Substrate Inhibition Growth Kinetics for Cutinase Producing *Pseudomonas cepacia* Using Tomato-peel Extracted Cutin

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Using tomato-peel extracted cutin, an economically viable substrate, cutinase production by *Pseudomonas cepacia* was studied at different initial substrate concentrations (2–20 g L^{-1}). The highest volumetric enzyme activity was observed at 10 g L^{-1} of cutin, which was inhibited at further higher concentrations. Various 3-, 4- and 5- parametric Monod-variant models were chosen to analyze the inhibition kinetics. The model parameters as well as goodness of fit were estimated using non-linear regression analysis. The 4- parameter Webb model was the best-fit model (R^2 = 0.933), followed by the 3-parameter Andrews model (R^2 = 0.92). Parameter sensitivity analysis revealed that the maximum specific growth rate was the most sensitive parameter for both the models, and the Webb constant was the least sensitive. Finally, based on a strong evidence ratio 190.65 from Akaike's information content criteria analysis as well as extra sum of square F test ($P > 0.05$), it was found that 3-parameter Andrews model gave the best fit.

Key words:

cutinase, *Pseudomonas cepacia*, substrate inhibition model, Akaike's information content criteria, parameter sensitivity

Introduction

Cutinase [EC 3.1.1.74] is a key enzyme known to hydrolyze cutin – a water insoluble polymeric network of esterified hydroxyl and epoxy fatty acids (C16 and C18), and the predominant component embedded in plant cuticles[1] protecting it from desiccation as well as microbial and insect attack. Cutinase is believed to belong to a group of intermediate enzymes between lipase and esterase, which are able to hydrolyze cutin polymer, soluble esters and emulsified triacylglycerol – as efficiently as pancreatic lipases.[2] The absence of any significant structural rearrangements upon binding to a non-hydrolysable substrate analogue represents an important feature of cutinase.[3] Because of no or little interfacial activation and being active on both soluble and emulsified triglycerides, this enzyme is gaining more attention over lipases in recent years.[1]

Cutinases have potential use in the dairy industry for hydrolysis of milk fat, oleochemical industry for synthesis of structured triglycerides polymers and surfactants[4], as ingredients for personal-care products, pharmaceuticals, and in agricultural chemicals preparation to increase the pharmacological effect.[5]

Extensive studies have been carried out on the production, purification and characterization of cutinase from phytopathogenic fungi.[6–9] On the other hand, there are only a few isolated studies on bacterial cutin degradation ability or bacterial cutinase[10–12], which are even better in terms of thermal or pH stability when compared to their fungal counterpart. Unlike fungal cutinase, which loses its activity at 45 °C, bacterial cutinase could be stable at as high as 70 °C.[13] Microbial production and specificity of enzymes have been shown to be dependent on the nature and concentration of substrate.

Authors have tried to find a cost-effective cutin substitute to induce cutinase production.[14] Recently, Dutta *et al.*[1] reported efficient induction of bacterial cutinase with cutin hydrolysate. However, the production of cutinase is often highly regulated by the growth conditions.[15] Substrate inhibition remains a very general phenomenon in hydrolysis of water-insoluble substrates by extracellular microbial enzyme systems.[16] As substrate consumption is coupled to the cell growth and enzyme production, the assessment of a proper substrate inhibition growth model for cutinase producing microbial culture would be an integral and essential roadmap to eval-

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uate, predict and optimize the biological process system.

In the present work, kinetics of cutinase production using laboratory-isolated cutin from tomato peels as inducer/substrate and carbon source have been studied. At different initial cutin concentrations, cell growth and cutinase production by *Pseudomonas cepacia* (NRRL B 2320) were measured. Experimental data were analyzed with a set of well-known substrate inhibition models and the selected models were rigorously compared for their goodness of fit, parameter sensitivity, and different statistical and information content criteria.

Materials and methods

Preparation of cutin

Cutin as substrate for cutinase was prepared in the laboratory from fresh tomato peels using the method described by Chen *et al*.[12] In brief, the tomato peels collected from fresh tomatoes were boiled in oxalic acid/ammonium oxalate buffer for 3–4 h. After cooling to room temperature, the peels were digested with enzymes (cellulase and pectinase) to remove pectin and cellulose, subjected to extensive solvent extraction with methanol-chloroform in soxhlet apparatus to remove the embedded waxes, and then dried in an oven at 40 ºC. These dried peels were ground to powder (< 20 mesh) to obtain cutin.

