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A KINETIC MODEL FOR GROWTH AND BIOSYNTHESIS OF MEDIUM-CHAIN-LENGTH POLY-(3-HYDROXYALKANOATES) IN *Pseudomonas putida*

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Abstract - A kinetic model is presented giving a mathematical description of batch culture of *Pseudomonas putida* PGA1 grown using saponified palm kernel oil as carbon source and ammonium as the limiting nutrient. The growth of the micro-organism is well-described using Tessier-type model which takes into account the inhibitory effect of ammonium at high concentrations. The ammonium consumption rate by the cells is related in proportion to the rate of growth. The intracellular production of medium-chain-length poly-(3-hydroxyalkanoates) (PHA_{MCL}) by *P. putida* PGA1 cells is reasonably modeled by the modified Luedeking-Piret kinetics, which incorporate a function of product synthesis inhibition (or reduction) by ammonium above a threshold level.
Keywords: Ammonium; Kinetic; Medium-chain-length PHA; *P. putida*; Substrate-inhibition.

INTRODUCTION

Poly-(3-hydroxyalkanoates) (PHA) are natural polyesters accumulated intracellularly by various types of microorganisms. When nutrient supplies such as nitrogen, oxygen, phosphorus, sulfur or magnesium are imbalanced, it is advantageous for bacteria to store excess carbon by polymerizing soluble carbon intermediates into water-insoluble molecules like PHA inside their cells (Madison and Huisman, 1999). PHA is classified into two major families, i.e., short-chain-length PHA (PHA_{SCL}) and medium-chain-length PHA (PHA_{MCL}). Typical examples of PHA_{SCL} are poly-(3-hydroxybutyrate) (PHB) and poly-(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV). A well known producer of PHA_{SCL} is *Wautersia eutropha* (formerly known as *Alcaligenes eutrophus*). The class of PHA_{MCL} is characterized by monomers with a carbon atom length ranging from 6 to 18, and is primarily produced by the fluorescent pseudomonads (Huisman et al., 1989). More than 100 different monomers have been reported to occur in PHA_{MCL} (Steinbuchel and Valentin, 1995). The PHA_{MCL} are also targeted for specific uses where chirality and elastomeric properties are important. The constituent monomers that display different functional groups in their side chain are a valuable source of chiral synthons yet to be exploited (Kessler et al., 2001).

Only the PHA_{SCL} have been commercially produced up to 500 tons $year^{-1}$, which was manufactured by Monsanto (Kellerhals et al., 2000).

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The PHA_{MCL} are yet to make a significant impact as a viable choice due to the fact that it is very expensive to produce this polymer in bulk amounts even for material testing purposes. The final PHA_{MCL} yield and content obtained are lower compared to those of PHA_{SCL}, which hampered development of its applications (Lee et al., 2000). Much of the research effort was directed to improve its yield and productivity using fermentation processes. Several PHA_{MCL} production strategies in the bioreactor such as batch and continuous (Durner et al., 2001; Jung et al., 2001), fed-batch (Beom, 2002) and high-cell-density processes (Lee et al., 2000) under various cultivation conditions have been described.

For the production of PHA_{MCL}, one of the most preferred feedstocks is highly reduced and long carbon chain molecules such as animal or vegetable oils or their free fatty acids. These substrates have high energy content, which is excellent for good cell growth and energy metabolism. It is suggested that these oils in the semi-purified form can be a cheaper substrate for PHA_{MCL} fermentation as compared to the purified, single-type fatty acids. However, most of the studies on PHA_{MCL} fermentation employed the latter as the carbon source, which gives more defined fermentation components. Relatively high yield and productivity have been reported for the use of pure, single type fatty acids as the fermentation feedstock (Durner et al., 2001). The usage of crude fatty acid mixtures or their oils, however, has not been a popular choice.

One of the pioneering studies on the utilization of crude mixture of fatty acids from plant oils for microbial PHA_{MCL} production was reported by Tan et al. (1997). Using ammonium-limited culture of *Pseudomonas putida* PGA1 in shake-flasks, they have shown that saponified palm kernel oil (SPKO) and its major free fatty acids, when used as the sole carbon and energy source in the fermentation, gave good biomass growth and PHA_{MCL} yield. Subsequently, kinetics of ammonium uptake and growth of *P. putida* PGA1 using SPKO as the sole carbon and energy source with ammonium as the limiting nutrient was studied by Annuar et al. (2006). They reported that the ammonium uptake by *P. putida* PGA1 cells can be described using a first-order kinetic model, indicating that the micro-organism's specific uptake rate of ammonium and its growth should increase as the ammonium ion concentrations become higher ($\leq$ 0.1-0.2 gL^{-1}). Further increase in the ammonium ion concentration above 0.2 g L^{-1} resulted in slightly lower specific growth rates of *P. putida* PGA1 (Annuar et al., 2006, 2007).

