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Energetic Analysis Of Pseudomonas Fluorescence Growth On Phenol In Aerobic Chemostat Culture

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ABSTRACT

Bioenergetic analysis of the growth of *Pseudomonas fluorescence* on phenol chemostat culture was carried out. The data were checked for consistency using carbon and available electron balances. Similar estimates were obtained using Pirt's model based on Monod approach and a modified model based on substrate consumption rate being rate limiting. Coupled with the covariate adjustment estimation technique, the best estimates were the maximum likelihood estimates (MLE) based on the complete data. For the aerobic growth of *P.fluorescence* growth on phenol, η_{max} = 0.262 and m_e = -0.017hr^{-1}. From the 95% confidence intervals, a maximum of about 25–27% of the energy contained in phenol is incorporated into the *P.fluorescence* biomass. While, the balance (73–75%) is evolved as heat with little or no energy needed for the maintenance of the respective organisms.

KEYWORDS

Pseudomonas fluorescence;
Energetic analysis;
Chemostat culture;
Biomass energetic yield;
Maintenance coefficient;
Pirt's model.

INTRODUCTION

Proper design and operation of biological systems has the potential of being the most cost effective way to dispose of toxic and hazardous chemicals since almost complete oxidation may be accomplished. The toxicity of phenol and the need to find ways of removing it from the environment has made the molecule a prime candidate for study. Many microbes are capable of utilizing phenol as a source of carbon and energy provided it is not present in too high a concentration[1-5]. Several studies have been carried out on the kinetics of phenol degradation by various microorganisms and on its inhibitory effects [4,6-8].

Most data in the literature on phenol biodegra-

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dation do not lend themselves to energetic analysis except the work of Solomon et al.[1] using the concept of carbon and available electron balances which have been widely used for data analysis[9-15]. The reason for this is that the data are incomplete as many variables required are either not measured or reported. Therefore, the main objective of this study was to carry out the analysis of complete data obtained on the aerobic degradation of phenol by indigenous pseudomonas fluorescence in chemo stat culture. The data collected by Agarry[16] would be used for the analysis and these include parameters that were measured at various dilution rates: biomass concentration, substrate consumption rates, carbon dioxide production and oxygen uptake rates. Through the application of a multivariate statistical procedure known as covariate adjustment technique (CAT)[1,12] the analysis should provide accurate estimates of the significant design and model growth parameters, true growth yields and maintenance coefficients. The parameters were estimated using two similar growth models that belong to two different classes. One is Pirt's model[17], which assumes that substrate uptake is a consequence of growth. The second model is a modified form of Pirt's model, which assumes that growth is a consequence of substrate uptake.

METHODS OF DATA ANALYSIS

Consistency tests

When phenol is oxidatively converted to biomass with concomitant carbon dioxide and water production as the only other end products, the growth process can be represented stoichiometrically as:

$$CH_mO_l + aNH_3 + bO_2 = y_cCH_pO_nN_q + cH_2O + dCO_2 \quad (1)$$

Where CH_mO_l and $CH_pO_nN_q$ represent the elemental compositions of the organic substrate(phenol in this case) and biomass respectively. The carbon and available electron balances on equation (1) yield[1]:

$$y_c + d = 1.0 \quad (2)$$

and

$$\eta + \varepsilon = 1.0 \quad (3)$$

respectively.

For chemostat operation, where $D = \mu$,

$$y_c = \sigma_b X / \{\sigma_s (S_o - S_1)\} \quad (4)$$

$$d = 12Q_{CO_2} / \{\sigma_s M_{CO_2} (S_O - S_1)\mu\} \quad (5)$$

$$\eta = \sigma_b \gamma_b X / \{\sigma_s \gamma_s (S_O - S_1)\} \quad (6)$$

$$\varepsilon = 48Q_{O_2} / \{\sigma_{b\,b} M_{O_2} \{\sigma_{s\,s} (S_O - S_1)\mu\} \quad (7)$$

Estimation of true yield and maintenance coefficient.