Microorganism and maintenance

The bacterium used in this study, *P. cepacia* NRRL B 2320 (also known as *Burkholderia cepacia*), was procured from Agricultural Research Service (ARS-Culture collection), USDA, Peoria, USA. The organism was grown on nutrient agar medium at 28 °C.

Seed culture medium

The medium used for the development of seed culture contained (g L^{-1}): glucose 6.0, beef extract 3.0, peptone 15.0, urea 6.0, KH_2PO_4 2.0, KCl 0.5, $MgSO_4 \cdot 7H_2O$ 5.0 (pH 7.0). The seed culture medium was inoculated with a loop full of pure culture grown on nutrient agar slant. The culture was then incubated for 10 h (to reach culture OD of 0.6~0.8 at 600 nm) at 28 °C and 180 rpm in a shake flask.

Kinetic experiment

The medium for cutinase production contained (g L^{-1}): beef extract 4.0, peptone 17.77, urea 5.0, KH_2PO_4 3.0, KCl 0.635, $MgSO_4 \cdot 7H_2O$ 5.546 and crude cutin preparation at 2–20 g L^{-1}, and the initial pH was adjusted at 7.0. A 2 % v/v of inoculum from the seed culture was added to 50 mL of the medium in sets of 250 mL Erlenmeyer flasks. The flasks were incubated in a shaker incubator set at 28 °C and 180 rpm. Samples (culture medium) were withdrawn at regular intervals and measured for cutinase activity. For each initial mass concentration of cutin in batch experiment, the specific growth rate (μ) was calculated from the slope of plot drawn between ln (γ_x) vs time; where, γ_x is the dry cell mass obtained at a particular time. The experiments were conducted in duplicate and an enzymatic assay was performed in duplicate for each sample.

Cutinase assay

Samples were centrifuged at 10,000 *g* for 10 min at 4±1 ºC. The supernatant was separated and used for cutinase assay. The activity was measured by two different sets of assay. Firstly, a non-specific yet simple spectrophotometric assay, which does not distinguish between esterase and cutinase, was used to measure the release of *p*-nitrophenol (*p*NP) from *p*-nitrophenylbutyrate (*p*NPB).[12] The production of cutinase was further confirmed by using cutinase-specific substrate *p*-nitrophenyl (16 methyl sulphone ester) hexadecanoate (*p*-NMSH).[17]

For the nonspecific assay, an aliquot of (20 µL) culture supernatant was added to 980 µL of reaction mixture, which was prepared by adding 1 mL of *p*NPB solution (23 mmol L^{-1} in tetrahydrofuran) to 40 mL of potassium phosphate buffer (50 mmol L^{-1}) containing sodium deoxycholate (11.5 mol L^{-1}). The release of *p*-nitrophenol was monitored at 410 nm for 15 min at 37 ºC. One unit of enzyme activity was defined as the amount that releases one µmol L^{-1} of *p*-NP min^{-1} under assay conditions. The production of cutinase was confirmed by using cutinase-specific substrate *p*-NMSH according to Degani *et al.*[17] with minor modifications (incubation period of 4 h instead of 15 min).

Microbial biomass measurement

Due to the presence of cutin, the cell dry-weight concentration could not be measured directly, and therefore, the intracellular protein concentration was measured.[18] Briefly, the cell samples were centrifuged at 10,000 *g* for 10 min at 4 ºC and the pellets were re-suspended with an equal volume of distilled water and again centrifuged. After repeating the washing step two more times, the cells pellet was subjected to alkaline lysis (heating at 100 ºC for 20 min in original volume using 0.4 N NaOH). The total protein content in the samples was determined by Lowry's method[19] using bovine serum albumin (Sigma) as standard. The relationship with

cell dry weight and intracellular protein was established as:

$$\text{cell dry weight (g L}^{-1}) = \\ = \text{intracellular protein content (in g L}^{-1}) \cdot 19.03 \quad (1)$$