In cultivations using an automated bioreactor, PHA_{MCL} accumulation by *P. putida* PGA1 is encouraged under ammonium-limited conditions with SPKO as the sole carbon and energy source (Annuar et al., 2007). The amount of PHA_{MCL} accumulated and its specific production rate, q_{PHA}, were influenced by the residual ammonium concentration level in the culture medium. It was observed in both batch and fed-batch fermentations that when the residual ammonium becomes exhausted (<0.05 g L^{-1}), the PHA_{MCL} accumulation and q_{PHA} were significantly reduced (Annuar et al., 2007). However, this effect can be reversed by feeding low amount of ammonium to the culture, resulting in significantly improved PHA_{MCL} yield and productivity. It is concluded that the feeding of residual ammonium concentration in the culture medium during the PHA_{MCL} accumulation has a positive effect on sustaining the PHA_{MCL} biosynthetic capability of the organism. Uptake of SPKO by the micro-organism follows zero-order kinetics, indicating a mass transfer limitation of the free fatty acids by the *P. putida* PGA1 cells (Annuar et al., 2007).

Several kinetic models have been proposed for the growth and PHA_{SCL} production by strains of *W. eutropha* under chemolithoautotrophic and heterotrophic growth conditions using laboratory-scale automated bioreactor (Heinzle and Lafferty, 1980; Mulchandani et al., 1989; Belfares et al., 1995). On the other hand, no formal kinetic models have been reported for growth and PHA_{MCL} production by microorganisms, especially by the main producer of PHA_{MCL}, i.e., *Pseudomonas* sp.

In this short communication, a kinetic model for growth and PHA_{MCL} production by *P. putida* PGA1 is presented which complements the earlier studies of Annuar et al. (2007). The present study evaluated several kinetic models for growth of *P. putida* PGA1 on SPKO with ammonium as a limiting nutrient. Two classes of growth models were tested on published experimental data of Annuar et al. (2006), i.e., models incorporating a substrate inhibition parameter and models that consist of only growth parameters. This was followed by the development of a simple mathematical model *via* partial adoption of a published model of Heinzle and Lafferty (1980), which reasonably describes the limiting substrate consumption (i.e., ammonium) and PHA_{MCL} production in *P. putida* PGA1 in a batch fermentation. The simulation results for growth, ammonium consumption and PHA_{MCL} biosynthesis in *P. putida* PGA1 were compared with the published experimental data (Annuar et al., 2007).

MATERIALS AND METHODS

Microorganism

Pseudomonas putida PGA1 strain was a gift from Professor G. Eggink of the Agrotechnological Research Institute, Wageningen, The Netherlands.

Medium Composition

In all studies, a defined mineral medium was used with $NaNH_4HPO_4.H_2O$ providing the limiting ammonium nutrient. SPKO was supplied as the sole carbon and energy source. The exact composition of the mineral medium and trace elements used was detailed in Annuar et al. (2007). Saponification of palm kernel oil (PKO) was carried out according to Tan *et al*. (1997). PKO is the extract from the nut of the oil palm (*Elaeis guineensis Jacq*.) fruit. The oil consists of a mixture of C6–C18:2 fatty acids with approximately 82% saturated fatty acids and 18% unsaturated fractions. Detailed fatty acid composition of palm kernel oil was reported by Elson (1992).

Shake-Flasks Studies

The different growth models for *P. putida* PGA1 were evaluated using experimental data obtained from shake-flasks cultivation. The corresponding data and details of the experimental conditions for this cultivation which include the cultivation conditions, growth and ammonium assays, data analyses and numerical calculations have been described elsewhere (Annuar et al., 2006).

Bioreactor Studies

Experimental data for comparison with simulation results were obtained from published work of Annuar *et al*. (2007), which also elaborated on the bioreactor specifications and experimental conditions (fatty acid compositions of SPKO, cultivation conditions, analytical methods, data analyses and calculations). Data from batch fermentation was used for comparison with the simulation results. The main geometric characteristics of the stirred tank bioreactor and the initial conditions of the experiment are reproduced in Tables 1 and 2, respectively. In the bioreactor studies, the temperature and the pH were maintained at 30 (±0.5) °C and 7.0 (±0.05), respectively, with an agitation rate of 600 rpm and an aeration rate of 0.5 vvm of filtered air. Silicone anti-foaming agent (BDH) was included in the aqueous medium at 1.0 g L^{-1}.

Experimental Data Regression and Estimation of Growth Model Kinetic Parameters

Mathematical models describing growth only and those that incorporated growth inhibitions by the substrate were fitted to the shake-flask cultivation data using non-linear regression function of Polymath 6.0 software. The program uses the Levenberg-Marquardt (LM) algorithm, a technique that uses an iterative solution method to calculate the kinetic parameter values.

Table 1: Dimensions of the stirred tank bioreactor (Biostat®B 3-liter fermenter, B. Braun Biotech International) and its components.