Pirt's model [17] for growth processes has been written in the following forms:

$$r_S = \mu / Y^{max}_{X/S} + m_S \quad (8a)$$

$$r_{O_2} = \mu / Y^{max}_{X/O_2} + m_{O_2} \quad (9a)$$

$$r_{CO_2} = \mu / Y^{max}_{X/CO_2 +} m_{CO_2} \quad (10a)$$

based on substrate consumption, oxygen uptake and carbon dioxide production rates respectively. These equations have been reparametrized in energetic terms and shown to be correspondingly equivalent [1] to:

$$\mu / \eta = \mu / \eta_{max} + m_e \quad (8b)$$

$$\mu (\eta + \varepsilon) / \eta = \mu / \eta_{max} + m_e \quad (9b)$$

$$\mu (y_c + d) / \eta = \mu / \eta_{max} + m_e \quad (10b)$$

Using equations (8b) to (10b), combined estimates of the true biomass energetic yield, η_{max}, and maintenance coefficient, m_e, can be obtained by application of the covariate adjustment technique [1,14]. Nonetheless, the above equations are based on Monod kinetics, which is,

$$\mu = \mu_{max} S / (K_S + S) \quad (11)$$

that require a well defined substrate consumption rate. However, in many cases, growth of microbes is a consequence of substrate consumption and not vice versa [1,18]. Posten [1] showed that in this approach as S tends to 0, $\mu = 0$ and yet a finite quantity of substrate consumption, m_e, is required that is due to maintenance. Hence, there is a substrate consumption even for $S = 0$ which is physiologically impossible. Also the substrate consumption is the limiting step and the microorganism's growth actually follows substrate availability; therefore, instead of equation (11), a model of the form:

$$r_S = r_S^{max} S / (K_S + S) \quad (12)$$

Makes more biological as well as mathematical sense. Therefore, in place of equations (8a) to (10a), the following would become valid:

$$\mu = Y^{max}_{X/S} r_S - m_s \quad (13a)$$

$$\mu = Y^{max}_{X/O_2} r_{O_2} - \mu_{mO_2} \quad (14a)$$

$$\mu = Y^{max}_{X/CO_2} r_{CO_2} - \mu_{mCO_2} \quad (15a)$$

The equations (13a) to (15a) have been

TABLE 1 : Calculated kinetic parameters and transfer rates for the continuous degradation of phenol by *P.fluorescence*

D (hr^{-1})	Qo_2 (mg/l/hr)	Qco_2 (mg/l/hr)	r_s ($gg^{-1}hr^{-1}$)	ro_2 ($ggm^{-1}hr^{-1}$)	rco_2 ($ggm^{-1}hr^{-1}$)
0.05	8.357	9.723	0.096	0.161	0.187
0.06	9.643	11.491	0.120	0.193	0.230
0.07	10.929	13.259	0.141	0.223	0.271
0.08	12.857	15.027	0.165	0.268	0.313
0.10	15.429	18.563	0.205.	0.321	0.387
0.11	17.357	20.330	0.229	0.369	0.433

reparametrized in energetic terms and are shown to be correspondingly equivalent[1] to:

$$\mu = \mu\ \varsigma_{max} / \eta + m' \quad (13b)$$

$$\mu = \mu(\eta + \varepsilon)\ \eta_{max} / \eta + m' \quad (14b)$$

$$\mu = \mu\ (y_c + d)\ \eta_{max} / \eta + m' \quad (15b)$$

where $m' = - m_e\ \eta_{max}$ .The values of m' has the same dimension as μ mathematically and hence cannot be referred to as the maintenance. They may be described as specific death rates and physiologically as energy not available for growth[1] . Equations (13b) to (15b) were also used to estimate η_{max} and m_e.

RESULTS AND DISCUSSION

The calculated values of phenol consumption rates (Q_s), oxygen uptake rate (Qo_2), and carbon dioxide production rate (Qco_2)(TABLE 1) were used for the estimation of the biomass energetic yield (η) and carbon yield (y_c) for P.fluorescence using the carbon and available electron balances as given in equations4–7.For the estimation, the average values of $\sigma_b = 0.462$ and $Y_b = 4.291$ which have been calculated from the measured composition of Pseudomonas species obtained by Erickson et al.[19] were used. The instantaneous available electron and carbon balances results obtained for *P.fluorescence* are presented in TABLE 2. From the TABLE, it could be seen that the biomass energetic yield (η) and carbon yield (g_c) for *P.fluorescence* are low (i.e. less than 1) which thus agree with the available electron and carbon balance equation. It could also be seen from the respective TABLES that both the biomass energetic yield (η) and carbon yield (γ_c) decreased as the dilution rate increased for P.fluorescence.

TABLE 2: Examination of data consistency using instantaneous available electron and carbon balances for the growth of *Pseudomonas fluorescence* in phenol-limited chemostat culture.