Substrate inhibition growth models for cutinase production

In this particular study, a large set of different widely published substrate inhibition models that have evolved from or are an extension of Monod's kinetics, were used to analyze experimental data (Table 1). The *Andrews* model explains the inhibitory effects of substrate at higher concentrations, which however, reduces to the conventional Monod's equation when the inhibition constant becomes very high.[20] The *Luong* model is a generalization of Monod's kinetics that includes an additional term to account for complete growth inhibition above a critical substrate concentration. The relation between the specific growth rate and the initial substrate concentration could either be linear ($n = 1$), a concavity upward ($n > 1$) or a concavity downward ($n < 1$) depending on the value of the constant parameter (n).[21] The *Han and Levenspiel* model is an extension of the Monod's model to account for cell, product and substrate inhibition, and is also capable of explaining the type of inhibition as competitive, non-competitive or uncompetitive depending upon the values of the two constant parameters (n, m). This model also assumes a value for critical inhibitory concentration of substrate, above which growth completely ceases.[22] The *Haldane* growth model incorporating both the substrate affinity constant and the substrate-inhibition constant is widely accepted for representing the growth kinetics under inhibitory substrates. The kinetics follows simple Monod's model when the inhibition constant is infinitely large.[23] The *Moser* model is a modified Monod equation with a power function of substrate concentration. The value of the power determines the degree of inhibition. However, it does not indicate critical substrate concentration or inhibition constant.[24] The *Aiba* growth inhibition model depicts a decrease in specific growth rate with an increase in product concentration. The exponential term to take care of the product inhibition could be well replaced with substrate concentration. However, it fails to give the critical value of inhibitory substrate/product concentration.[25]

Table 1 – *List of substrate inhibition models initially selected for this study*

Model	Equation	Ref.
Andrews	$\mu = \frac{\mu_{\max}\gamma_s}{\left(K_s + \gamma_s\right)\left(1 + \frac{\gamma_s}{K_I}\right)}$	20
Aiba	$\mu = \frac{\mu_{\max}\gamma_s}{K_s + \gamma_s}\exp\left(\frac{-\gamma_s}{K_I}\right)$	25
Moser	$\mu = \frac{\mu_{\max}\gamma_s^n}{K_s + \gamma_s^n}$	24
Edward	$\mu = \mu_{\max}\gamma_s\left[\exp\left(\frac{-\gamma_s}{K_I}\right) - \exp\left(\frac{-\gamma_s}{K_s}\right)\right]$	27
Webb	$\mu = \frac{\mu_{\max}\gamma_s\left(1 + \frac{\gamma_s}{K}\right)}{\gamma_s + K_s + \frac{\gamma_s^2}{K_I}}$	28
Luong	$\mu = \frac{\mu_{\max}\gamma_s}{\left(K_s + \gamma_s\right)}\left[1 - \frac{\gamma_s}{\gamma_s^*}\right]^n$	21
Yano	$\mu = \frac{\mu_{\max}\gamma_s}{K_s + \gamma_s + \frac{\gamma_s^2}{K_I}\left(1 + \frac{\gamma_s}{K}\right)}$	26
Haldane	$\mu = \frac{\mu_{\max}\gamma_s}{K_s + \gamma_s + \frac{\gamma_s^*}{K_I}}$	23
Han-Levenspiel	$r = k\left[\left(1 - \frac{\gamma_s}{\gamma_s^*}\right)^n\right]\frac{\gamma_s C}{\gamma_s + K_M\left[\left(1 - \frac{\gamma_s}{\gamma_s^*}\right)^m\right]}$	22

The *Yano* model was originally proposed for the kinetics of amylase production at high sugar concentration.[26] The *Edward* model gives the protective diffusional-limitation of high and inhibitory substrate concentrations.[27] The *Webb* model is the modified Yano model, where $(1 + \gamma_s/K)$ term is present in the numerator rather than the denominator.[28]

The parameters of different models were estimated from the experimental results using MATLAB v.7.1. Since the models had non-linear coefficients, the parameters were estimated iteratively with non-linear least square algorithm.

Statistical comparison of model for acceptability

The various substrate inhibition models chosen for this study were different in terms of complexity (degree of freedom). Therefore, it was essential to test whether experimental data could be more consistent with one mechanism (model) than with the other. Kinetic models, where improved fitting (lower model sum of square or highest regression coefficient) comes at the cost of increasing complexity, must be compared either using the extra sum-of-square F test[29] for nested models or Akaike information criterion (AIC) for both nested and non-nest-

ed pairs.[30–31] In our study, the best-fit 3-parameter and 4-parameter models were compared using both *F* test and AIC.

