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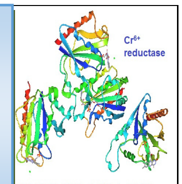

Modelling the Growth Kinetics of Callus Cultures from the Seedling of *Jatropha curcas* L. According to the Modified Gompertz Model

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ABSTRACT

One of the most important preliminary investigation of callus attributes is the growth characteristics. Most often than not, callus growth curve is sigmoidal in characteristics. In this work, we model callus growth from the seedling of *Jatropha curcas* L. according to the modified Gompertz model from published literature to acquire essential growth constants. These growth constants can be obtained with better precision using model such as the modified Gompertz. Parameters obtained from the fitting exercise were maximum callus growth rate (μ_m), lag time (λ) and maximal callus production (Y_{max}) of 0.193 d^{-1}, 2.91 days and 0.38 g callus/25 mL culture, respectively. Growth parameter constants extracted from the modelling exercise will be helpful for additional secondary modelling implicating the consequence of media conditions as well as other factors on the growth of callus from this plant.

INTRODUCTION

Plants as a result of stresses, produce disorganized cell masses, for example, tumors or callus upon pathogen infections or injury. The phrase "callus" emanates from the Latin word callum, meaning hard, and in medicine, it means dermal tissue thickening [1,2]. These days, unorganized cell masses are jointly known as callus, and the same word is utilized more generally. Callus can be made from just one differentiated cell, and many callus cells really are totipotent, which means they are able to bring about whole plant regeneration [3–5]. Under particular circumstances, callus cultures can go through a process in which embryos are produced by adult somatic cells or a process more commonly known as somatic embryogenesis [3]. An immediate use of callus is micropropagation where stock plant materials are multiplied to produce progeny plants in large numbers using modern methods of plant tissue culture. Micropropagation is used to multiply plants that have been bred through conventional plant breeding methods or genetically modified, plants from a seedless stock plant and plants that are not easily vegetatively produced. Other uses of callus include the production of medicinally important compounds, bioactive plant metabolites or vaccines and useful proteins in biadiognostics or other applications [6–12].

Callus growth, like bacterial growth, is a linked process that displays unique phases where the specific growth rate, which initially has a value of zero producing a lag time (λ) then accelerates in a certain time period to a maximal value. The growth curves also include a final phase where an asymptote (A) is achieved where the rate gets to zero. Eventually, callus growth reaches a stage where the cells started to die and entering the death phase. The overall profile of the growth rate appears sigmoidal curve [13]. One of the most important parameters of the growth curve is μ_{max} (or μ_m). In biological systems, this value is used to develop secondary models such as the effects of product, pH, temperature, substrate on the growth rate of the organism. The μ_{max} or μ_m is usually given by the slope of the line at the exponential phase [14]. The most popular method for estimating this value is through conversion of the exponential phase of a linearized form usually via transforming the y values into logarithm or natural logarithm and then determining the slope of this curve using linear regression. A better method, but often neglected, is to model all of the set of data with non-linear regression growth model and then getting the values of μ_{max}, λ, and A from the model [15].

The modified Gompertz model is one of the classical growth models that include model such as the Verhulst [13,16]. The Gompertz function, named in 1844-1845 by Pierre François Verhulstis, is based on an exponential relationship between specific growth rate and population density (Eqn X). The initial stage of growth is approximately exponential; then, as saturation begins, the growth slows, and at maturity, growth stops. Gibson et al. [17] were the first to use the Gompertz equation to fit microbial growth curves and the equation was successfully used to describe the exponential and stationary phases of the microbial growth curves that are sigmoidal. However, the model was not adequate to describe the lag phase. The model was modified by Gibson et al. [17] to incorporate the lag phase, and have been successfully used in modelling many microbial growth curves to the point where its dominance in mathematically modelling bacterial growth and product formation curves have been acknowledged [13,15,18].

The asymmetrical sigmoidal shape of the modified Gompertz represents and may offer greater flexibility than the logistic. Sigmoidal models such as the logistic and Gompertz differ chiefly at the point of inflection between the lower and the upper asymptotes with the logistics and Gompertz models having the distance of 1/2 and 1/e between the lower and the upper asymptotes, respectively [18]. In an essence, other growth models provide flexible slope function and variable point of inflection between the lower and upper asymptotes. These functions are either special or simpler cases of a parent growth model. For instance, the Richard model incorporates the logistics, Gompertz or von Bertalanffy growth models [13,17,18]. The model has its drawbacks and is not perfect with several main issues. Firstly, in the static version, $y_{(t=0)}$ is not equal to y_o. Secondly, an inflection point is the intrinsic property of the sigmoidal curve causing the model to have a systematic problem in describing the exponential phase (Baranyi et al., 1993). Finally, the model tends to over-estimates its parameter values [19–21]. Despite this, the modified Gompertz model has been extensively used to model the growth of bacteria and bacterial secondary products production such as biohydrogen, methane, lactic acid, biofuel and bacterioricin to name a few [22–26] including callus growth [27–29].