$D = \mu$	y_c	d	$y_c + d$	H	ε	$\eta + \varepsilon$
0.05	0.314	0.692	1.006	0.288	0.701	0.989
0.06	0.302	0.682	0.984	0.277	0.674	0.951
0.07	0.299	0.681	0.980	0.274	0.662	0.936
0.08	0.293	0.677	0.970	0.269	0.683	0.952
0.10	0.295	0.673	0.968	0.271	0.659	0.930
0.11	0.290	0.672	0.962	0.266	0.676	0.942

Consistency tests (checks) were made for *P.fluorescence* using equations2-3. It has been established [20] that in consistency analysis allowance has to be made for deviation from the ideal. The parameters by which consistency is defined should satisfy $0.94 \leq (y_c + d) \leq 1.06$ and $0.93 \leq (\eta + \varepsilon) \leq 1.07$. The results of the data consistency tests are as shown in TABLE3. Thus, it could be seen from the TABLE that the consistency equations are generally satisfied. Also, it could be seen from the TABLE that the $(y_c + d)$ and $(\eta + \varepsilon)$ values generally decreased as the dilution rate increased. Generally, therefore, the consistency tests suggest that in phenol-limited chemostat culture, *P.fluorescence* was able to oxidatively metabolized phenol to carbon dioxide and water with concomitant biomass production.

However, Pirt's model for growth as given in equations (8a)-(10a) were used to estimate the true yields and maintenance coefficients in terms of substrate, oxygen and carbon dioxide. The calculated specific rates of phenol consumption (r_s), oxygen uptake (ro_2), and carbon dioxide production (rco_2) obtained for P.fluorescence were plotted as a function of dilution rate (D) . These resulted in straight lines (not shown). The slopes and intercepts of these straight lines give the true yield and maintenance coefficients respectively. The estimated values are given in TABLE 3.

The Pirt's model was reparametrized which produced multiresponse models with common parameters as given in equations (8b) – (10b), and application of covariate adjustment technique [1] to these equations resulted in a unit variate linear model with covariates This allows a combined point and interval estimates of biomass energetic yield and maintenance coefficient to be obtained using standard mul-

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TABLE 3 : Estimates of true biomass growth yields and maintenance coefficient for the growth of indigenous Pseudomonas species in phenol-limited chemostat culture using Pirt's model (Equations 8a –10a)

Organism	$Y^{max}_{x/s}$ gg^{-1}	Y^{max} x/$o_2$gg^{-1}	Y^{max}x/co_2gg^{-1}	$M_s$gg^{-1}hr^{-1}	Mo_2 gg^{-1}hr^{-1}	Mco_2 gg^{-1}hr^{-1}
P.aeruginosa	0.540	0.341	0.284	-0.0133	-0.0353	-0.0481
P.fluorescence	0.463	0.294	0.247	-0.0101	-0.0107	-0.0131

tiple regression programs. Therefore, using equations (8b)–(10b), various estimates of the true biomass energetic yield and maintenance coefficients based on the data in TABLE 1 was obtained for P.fluorescence as presented in TABLE 4. The first three estimates in the TABLE are the individual least square estimates using substrate and biomass data and equation 8b and oxygen and biomass data and equation 9b and carbon dioxide and biomass data and equation10b respectively. These estimates are quite comparable but differ because of measurement errors..

When all the measured data were used (i.e. Q_s, Qo_2, Qco_2, μ were used) the best estimate was the maximum likelihood estimate(MLE) which corresponded to when one covariate (Z_1) was included. This was based on the lowest value of J which in this case J = 1.841 x10^{-6} .The respective combined point estimates for η_{max} and m_e were 0.262 and 0.0144hr^{-1} with the corresponding 95% confidence intervals (0.253, 0.271) and (-0.0247, -0.0042) hr^{-1}. When the carbon dioxide data were excluded (i.e. Q_s , Qo_2 , μ were used) then the respective best point and interval estimates for η_{max} were 0.262 and (0.250, 0.274) and the m_e are –0.0166hr^{-1} and (-0.0311, -0.0020)hr^{-1}. With the oxygen data excluded (i.e. Q_s , Qco_2 , μ, were used) , η_{max} = 0.264 with interval (0.254, 0.273) and m_e = -0.0129hr^{-1}.with interval (-0.0239, -0.0020)hr^{-1}. When substrate measurements were excluded (i.e. Qo_2 ,Qco_2, μ were used), η_{max} = 0.275 with interval (0.264, 0.288) and m_e = -0.0070 with interval (-0.0201, 0.0062)hr^{-1}.