The extra sum-of-square *F* test, as an alternate view for ANOVA analysis, assumes the simpler model to be correct (null hypothesis) if the relative increase in the model sum of squares and in the degree of freedom are approximately equal. Otherwise, if the relative increase in the model sum of squares largely exceeds the relative increase in the degree of freedom, the complex model (alternate hypothesis) is justified. The *F* ratio is given by Eq. 2.

$$F = \frac{\left(SS_{simple} - SS_{alternate}\right)/SS_{alternate}}{\left(df_{simple} - df_{alternate}\right)/df_{alternate}} \qquad (2)$$

Where, "*SS"* represents the sum of square differences between experimental and model data, and "*df"* represents their degree of freedom. Subscripts "*simple*" and "*alternate*" represent the two models of different complexity. The probability value for the *F* distribution is compared with the confidence level cutoff probability.

The Akaike information criterion (AIC) is defined by the following equation:

$$\mathrm{AIC} = p \ln\left(\frac{SS}{p}\right) + 2b \qquad (3)$$

Where, "*p*" is the number of data points, "*b*" is the number of parameters "*prm*" to be fitted by the regression plus one ($b = prm + 1$). When there are few data points, the corrected AIC ($\mathrm{AIC_c}$) is used.

$$\mathrm{AIC_c} = AIC + \left(\frac{2b(b+1)}{p-b-1}\right) \qquad (4)$$

The model with lower $\mathrm{AIC_c}$ value is more likely to be correct and the probability (*pAIC*) that the more complex model is correct is given by

$$pAIC = \left(\frac{e^{-0.5\Delta_{AICc}}}{1+e^{-0.5\Delta_{AICc}}}\right) \qquad (5)$$

Where, ΔAIC_c is the difference between $\mathrm{AIC_c}$ values of the complex and simple models.

Results and discussion

Growth and enzyme activity profiles at varying substrate concentrations

The growth profiles of *P. cepacia* at different initial substrate concentrations are shown in Fig. 1. It was observed that the growth of *P. cepacia* was very low at the initial cutin concentration of 2 g L^{-1}, but continued to improve up to an initial substrate concentration of 10 g L^{-1}. Irrespective of initial cutin concentrations in different sets, there was a consistent lag period of about 4 h before the onset of logarithmic growth, and the stationary phase was reached at about 96 h.

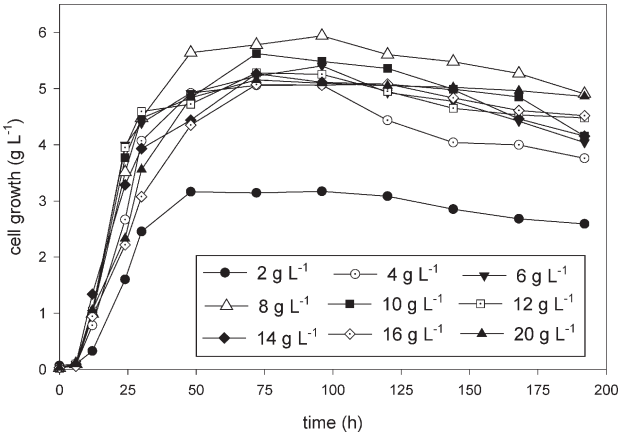

Fig. 1 – *Growth profile of P. cepacia at different initial substrate concentrations*

Extracellular enzyme activity measured at different time intervals from batch experiments with varying initial substrate concentrations is presented in Fig. 2. The culture enzyme activity continues to increase until 96 h, when the highest enzyme activity was recorded (344 U mL^{-1}) with initial substrate, cutin concentration of 10 g L^{-1}. The activity was 3.1 U mL^{-1} with *p*-NMSH assay. This observation suggested the possibility of substrate inhibition when cutin concentration increased above 10 g L^{-1}. However, there is no available report that quantitatively relates cutinase production to cutin as the sole carbon source. Fett *et al.*[10] have observed that cutin hydrolysate failed to induce cutinase production with *T. fusca,* and higher concentrations of cutin hydrolysate were inhibitory towards microbial