Design parameters	Specifications
Total volume	3 litres
Diameter of inner tank	130 mm
Height of tank	240 mm
Number of baffles	4
Baffle width	10.5 mm
Type of impellers	Rushton disc turbine
Number of impellers	2
Distance between impellers	79.5 mm
Distance of lower impeller from bottom plate	25 mm
Impeller diameter of disc	53 mm
Number of blades	6
Impeller blade width	10.5 mm
Impeller blade length	14.5 mm
Diameter of single ring sparger	48 mm
Number of holes	14
Distance of ring sparger from bottom plate	20 mm
Diameter of oxygen electrode	12 mm

Table 2: Initial conditions for the batch fermentation of *P. putida* PGA1 in a stirred tank bioreactor.

Fermentation mode	Working volume (L)	Initial SPKO concentration (g L^{-1})	Initial ammonium (S) concentration (g L^{-1})	Initial total biomass (X) concentration (g L^{-1})	Initial residual biomass (R) concentration (g L^{-1})	Initial PHA_{MCL} (P) concentration (g L^{-1})
Batch	1.2	6.8	0.4	0.115(±0.050)	0.11 (±0.04)	0.009(±0.002)

Simulation of Batch Fermentation

Simulation of the batch fermentation in the bioreactor was performed using the differential equations solver of Polymath 6.0 software. A set of ordinary differential equations (ODE) (eqs. 1, 8 and 9, see Results and Discussion section) was solved using the Runge-Kutta-Fehlberg (RKF45) algorithm.

RESULTS AND DISCUSSION

Simple models are necessary in order to have a solid basis for the design of fermentation processes, for economic calculations, and for the control of fermentation processes. Modeling requires simplifications of the complex biological system, which are at the same time a major goal of modeling. In this study, a semi-empirical model proposed on the basis of a simple mechanistic description from the work of Heinzle and Lafferty (1980) was partially adopted. They presented a structured model describing batch culture of *Wautersia eutropha* strain H16 (formerly classified as *Alcaligenes eutrophus* H16) under chemolithoautotrophic growth conditions. In their work, growth and storage of poly-*β*-hydroxybutyrate (PHB), i.e., PHA_{SCL} are described as a function of the limiting substrate S (i.e., ammonium), the residual biomass R (i.e., PHA-free biomass), and the product P (PHB). Their bacterial ammonium consumption and PHA_{SCL} biosynthesis models were fitted to the experimental data obtained from the reported work of Annuar *et al.* (2007); which details the dynamics of the PHA_{MCL} fermentation of *P. putida* PGA1 grown on 6.8 g L^{-1} SPKO and 0.4 g L^{-1} ammonium as carbon and nitrogen sources, respectively. The growth, ammonium consumption, and PHA_{MCL} production profiles are reproduced in Figure 1.

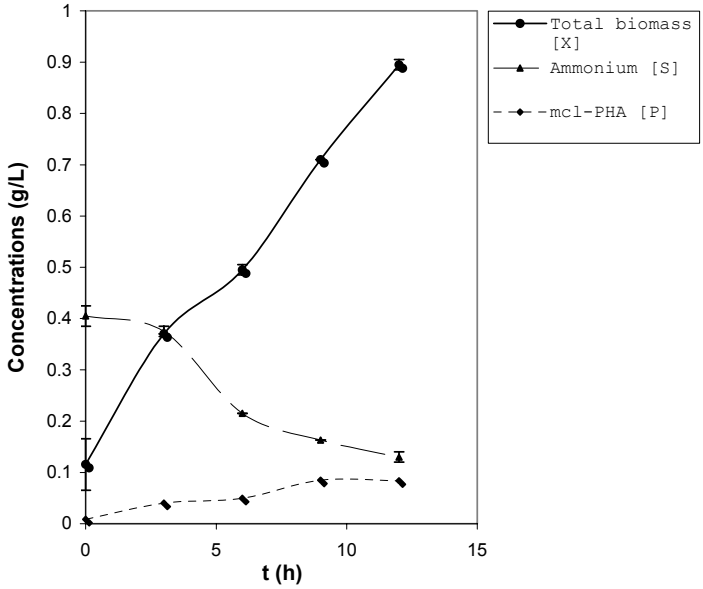

Figure 1: Growth and accumulation of PHA_{MCL} in *Pseudomonas putida* PGA1 in batch fermentation under ammonium-limitation with SPKO as sole carbon and energy source.

Rate of Cell Growth (r_R)

The total dry biomass (X) of *P. putida* PGA1 consists of two parts, namely PHA_{MCL} (P) and residual biomass (R), where R is calculated as the difference between the total dry biomass and PHA_{MCL} concentration (X=R+P). R is the catalytically active fraction of biomass, which includes proteins and nucleic acid.