For the organisms studied, even though the respective values of these combined point estimates were different from one another, all the 95% confidence intervals were overlapping and included all the point estimates. Generally, based on the least measure of goodness of fit value, the best estimate was obtained when J = 1.841 x10^{-6} which was for the case when all the measurements were used and corresponded to the maximum likelihood estimate (MLE) value of η_{max} = 0.262 with 95% confidence intervals (0.253, 0.271) and m_e = 0.0144 hr^{-1} with interval (-0.0247, -0.0042)hr^{-1} .

In earlier applications of this procedure [11,12], the best combined estimates were always assumed to be obtained when all the measured data were used. The

TABLE 4 : Estimates of true biomass energetic yields and maintenance coefficient for the growth of indigenous Pseudomonas fluorescence in phenol-limited chemostat culture using Pirt's model (Equations 8b –10)

Data	Covariates included	η_{max} Point	η_{max} Interval	m_e Point	m_e Interval	J
Q_s, μ	-	0.254	(0.244, 0.264)	-0.021	(-0.033, -0.008)	-
Qo_2, μ	-	0.279	(0.260, 0.302)	-0.008	(-0.030, -0.014)	-
Qco_2, μ	-	0.272	(0.265, 0.280)	-0.007	(-0.016, -0.001)	-
Q_s, Qo_2,Qco_2, μ	-		(0..256, 0.281)	-0.012	(-0.026, 0.002)	3.371 x 10^{-6}
	Z_1		(0..253, 0.271)	0.014	(-0.025, 0.004)	1.841 x10^{-6}
	Z_2		(0.244, 0.270)	-0.021	(-0.037, -0.005)	4.514 x10^{-6}
	Z_1,Z_2	0.292	(0.194, 0.590)	-0.041	(-0.182, 0.100)	3.495 x10^{-4}
		0.266	(0.253, 0.281)	-0.014	(-0.031, 0.002)	
Q_s , Qo_2, μ	-	0.262	(0.250, 0.274)	-0.017	(-0.031, -0.002)	4.688 x10^{-6}
	Z_1	0.264	(0.254, 0.273)	-0.013	(-0.024, -0.002)	3.741 x10^{-6}
Q_s, Qco_2, μ	-	0.253	(0.243, 0.264)	-0.021	(-0.034, -0.007)	2.100 x 10^{-6}
	Z_1	0.276	(0.263, 0.291)			3.101 x 10^{-6}
Qo_2, Qco_2 , μ	-	0.275	(0.264, 0.288)	-0.007	(-0.022, 0.008)	3.853 x10^{-6}
	Z_1			-0.007	(-0.020, 0.006)	3.040 x10^{-6}

TABLE 5 : Estimates of true biomass energetic yields and maintenance coefficient for the growth of indigenous *Pseudomonas fluorescence* in phenol-limited chemostat culture using modified Pirt's model (Equations 13b–15b)

Data	η_{max}		m ' (hr^{-1})		m_e (hr^{-1})	
	Point	Interval	Point	Interval	Point	Interval
Q_s, μ	0.254	(0.244, 0.264)	0.005	(-0.002, 0.008)	-0.021	(-0.033, -0.009)
Qo_2, μ	0.278	(0.257, 0.299)	0.003	(-0.003, 0.009)	-0.009	(-0.031, 0.012)
Qco_2, μ	0.272	(0.264, 0.280)	0.002	(-0.000, 0.004)	-0.007	(-0.016, 0.001)
Average	0.268	(0.255, 0.281)	0.003	(-0.001, 0.007)	-0.012	(-0.026, 0.004)

results obtained for *P.fluorescence* have supported this assumption and were also in agreement with the report of Solomon et al.[1] who obtained the best combined estimates of $\eta_{max} = 0.432$ and $m_e = 0.0684 hr^{-1}$ for the growth of *Pseudomonas cepacia* G4 on phenol when all the measured data (Q_s , Qo_2 , Qco_2, D) were used. Layokun et al.[20] also obtained the best combined estimate of $\eta_{max} = 0.673$ and $m_e = 0.00$ for the growth of *Pseudomonas aeruginosa* on n- hexadecane when all the measured data (Q_s , Qco_2 , Qo_2 , μ) were used. The estimates of η_{max} and m_e from equations (13b)-(15b) using the data in TABLE 1 are presented in TABLE 5. For these cases only, the individual estimates have been made because the covariate adjustment technique was not suitable. Instead of a multiresponse situation with constant independent variable (as in equations (8b-10b) a constant response for varying independent variables was obtained. Nonetheless, there was good agreement between the corresponding individual estimates for the two cases. The most reliable estimate in TABLE 5 was the average which gave $\eta_{max} = 0.268$ and $m_e = -0.0216 hr^{-1}$ with the respective 95% confidence interval of (0.255, 0.281) and (-0.0264, 0.0035)hr^{-1}. The estimates of m_e in TABLE 4 are statistically significantly lower than zero and therefore negligible. Hill and Robinson [4] reported that the maintenance coefficient for phenol degradation is negligible.