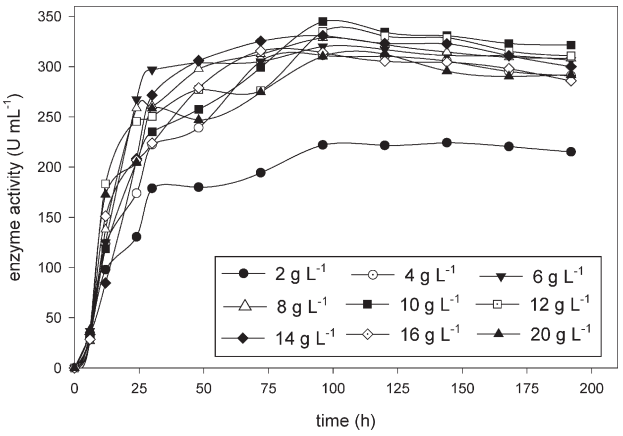

Fig. 2 – *Enzyme activity profile obtained at different initial substrate concentrations*

growth. A very logical reasoning could be that, at low concentrations, the effect of the nutrient was negligible on gross measures of metabolic activity, such as specific growth rate, respiration rate and rate of protein/enzyme synthesis, etc. However, at higher concentrations of cutin, the mass and oxygen transfer in the media had been hindered due to the insoluble nature of cutin, thus, cell growth as well as enzyme production had decreased. It was observed that the same cutin concentration (>10 g L^{-1}) was responsible for attenuation of the specific growth rate as well as enzyme production. At this point, either some other environmental factor was limiting the nutrient supply or the cells themselves had reached their own limit for the cultural conditions.

Specific growth rate and enzyme activity at different substrate concentrations

The cell growth increase with increasing concentration of cutin, the specific growth rate and maximum enzyme activity as a function of initial substrate concentration are presented in Fig. 3. The specific growth rate increased from 0.11 h^{-1} to 0.21 h^{-1} before dropping down again to 0.17 h^{-1} at a substrate concentration of 20 g L^{-1}. The decrease in specific growth rate (μ) and enzyme activity at and above initial substrate concentration of 10 g L^{-1} was due to the presence of substrate inhibition kinetics. In fact, the maximum specific growth rate and the maximum enzyme activity measures were highly correlated with Pearson product moment correlation of 0.94 ($P < 0.001$), suggesting cutinase production to be highly growth-associated. A similar observation has also been reported by Calado *et al.*[32], where cutinase production was highly growth-associated while using galactose (10–40 g L^{-1}) as the sole carbon source. Characterization of crude and purified cutinase from *P. cepacia* NRRL B 2320 has been described elsewhere.[33]

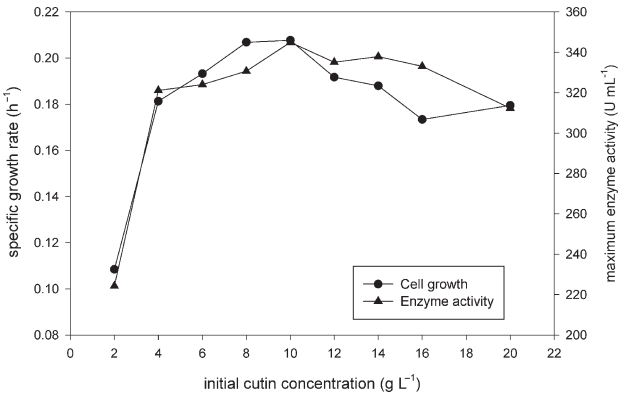

Fig. 3 – *Specific growth rate and maximum enzyme activity as a function of initial substrate (cutin) concentration*