The limiting substrate ammonium (S) is essential to produce R and limits its synthesis at low concentrations. The synthesis of R is described as follows,

$$dR/dt = r_R = \mu.R \quad (1)$$

where r_R is the rate of synthesis of R and μ is the specific rate of synthesis of R.

The maintenance requirement for the limiting substrate is assumed to be small enough to be neglected. Several growth models were tested to describe the specific growth rate of *P. putida* PGA1 (Table 3). The growth models were divided into those that incorporate limiting substrate-inhibition kinetics and those that contained only growth kinetic parameters. All the models were fitted to the experimental data from shake-flask culture with varying initial ammonium concentrations (Annuar et al., 2006). The estimated values for the model kinetic parameters and fitting constant as returned by the fitting algorithm are shown in Table 3, along with the post-regression statistics. It is clear that growth models that incorporate the substrate inhibition parameter (R^2: 0.9867-0.9955) gave better fits to the experimental data compared to the models with only growth parameters (R^2: 0.9094-0.9569). Among the three models that take into account the substrate inhibition factor, the Tessier-type model showed the best fit of the experimental data (R^2: 0.9955), as compared to the Andrews (R^2: 0.9901) and Aiba (R^2:0.9867) models. The graphical outputs showing the fits of the experimental data by the models with growth kinetic parameters only and by the growth models incorporating the substrate-inhibition kinetic are shown in Figure 2(a) and 2(b), respectively.

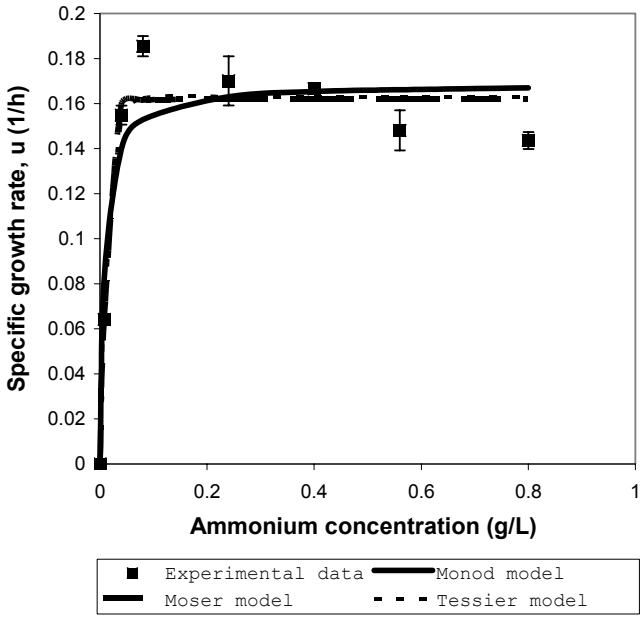

Figure 2a): Fitting of the experimental data with growth models (Monod, Moser and Tessier) without substrate inhibition kinetics.

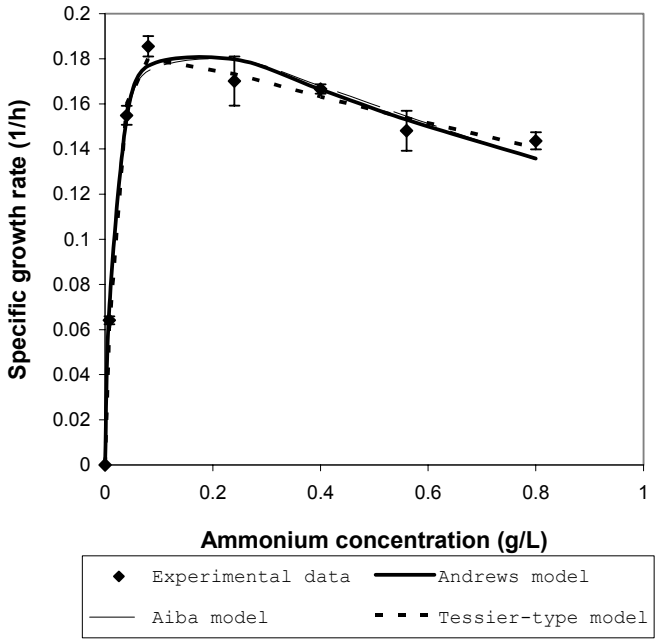

Figure 2b): Fitting of the experimental data with growth models (Andrews, Aiba and Tessier-type) incorporating substrate inhibition kinetics.

Table 3: Values of the kinetic parameters for growth models (with and without the substrate-inhibition kinetic parameter) as returned by the numerical calculations.