Modelling of the growth curves can yield important parameters that can be used for further optimisation works for callus such as determination of specific growth rate, lag period and maximum callus formation. In this study, the callus cultures from the seedling of *Jatropha curcas* L. was modelled according to the modified Gompertz model.

MATERIALS AND METHODS

Acquisition of Data

In order to process the data, graphs were scanned and electronically processed using WebPlotDigitizer 2.5 [30]. The software helps to digitize scanned plots into a table of data with good enough precision [31]. Data were acquired from a published work where callus cultures initiation was carried out from the leaf and hypocotyl explants of the seedling of *Jatropha curcas* L. [32] from Figure 1 and then replotted (**Fig.** 1).

Fitting of the data

To find out regardless of whether there is a statistically substantial distinction between models with many numbers of parameters, according to the quality of fit, data was statistically examined by the coefficient of determination (R^2).

The modified Gompertz model (**Eqn**. 1) is expressed as follows:

$$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\} \quad (1)$$

A= Callus growth lower asymptote;
μ_m= maximum specific callus growth rate;
λ=lag time
y_{max}= Callus upper asymptote;
e = exponent (2.718281828)
t = sampling time

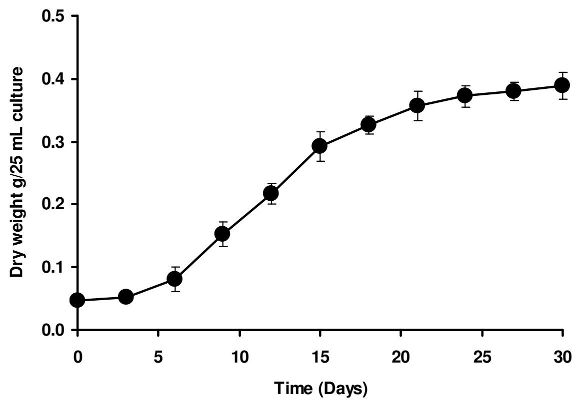

Fig. 1. Refitting of the callus growth curve of *Jatropha curcas* L.

RESULTS AND DISCUSSION

The callus production from this plant was sigmoidal in shape with a lag phase (**Fig.**1). The callus production over time profile was fitted to the modified Gompertz model. The resultant fitting shows visually acceptable fitting with an adjusted coefficient of determination (R^2) of 0.99 indicating good fitting (**Fig.** 2). Parameters obtained from the fitting exercise were maximum callus growth rate (μ_m), lag time (λ) and maximal callus production (Y_{max}) of 0.193 d^{-1}, 2.91 days and 0.38 g callus/25 mL culture, respectively (**Table** 1).

Table 1. Callus production coefficients of *Jatropha curcas* L. fitted to the modified Gompertz model.

Constants	Values (95% confidence interval)
Asymptote (callus g/25 mL culture)	0.38 (0.37 to 0.40)
μ_m (h^{-1})	0.193 (0.178 to 0.208)
lag (days)	2.91 (2.267 to 3.554)

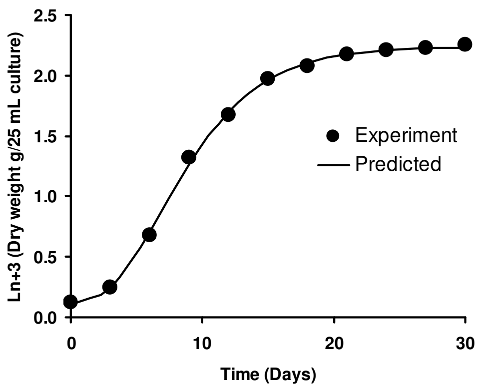

Fig 2. Callus production of *Jatropha curcas* L. fitted to the modified Gompertz model.

The study carried out here attempt to optimize callus formation using mathematical model as callus growth has not been modelled properly using any primary growth models. Other growth models that are available including Baranyi-Roberts [31,33] and Logistic, modified Gompertz [28,28,34–36,36–38], Richards, Schnute [13,39], Von Bertalanffy [40,41], Buchanan three-phase [42–48] and more recently the Huang model [49]. The use of other growth models need to be statistically weighed in against the modified Gompertz model in the future [28,50], and this is currently being carried out. Despite this, the modified Gompertz model is the most popular model as it is the simplest (having three parameters) and allows comparison with published results to be carried out. It is anticipated that many more works on plant secondary products utilizing plant's callus and tissue culture [1–3,7,8,51–56] can benefit from this work.

Parameters obtained from the fitting exercise would be later used for further secondary modelling. These mechanistic models are aimed to reach a better understanding of the chemical, physical, and biological processes. Compared to empirical model, mechanistic models including the modified Gompertz are more powerful since they tell you about the underlying mechanism or processes that drive the change in growth rates observed [57].

CONCLUSION

In conclusion, the callus growth profile has been successfully modelled using the modified Gompertz model. Parameters obtained from the fitting exercise were maximum callus growth rate (μ_m), lag time (λ) and maximal callus production (Y_{max}) of 0.193 d^{-1}, 2.91 days and 0.38 g callus/25 mL culture, respectively. The use of the modified Gompertz growth model to obtain useful growth constants is novel for this plant.

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