Exploration of kinetic models for growth of *P. cepacia*

Even if the patterns of microbial growth throughout the experimental period were similar to each other irrespective of initial cutin concentration, the variation in the experimental specific growth rate for *P. cepacia* was dependent on the initial substrate concentration. Similar results were reported by Agarwal *et al.*[18], where they found that for all initial concentrations of cellulose in shake flask there was a lag of about 2 h, but the time to reach stationary phase increased with increasing initial cellulose concentration. The authors have noted a decreasing trend in the specific growth rate at higher substrate concentration, indicating substrate inhibition. The variation in adaptation of the culture at different initial substrate concentrations due to high substrate concentrations is most probably tolerated by a development of cell adaptation mechanisms during the lag and early exponential growth phases of a batch culture. The substrate inhibition models chosen in this study were regressed with the experimental data, and the model predicted values by MATLAB v.7.1 are presented in Fig. 4. It can be observed that, except the Haldane and Moser's models, most of the other kinetic models had very closely resembling experimental and predicted specific growth rate profiles in the substrate concentration regime. The parameters were estimated using the non-liner regression of various models as presented in Table 2. Among the several models used to fit the present experimental data for specific growth rates, it was observed that the Webb model had the highest R^2 value of 0.933, followed by the Andrews model (R^2 = 0.92), the Aiba model (R^2 = 0.91), and Luong model (R^2 = 0.90) showing an apparent good fit between experimental and model predicted values. The root mean square errors (RMSE) between experimental and model pre-

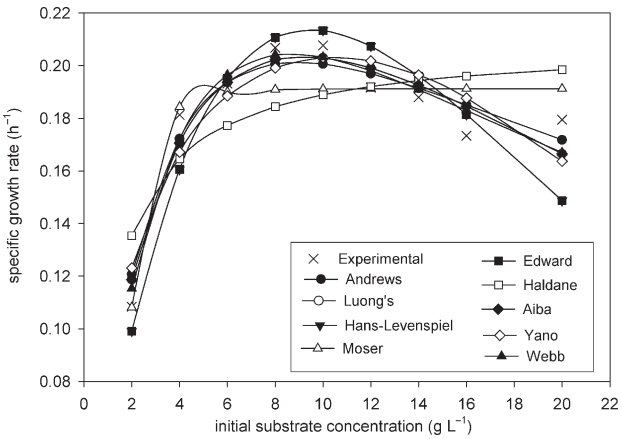

Fig. 4 – *Experimental and the various substrate inhibition models predicted specific growth rate profiles as a function of initial substrate (cutin) concentration*

Table 2 – *Estimated biokinetic parameters from different models*

Model	Parameter	df	Estimated parameters									R^2	RMSE
			μ_{max}	K_S	K_I	γ_s^*	K	n	m	k	K_m		
Andrews	3	6	0.72	7.94	10	–	–	–	–	–	–	0.92	0.009
Aiba	3	6	0.44	4.88	26.52	–	–		–	–	–	0.909	0.010
Moser	3	6	0.191	15.75	–	–		4.35	–	–	–	0.855	0.013
Edward	3	6	0.061	0.051	9.49							0.73	0.017
Webb	4	5	0.52	6.63	11.28	–	26487.93	–	–	–	–	0.933	0.009
Luong	4	5	0.44	4.87	–	6039.2	–	227	–	–	–	0.909	0.011
Yano	4	5	0.21	1.79	10062.31	–	2.269	–	–	–	–	0.836	0.015
Haldane	4	5	0.21	1.10	5364.48	–	–	–	–	–	–	0.569	0.022
Han-Levenspiel	5	4	0.14	–	–	8002.22	–	834.5	6	478.1	1061.98	0.73	0.025

df – degree of freedom for a model

dicted values were 0.0097, 0.0096, 0.010, and 0.01, respectively, for the four different models. Due to its simpler formulation compared to other Monod model derivatives, the Andrews equation remains the most widely used.[34] In this study, the Andrews model predicted the substrate inhibition to start at cutin concentration 10 g L^{-1}, whereas the Webb model predicted the value to be 11.28 g L^{-1}. The close resemblance of predicted values between the two models is logical because for high value of constant '*K*' the Webb model reduces into the Andrews model. The highest maximum specific growth rate (μ_{max}) was predicted by the Andrews model (0.72 h^{-1}), followed by the Webb model (0.518 h^{-1}), the Luong and Aiba models (0.44 h^{-1}). The predicted μ_{max} by all other models, particularly the Moser and Edward models were much lower and certainly insensible. By modeling growth of *Cellulomonas cellulans* during production of cellulase using water-insoluble substrate cellulose, Agarwal *et al.*[18] had found that the Han Levenspiel model was the best fit to experimental data. Theodore and Panda[35] observed that growth of *Trichoderma harzianum* during production of β-1,3glucanase could better be represented with the Luong model. Agarrey and Solomon[36] studied the degradation of phenol by *Pseudomonas fluorescence* and observed that the experimental data were a good fit to the Haldane model. It was observed that different models could be applicable for different systems.