	Values of kinetic parameters and fitting constant (± 95% confidence interval)				
Models for growth only	μ_{max} (h^{-1})	K_S (gL^{-1})	n (-)	**Correlation coefficient (R^2)**	**Variance (σ)**
Monod $\mu = \mu_{max}.S/(K_S+S)$ (2)	0.1687 (±0.0158)	0.0083 (±0.0060)	-	0.9093874	0.0004289
Moser $\mu = \mu_{max}.(S^n/K_S+S^n)$ (3)	0.1620 (±0.0156)	1.21×10^{-6} ($\pm2.858\times10^{-6}$)	2.9098 (±0.4895)	0.9574338	0.0002417
Tessier $\mu = \mu_{max}.(1-e^{-S/K}S)$ (4)	0.1632 (±0.0152)	0.0149 (±0.0094)	-	0.9568672	0.0002041
Models for growth with substrate inhibition	μ_{max} (h^{-1})	K_S (gL^{-1})	$K_{I,S}$ (gL^{-1})	**Correlation coefficient (R^2)**	**Variance (σ)**
Aiba $\mu = \mu_{max}[S/(K_S+S)]\exp(^{-S/K}{}_{I,S})$ (5)	0.2218 (±0.0232)	0.0169 (±0.0069)	1.6890 (±0.6017)	0.9867078	7.549×10^{-5}
Andrews $\mu = \mu_{max}/[(1+K_S/S)(1+S/K_{I,S})]$ (6)	0.2336 ($\pm3.538\times10^{-6}$)	0.0189 ($\pm1.463\times10^{-6}$)	1.1750 ($\pm7.832\times10^{-5}$)	0.9901356	5.602×10^{-5}
Tessier-type $\mu = \mu_{max}[\exp(-S/K_{I,S})-\exp(S/K_S)]$ (7)	0.1894 ($\pm6.109\times10^{-5}$)	2.6812 (±0.0057)	0.0205 ($\pm3.588\times10^{-5}$)	0.9955221	2.543×10^{-5}

*Equations (2) to (7) were obtained from Moser (1985).
Symbols: μ_{max}: maximum specific growth rate;
K_S: substrate constant;
$K_{I,S}$: substrate-inhibition constant;
n: fitting constant.

The R^2 (correlation coefficient) is frequently used to judge whether the model represents correctly the data, implying that, if the correlation coefficient is close to one, then the regression model is correct. However, many examples exist where the correlation coefficient is close enough to one but the model is still not appropriate. Hence, the residual plot should be used together with R^2 for judging the appropriateness of the model, while R^2 can be used for comparing various models representing the same dependent variable. The residual plot shows the difference between the calculated and measured values of the dependent variable as a function of the measured values. This is shown in Figure 3 for the fitting of a Tessier-type model to the experimental data. The residuals are randomly distributed around the line of error=0 with zero mean, indicating that the Tessier-type model represents the data correctly (Figure 3). The residual plots for other tested growth models also showed similar random nature of their residuals distribution (data not shown). If the residuals show a clear trend, this indicates that an inappropriate model is being used. This information combined with the high R^2 showed that Tessier-type model describes best the growth of *P. putida* PGA1 with ammonium as the limiting substrate. Another useful indicator for the comparison of various models representing the same dependent variable is the variance (σ). A model with smaller variance represents the data more accurately than a model with larger variance. It is also found that all the models representing substrate inhibition kinetics have consistently much lower σ as compared to those representing the growth kinetics as a function of substrate concentration only (Table 3). This indicated that the substrate-inhibition kinetic model should be able to represent growth data more accurately. Different levels of high ammonium concentration in the aqueous medium were found to exert a substrate-inhibition effect towards growth of *W. eutropha* strains (Suzuki et al., 1986; Mulchandani et al., 1989; Belfares et al., 1995; Beaulieu et al., 1995) and *P. putida* PGA1 (Annuar et al., 2006, 2007).

On the other hand, for the regression model to be stable and statistically valid, the confidence intervals must be much smaller (or at least smaller) than the respective parameter values (in absolute values). The confidence intervals at 95% for all tested growth models are less than the estimated parameter values returned by the fitting algorithm (Table 3). The Tessier-type model has significantly much smaller confidence intervals for all its kinetic parameters.

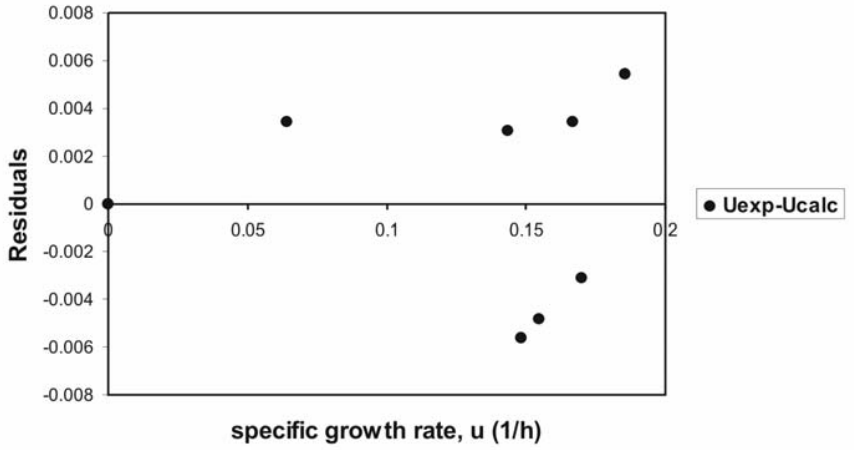

Figure 3: Residual plots for the fitting of the Tessier-type model to the growth data from shake-flask cultivation. (Residual points were obtained by subtracting calculated values, (Ucalc) from experimental data (Uexp)).

