$] | d [$\mu$] / d[1/S] | d[$\mu$]/ d[S] |

Using Cassava Whey as the sole carbon source, phase was reported to have an R2 value of 0.96 and a growth rate of 0.065 biomass/day. This supports Matsakas et al. [27]'s findings that most monocultures' substrate adaption kinetics may limit growth performance and substrate consumption. The mineralization limits of a variety of agrowaste for limiting nutrients may limit the growth dynamics of a monoculture, especially when employing *A. niger* as a feedstock. The paper emphasized the positive correlation between the substrate and the feed stock utilized in the synthesis of oxalic acid, which was comparable to the finding of Olorunnisola et al. (2018), who found a match between process parameters and microbial performance prior to growth investigations in their study. These observations corresponded to those made in prior papers [23,28]. The positive tolerance of the substrates seen in this investigation and characterized by Andrew's and Leudeking- Piret Models indicated that the cassava whey and yeast extract-based media used in the study were positive and tolerated. Ravi et al. [29] were able to use Haldane's Model to predict growth and process parameters in monocultures using substrate utilization constants in a similar investigation. Mullai et al., [30] claimed that a large fraction of substrate in a solid-state fermentation can limit microbial performance and product creation rate. The time it takes for most Ascomycota and Zygomycota-based inocula to sprout and prosper in a substrate differs from one substrate to the next, necessitating substrate research prior to growth kinetics, which is consistent with the current work. Monoculture cultivation and fermentation have limits. Other research has identified the role of mixed cultures in mitigating the issues that monoculture systems face [31,32,25,33].The congruency of model parameters obtained in this study is likewise consistent with a trend previously described by other researchers. The substrate consumption rates (14.28 and 11.16 mg/g/day) for the cassava whey and yeast extract with an $R^2$ value of 0.96 an d 0.94 respectively indicated that there was a healthy utilization of Cassava whey and yeast extract for the generation of oxalic acid and that the process was growth associated.

## 4. CONCLUSION

Biotechnologists are still working to gain a complete understanding of microbial cell factories. The models utilized in this study to represent the fermentation process appear to be useful in determining oxalic acid synthesis, biomass, and substrate consumption. According to the findings, the Luedeking-Piret model best characterized oxalic acid production, indicating that substrate consumption was linked to product generation by *A. niger*. As a result, the models created could be beneficial for managing the growth, oxalate generation, and substrate consumption kinetics of this strain at a large fermentation scale.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Jang YS, Kim B, Shin JH, et al. Bio-based production of C2-C6platform chemicals. Biotechnol Bioeng. 2012;10: 2437–59.
2. Sauer M, Porro D, Mattanovich D, Branduardi P. Microbial production of organic acids: Expanding the markets. TrendsBiotechnol. 2008;26:100–8.
3. Fatima S, Hameed A, Noman M, Ahmed T, Shahid M, Tariq M, Sohail I, Tabassum R. Lignocellulosic biomass: A sustainable bioenergy source for the future. Protein Pept Lett. 2018;25(2):148–163.
4. Alonso S, Rendueles M, Dı́az M. Bio-production of lactobionic acid: current status, applications and future prospects. Biotechnol Adv. 2013;31:1275–91.
5. Klement T, Bu¨chs J. Itaconic acid - a biotechnological process in change. Bioresour Technol. 2013;135:422–31.
6. Xu Q, Li S, Huang H, Wen J. Key technologies for the industrial production of fumaric acid by fermentation. Biotechnol. Adv. 2012;30:1685–1696.
7. Hermann BG, Blok K, Patel MK. Producing bio-based bulk chemicals using industrial biotechnology saves energy and combats climate change. Environ Sci Technol. 2007;41:7915–21.
8. Smidt M. A sustainable supply of succinic acid. Euro Biotech News. 2011;10:11–12.
9. Son, SM, Moon KD, Lee CY. Kinetic study of oxalic acid inhibition on enzymatic browning. J. Agric. Food Chem. 2000;48: 2071–2074.
10. Zheng X, Tian S. Effect of oxalic acid on control of postharvest browning of litchifruit. Food Chem. 2006;96:519–523.
11. Huang H, Jing G, Guo Zhang D, Yang B, et al. Effect of oxalic acid on ripening

attributes of banana fruit during storage. Postharvest Biol. Tec. 2013;84:22–27.

12. Aghaie E, Pazouki M, Hosseini M, Ranjbar M, Ghavipanjeh F. Response surface methodology(RSM) analysis of organic acid production for Kaolin beneficiation by *Aspergillus niger*, Chem. Eng. J. 2009;147: 245–251.