Sensitivity analysis of estimated kinetic parameters

Sensitivity analysis, using the methodology described by Sobie[37], was performed to evaluate how the change in model parameter estimate would affect the model regression coefficients. In this study, a set of input parameters for both the Webb and Andrews models were varied within ±50 % of their estimates, while keeping all other parameters "on hold". The sensitivity analysis results for all the parameters are presented in Fig. 5. It is apparent from the figure, that the maximum specific growth rate (μ_{max}) was the most sensitive parameter for both the models. Any variation of μmax (increase or decrease) severely compromised the regression coefficient for the Andrews model ($R^2 < -10$) or the Webb model ($R^2 < -50$), particularly with higher end values of μmax. The very high sensitivity of maximum specific growth rate suggests requirement of its precise measurement/estimate for further predictability of the model.[38] For any downward change in K_I or upward change in K_S from their respective optimal estimates, sensitivity profiles for both the models were nearly identical. However, the Andrews model quickly turned out to be more sensitive than the Webb model for any upside variations in K_I values or downside variations in *K*S values from their standard estimate. It was apparent from the figure that the regression coefficient of the Webb model was least sensitive to the Webb constant (*K*), and in such a situation, inference of its meaningful estimate would be hard or even impossible.[39] While measuring parameter sensitivity using change for initial guess, Banerjee and Ghoshal[40] have reported the poor repeatability Webb model fitting to substrate (phenol) inhibition kinetics. This could be due to the exorbitantly high absolute value of the estimated parameter (more than two orders of magnitude) compared to experimental substrate concentration.