Rate of Ammonium Consumption (r_S)

An examination of the experimental data (Figure 1) indicated that ammonium concentration was almost completely consumed in the growth phase due to the bacterial metabolism and this corresponded to an increase in the residual biomass growth rate. Furthermore, specific ammonium uptake rates were found to be positively correlated with specific growth rates of the micro-organism, with the optimal rates occurring at 0.1 g L^{-1} ammonium ion in aqueous medium (Annuar et al., 2006). The rate of ammonium consumption is related to the rate of growth as shown in following equation

$$dS/dt = r_S = -r_R/Y_{R/S} \quad (8)$$

which is adequate to describe the relationship between the rate of synthesis of R (r_R) and the rate of consumption of the limiting substrate S (r_S). The value of biomass yield coefficient on ammonium ($Y_{R/S}$) is taken from the work of Heinzle and Lafferty (1980) at 1.48±0.14 (Table 4). In their work, *W. eutropha* H16 was grown on fully synthetic medium containing ammonium (in the form of $(NH_4)_2SO_4$) as nitrogen source and gaseous CO_2 as the sole carbon and energy source.

Rate of PHA_{MCL} Production (r_P)

The rate of synthesis of PHA_{MCL} (r_P) is assumed to be the sum of a growth associated term ($r_{P,1}$) and a biomass associated term ($r_{P,2}$), following the suggestion of Heinzle and Lafferty (1980). They formulated a rate expression for P as:

$$dP/dt = r_P = r_{P,1} + r_{P,2} \quad (9)$$

As shown in Figure 1, the r_P is correlated with r_R during the growth phase. The first term in the expression of the rate of synthesis of P, i.e., $r_{P,1,}$ is a function of PHA_{MCL} yield coefficient on residual biomass ($Y_{P/R}$) and r_R (Eq. 10).

$$r_{P,1} = Y_{P/R} \, . \, r_R \quad (10)$$

The value of $Y_{P/R}$ is determined to be within a range of 0.105-0.16 (Heinzle and Lafferty, 1980). For the purpose of the modeling exercise in this work, average $Y_{P/R}$ value were used, i.e., 0.13.

The non-growth associated term of the synthesis of P ($r_{P,2}$) is assumed to be a function of the limiting substrate S, of the residual biomass R, and of the product P (Heinzle and Lafferty, 1980). At high contents of PHA_{MCL} in the cells, the rate of synthesis of P is decreased, which can be formally described as an inhibition. Following this, the second term in the expression of the rate of synthesis of P , $r_{P,2}$, is given by equation (11).

$$r_{P,2} = -k_1.P + k_2.R \quad (11)$$

The values of k_1 and k_2 fall within the range of 0.045-0.048 h^{-1} and 0.18-0.176 h^{-1}, respectively, as determined by Heinzle and Lafferty (1980). For the purpose of simulation in the present work, the average values of both (k_1=0.047 and k_2=0.18) were used in the rate expression (Eq. 11).

The crucial aspect of the model is the initialization of the non-growth associated production of P, and the inhibition of it by the limiting substrate ammonium. Heinzle and Lafferty (1980) proposed that this inhibition can be incorporated into the model by multiplying Eq. (11) by a function that is used to describe allosteric substrate inhibition, which yields the following equation:

$$r_{P,2} = [K_{I,P} / (K_{I,P}+S)] (-k_1.P + k_2.R) \quad (12)$$

$K_{I,P}$ is the inhibition constant representing the substrate concentration at half maximum rate of production of P. $K_{I,P}$ was determined to be between 0.036 and 0.047 g L^{-1} $(NH_4)_2SO_4$ (Table 4). Again here, the average value of 0.042 g L^{-1} for $K_{I,P}$ was employed in the simulation exercise.

Table 4 summarizes all the kinetic parameters required to solve the final set of equations [Eqs (1), (8), (9), (10), (11), and (12)].

Table 4: Evaluated parameters for the model equations.

Parameter	Value	Dimension	Equation no.
μ_{max}	0.19(±6.11x10^{-5})[a]	h^{-1}	(7)
K_S	2.68(±0.01)[a]	g L^{-1}	(7)
$K_{I,S}$	0.02(±3.59x10^{-5})[a]	g L^{-1}	(7)
$Y_{R/S}$	1.48(±0.14)[b]	-	(8)
$Y_{P/R}$	0.105-0.16[b]	-	(10)
k_1	0.045-0.048[b]	h^{-1}	(11)
k_2	0.18-0.176[b]	h^{-1}	(11)
$K_{I,P}$	0.036-0.047[b]	g L^{-1}	(12)

[a] Values of the kinetic parameters obtained in this study.
[b] Values of the kinetic parameters adopted from the study of Heinzle and Lafferty (1980).