13. Musiał I, Cibis E, Rymowicz W. Designing a process of kaolin bleaching in an oxalic acid enriched medium by *Aspergillus niger* cultivated on biodiesel-derived waste composed of glycerol and fatty acids. Appl. Clay Sci. 2011;52:277–284.

14. Betiku E, Emeko HA, Solomon BO. Fermentation parameter optimization of microbial oxalic acid production from cashew apple juice. Heliyon. 2016;2: e00082.

15. Rajeswari P, Jose PA, Amiya R, Jebakumar SR. Characterization of saltern based Streptomyces sp. and statistical media optimization for its improved antibacterial activity. Front Microbiol Jan 21; 2015.

16. Yun TY, Feng RJ, Zhou DB, Pan YY, Chen YF, Wang F, Xie JH. Optimization of fermentation conditions through response surface methodology for enhanced antibacterial metabolite production by *Streptomyces* sp. 1-14 from cassava rhizosphere. PloS One. 2018;13(11): e0206497.

17. Voll F, Krüger RL, de Castilhos F, Cardozo Filho L, Cabral V, Ninow J, Corazza ML. Kinetic modeling of lipase-catalyzed glycerolysis of olive oil. Biochem. Eng. 2011;56:107-115.

18. Xu LJ, Liu YS, Zhou LG, Wu JY. Modeling of Fusarium redolensDzf2 mycelial growth kinetics and optimal fed-batch fermentation for beauvericin production. J. Ind. Microbiol. Biotechnol. 2011;38:1187-1192.

19. Lin Y, Tanaka S. Ethanol fermentation from biomass resources: Current state and prospects. Appl. Microbiol. Biotechnol. 2006;69:627-642

20. Nielsen J, Nikolajsen K, Villadsen J. Structured modelling of a microbial system: I. A theoretical study of lactic acid fermentation. Biotechnol. Bioeng. 1991; 38:1-10.

21. Murat E, Ferda MA. A kinetic model for actinorhodin production by *Streptomyces* coelicolor A3 (2). Process. Biochem. 1999;34:625-631.

22. Ghosh S, Chakraborty R, Chatterjee G, Raychaudhuri U. Study on fermentation conditions of palm juice vinegar by response surface methodology and development of a kinetic model. Braz. J. Chem. Eng. 2012;29(3):461-472.

23. Song H, Eom M-H, Lee S. Modeling of batch experimental kinetics and application to fed-batch fermentation of Clostridium tyrobutyricum for enhanced butyric acid production. Biochem Eng J. 2010;53:71–76

24. Abu GO. Process and Phenomenal Microbiology: How microbes were created to create jobs for mankind. An inaugural lecture by professor Gideon Orkwagh Abu; Department of Microbiology, Faculty of Science, University of Port Harcourt; 2017.

25. Agbaji JE, Nwaichi EO, Abu GO. Optimization of bioremediation-cocktail for application in the eco-recovery of crude oil polluted soil. AAS Open Research. 2020;3.

26. Gupta A, Jana AK. Effects of wheat straw solid contents in fermentation media on utilization of soluble/insoluble nutrient, fungal growth and laccase production. 3 Biotech. 2018;8:35.

27. Matsakas L, Kekos D, Loizidou M, Christakopoulos P. Utilization of household food waste for the production of ethanol at high dry material content. Biotechnol Biofuels. 2014;7:2–9

28. Shi J, Sharma-shivappa RR, Chinn MS. Enzyme and microbial technology interactions between fungal growth, substrate utilization and enzyme production during shallow stationary cultivation of *Phanerochaete chrysosporium* on cotton stalks. Enzyme Microb Technol. 2012;51:1–8.

29. Ravi R, Philip L, Swaminathan T. Growth kinetics of an indigenous mixed microbial consortium during methylene chloride degradation in a batch reactor. Korean J Chem Eng. 2013;30:1770–1774.

30. Mullai P, Rene ER, Sridevi K. Biohydrogen production and kinetic modeling using sediment microorganisms of Picharavam Mangroves, India. Biomed Res Int. 2013;9.

31. Saheed O, Jamal P, Karim M. Cellulolytic fruits wastes: A potential support for enzyme assisted protein production. J Biol Sci. 2013a;13:379–385

32. Jamal P, Saheed OK, Alam MZ et al. Protein enrichment through synergistic activities of fruit wastes using white rot fungi under submerged state bio-

conversion. J Pure Appl Microbiol. 2014;8: 839–844

33. Olorunnisola. K. S., Jamal, P., and Alam, M.Z. Protein improvement of banana peel through sequential solid state fermentation using mixed-culture of Phanerochaete chrysosporium and Candida utilis. Biotech, 2018; 8(10): 1-10.

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