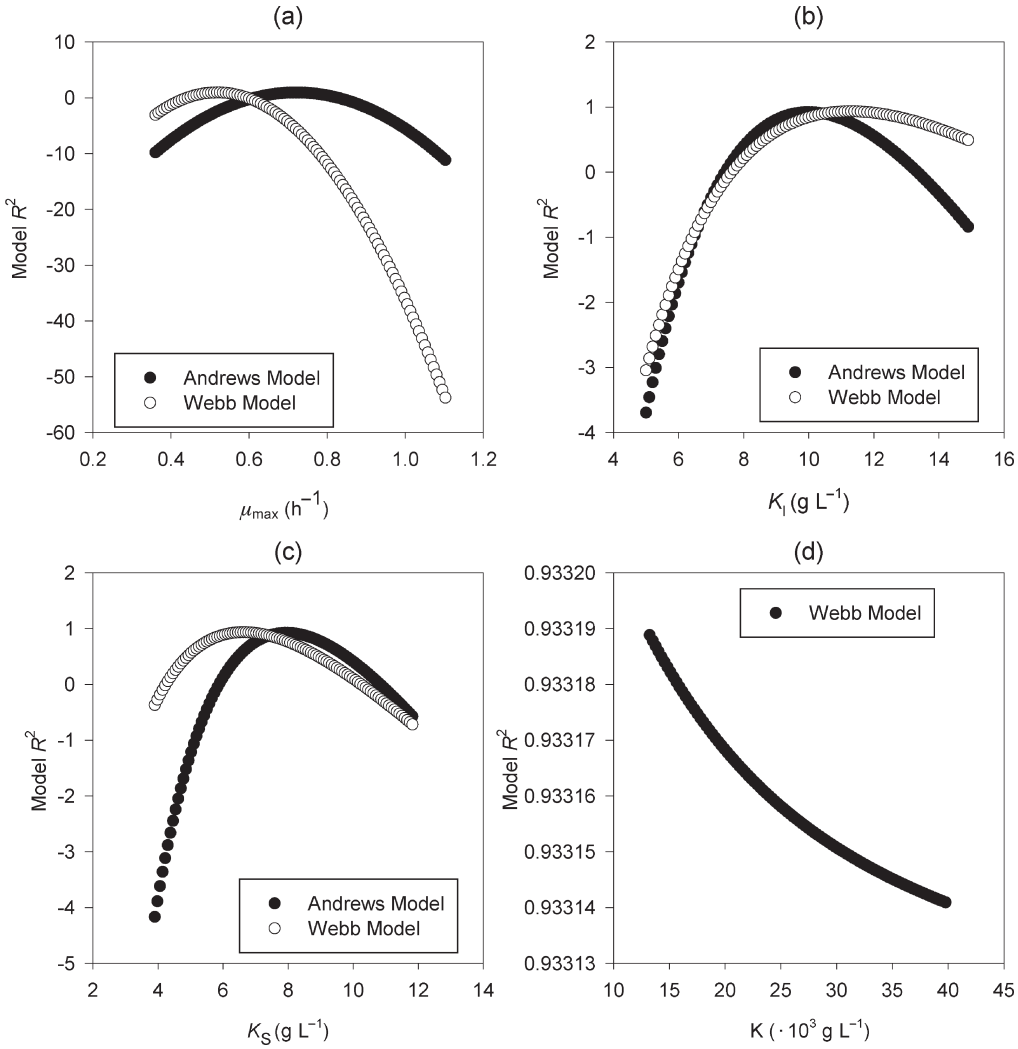

Fig. 5 – *Sensitivity analysis of a) maximum specific growth rate, b) substrate inhibition constant, c) Monod half saturation constant and d) Webb constant (only for Webb model) as estimated from the Andrews and Webb models toward model regression coefficients*

Comparison of model for acceptability

The best-fit 3-parameter, 4-parameter, and only one 5-parameter models had regression coefficients of 0.92, 0.933 and 0.73, respectively. The more complex Webb model had better fits than the simpler Andrews (3-parameter) model. Using the extra sum of square *F* test or Akaike's information content criteria, the two models were compared. The results are presented in Table 3. As the probability value for the extra-sum-square *F* test was > 0.05, at 95 % confidence we could not reject the null hypothesis (simpler model) in favor of the alternative that the more complicated model would be correct. In other words, there was no evidence supporting the alternate model, so the simpler model was accepted. In the AIC-based comparison as the ΔAIC_c was positive, we concluded that the change in SSE would not been as large as expected from a change in the number of parameters. The probability for choosing the correct model was > 99 % (here the simpler Andrews model), and associated with an overwhelming evidence ration of 190.65.

Table 3 – *Summary of model discrimination using Akaike's information criterion methodology and extra sum-of-square F test*

Models	Model specific information				Akaike's information criterion				Extra sum-of-square *F* test			
	prm	*p*	SS ($\cdot 10^{-5}$)	*df*	AIC_c	Δ_{AICc}	p_{AICc}	evidence ratio	X	∇	*F* ratio	*P* value
Andrews	3	9	55.4	6	–69.2	10.50	0.9948	190.65	0.18	0.2	0.906	0.384
Webb	4	9	46.9	5	–58.7		0.0052					

Conclusion

P. cepacia can utilize cutin in the range of 2 to 20 g L^{-1} as a sole carbon source for the production of cutinase. However, substrate inhibition was evident in the growth of *P. cepacia*. The 4-parameter Webb model and the 3-parameter Andrews model were the best fit among the different models tested. However, AIC and *F*-test analysis finally favored the acceptance of the 3-parameter Andrews model. Fed-batch culture strategy can be adopted to overcome substrate inhibition through intermittent addition of the substrate which maintains a substrate concentration at a low level or less than inhibitory level.

List of symbols

b – Variable of AIC analysis (number of parameters plus one)
C – Cell concentration in Han–Levenspiel model, g L^{-1}
df – Degree of freedom for a model with experimental data
K – Webb constant, g L^{-1}
K_I – Inhibition constant for cutin, g L^{-1}
K_M – Monod's constant, g L^{-1}
K_s – Half–saturation constant for cutin, g L^{-1}
n,m – Constant parameters
p – Number of experimental data points
p_{AIC} – Probability that model with given AICc value is correct
prm – Number of parameters to be estimated in a model
r – Cell growth rate, g L^{-1} h^{-1}
Δ_{AICc} – Difference in AIC_c value between two models
SSE – Sum of square of errors

Greek letters

γ_s – Initial cutin concentration, g L^{-1}
γ_s^* – Critical cutin concentration, g L^{-1}
μ – Specific growth rate, h^{-1}
μ_{max} – Maximum specific growth rate, h^{-1}

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