Simulation

Figure 4 shows the results of a numerical integration of the model with one set of parameters as given in Table 4. The time course of calculated concentrations is compared with experimental values. The comparisons demonstrate a good agreement between the data from simulations of the model with the experimental data. Thus, alongside the Tessier-type model incorporating substrate-inhibition kinetics to describe the batch growth of *P. putida* PGA1, the models for ammonium utilization and accumulation of PHA_{SCL} (PHB), initially proposed by Heinzle and Lafferty (1980) for *W. eutropha* under chemolithoautotrophic growth conditions, are equally applicable to simulate the ammonium consumption and PHA_{MCL} accumulation in *P. putida* under heterotrophic conditions. Ammonium utilization kinetics by *P. putida* PGA1 in a chemically defined medium can be modeled quite simply using a rate equation related directly by a yield coefficient to the growth kinetic model. The PHA_{MCL} formation rate can be described reasonably well by using a model that takes into account the growth-associated and non-growth associated product formation simultaneously. Using this general form of Luedeking-Piret kinetics, Heinzle and Lafferty (1980) modified the non-growth product formation rate equation to include a function of PHA_{MCL} biosynthesis inhibition by a certain level of ammonium in the medium; when the ammonium concentration exceeds the 0.036-0.047 g L^{-1} range, the rate of PHA_{MCL} biosynthesis in the cells is reduced. This is not an unfounded modification, as it is well known that PHA accumulation is increased significantly under threshold ammonium concentration in the medium. This ammonium level differs between different types of micro-organisms. The accumulation and storing of the intracellular polymer is the bacterial culture's response to the deficiency conditions (e.g. ammonium limitation) that do not permit continuation of its exponential growth. In this case, the intracellular stored carbon in the form of polymer helps the bacterial population to survive the starvation period until favorable growth conditions are restored in its environment.

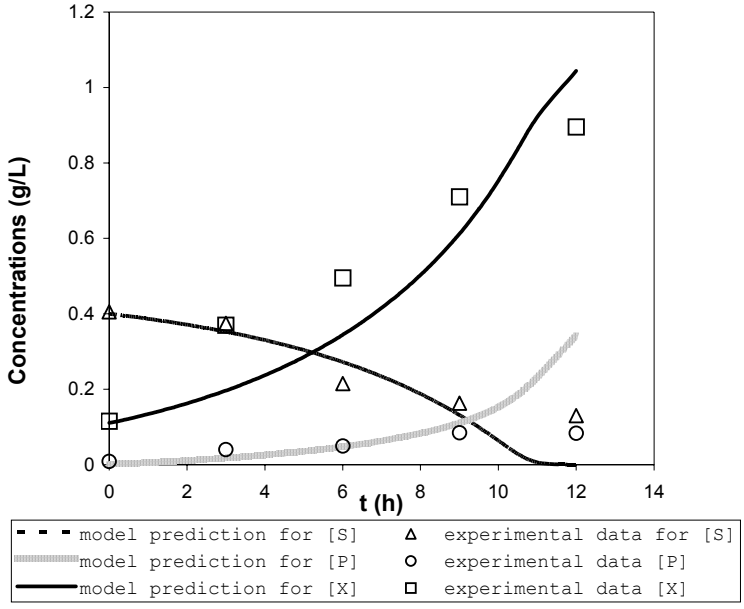

Figure 4: Comparison of model predictions (lines) with the experimental data (symbols). (X: total biomass concentration; S: ammonium concentration; P: PHA_{MCL} concentration)

Ammonium consumption and PHA_{SCL} (PHB) production models proposed by Mulchandani et al. (1989) were also compared to the current experimental data. Using *W. eutropha* ATCC 17697 culture grown in fructose as carbon substrate, their ammonium consumption rate equation took a form similar to the model of Heinzle and Lafferty (1980) with the biomass yield coefficient with respect to ammonium ($Y_{R/S}$) estimated at 1.46 g g^{-1}, which is essentially the same as the value in the current model. Hence, the ammonium utilization model of Muchandani et al. (1989) also describes well the experimental data (Figure 5). Their PHB production model, on the other hand, over-estimated the

biosynthesis of PHA_{MCL} by *P. putida* PGA1 cells. Although their rate equation also adopts the Luedeking-Piret kinetics, it does not take into account the possibility of PHA_{MCL} biosynthesis inhibition above a certain level of residual ammonium in the aqueous medium (Figure 5). Their product synthesis equation is solely a function of the culture's growth rate and biomass concentration, each related to the product balance by a constant (Mulchandani et al., 1989).

Alternative ammonium consumption and PHA_{SCL} (PHB) production models for *W. eutropha* DSM545 grown in mineral salts medium containing glucose as carbon source and $(NH_4)_2SO_4$ as nitrogen source were reported by Belfares et al. (1995). Their ammonium utilization rate equation also took a similar form to the two models tested earlier. However, due to significantly higher difference in the biomass yield coefficient with respect to ammonium (equivalent to $Y_{R/S}$), which was estimated at 6.25 g g^{-1} , in comparison to other $Y_{R/S}$ values used in this study, the rate of ammonium consumption was grossly over-estimated compared to the experimental data (Figure 5). On the other hand, the PHB production rate proposed by these authors was solely a function of cell growth with a biomass product yield coefficient (equivalent to $Y_{P/R}$) estimated at 0.13. As a result, it predicted a very much lower PHA_{MCL} production rate in *P. putida* PGA1 batch culture (Figure 5).

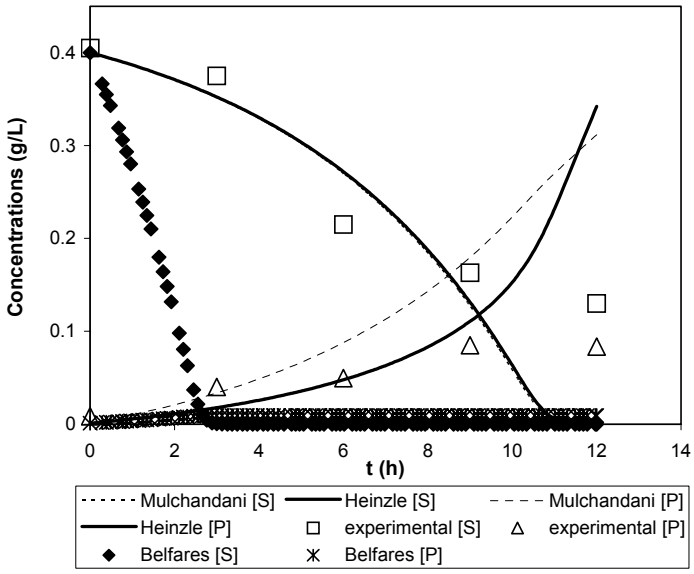

Figure 5: Comparison of simulation results for ammonium consumption and PHA production models as proposed by Mulchandani et al. (1989) and Belfares et al. (1995) to the experimental data.

CONCLUSIONS

The batch growth of *P. putida* PGA1 culture in a defined aqueous medium with a fatty acid mixture as carbon source can be described by using a Tessier-type model which incorporates the limiting substrate (ammonium) inhibition kinetics. Utilization of the ammonium is related vis-à-vis to growth kinetics. The PHA_{MCL} accumulation by *P. putida* PGA1 cells can be reasonably modeled using Luedeking-Piret kinetics that take into account the product synthesis inhibition (or reduction) by ammonium concentrations above a threshold level.

NOMENCLATURE

Abbreviations

PHA	poly-(3-hydroxyalkanoates)	(-)
PHA_{MCL}	medium-chain-length poly-(3-hydroxyalkanoates)	(-)
PHA_{SCL}	short-chain-length poly-(3-hydroxyalkanoates)	(-)
PHB	poly-(3-hydroxybutyrate)	(-)
PHBV	poly-(3-hydroxybutyrate-*co*-3-hydroxyvalerate)	(-)
PKO	palm kernel oil	(-)

SPKO	saponified palm kernel oil	(-)
LM	Levenberg-Marquandt	(-)
ODE	ordinary differential equations	(-)
RKF	Runga-Kutta-Fehlberg	

Symbols

P	product concentration	g L^{-1}
R	residual biomass concentration	g L^{-1}
S	limiting substrate concentration	g L^{-1}
X	total biomass concentration	g L^{-1}
k_1	constant 1	h^{-1}
k_2	constant 2	h^{-1}
$K_{I,S}$	substrate-inhibition constant	g L^{-1}
$K_{I,P}$	product-inhibition constant	g L^{-1}
K_S	substrate constant	g L^{-1}
n	fitting constant	(-)
r_R	rate of R synthesis	g L^{-1} h^{-1}
r_S	rate of S consumption	g L^{-1} h^{-1}
r_P	rate of P formation	g L^{-1} h^{-1}
$r_{P,1}$	first term of r_P equation	g L^{-1} h^{-1}
$r_{P,2}$	second term of r_P equation	g L^{-1} h^{-1}
R^2	coefficient of correlation	(-)
$Y_{R/S}$	growth yield coefficient	(-)
$Y_{P/R}$	product yield coefficient	(-)

Greek Symbols

μ	specific growth rate	h^{-1}
μ_{max}	maximum specific growth rate	h^{-1}
σ	variance	(-)

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