TABLE 6 is a summary of the yields and maintenance coefficients estimates. The true yields and maintenance coefficients in terms of oxygen and carbon dioxide were obtained using the modified model. The combined estimates, which seems to be an improvements on the estimates made from individual measurements are the values most likely to be used when true biomass energetic yield and maintenance coefficients are applied to the design of fermentors.

CONCLUSIONS

The advantage of combined estimates using covariate adjustment technique has been demonstrated by Solomon et. al [1] This analysis showed that with a combined use of material and energy balances and statistical procedure, discrimination may be made between various variables to identify those with more errors. The results demonstrated that the Pirt's model approach (based on Monod) which require well-defined substrate consumption as well as the modified approach which assumed that subas heat with little or no use for maintenance of the cells.

NOMENCLATURE AND ABBREVIATION

a Moles of ammonia per quantity of organic substrate 1g atom carbon (g-mol(g-mol carbon) $^{-1}$

b Moles of oxygen per quantity of organic substrate containing 1g atom carbon (g-mol(g-mol carbon) $^{-1}$

c Moles of water per quantity of organic substrate containing 1g-mol carbon (g-mol (g-mol carbon)$^{-1}$

d Moles of carbon dioxide per quantity of organic substrate

TABLE 6 : Summary of true biomass growth yields and maintenance coefficient for the growth of indigenous Pseudomonas fluorescence in phenol-limited chemostat culture.

Equations Used	η_{max} (-)	$Y^{max}x/s$ gg^{-1}	$Y^{max}x/o_2$ ggm^{-1}	$Y^{max}x/co_2$ ggm^{-1}	m_e hr^{-1}	m_s $gg^{-1}hr^{-1}$	mo_2 $gg^{-1}hr^{-1}$	mco_2 $gg^{-1}hr^{-1}$
Pirt's Model	0.262	0.463	0.294	0.247	-0.017	-0.010	-0.011	-0.013
Modified Model	0.268	0.462	0.292	0.247	-0.013	-0.010	-0.012	-0.013

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containing 1g atom carbon (g- mol(g-mol carbon)$^{-1}$, number of covariates included in model.

D Dilution rate (hr^{-1}).

J Measure of goodness of fit (dimensionless).

K_s Monod constant (mg/l).

mc_{o2} Maintenance requirement in terms of CO_2 (mol CO_2 g biomass^{-1} hr^{-1}).

m_e Maintenance requirement in terms of available electron (hr^{-1}).

m_{o2} Maintenance requirement in terms of O_2 (mol O_2 g biomass^{-1} hr^{-1}).

m_s Maintenance requirement in terms of organic substrate(g substrate g biomass^{-1} hr^{-1}).

m' A form of maintenance (hr^{-1}).

M_{co2} Molecular weight of CO_2(gg-mol^{-1}).

M_{o2} Molecular weight of O_2 (gg-mol^{-1}).

n Number of observations

Q_{CO2} Rate of CO_2 production ($mgL^{-1}hr^{-1}$).

Q_{O2} Rate of O_2 uptake ($mgL^{-1}hr^{-1}$).

rc_{o2} Specific rate of CO_2 production (g-mol g bio mass^{-1} hr^{-1}).

r_{o2} Specific rate of O_2 uptake (g-mol g biomass^{-1} hr^{-1}).

r_s Specific rate of substrate consumption (g substrate g biomass^{-1} hr^{-1}).

r_s^{max} Maximum specific substrate consumption rate (g substrate g biomass$^{-1}hr^{-1}$)

S Substrate concentration, subscripts 0 and 1 stand for inlet and outlet respectively (mg/L).

X Biomass concentration (mg/L).

y_c Fraction of organic substrate carbon incorporated into biomass (dimensionless).

Y^{max} True growth yield, X/S, X/O_2 and X/CO_2 represent yield based on substrate (biomass g substrate^{-1}), oxygen (g biomass mol O_2^{-1}), and car bon dioxide (g biomass mol CO_2^{-1}) respectively.

γ Reductance degree (equivalents of available electrons per gram atom carbon), subscripts b and s stand for biomass and substrate.

ε Fraction of substrate energy which is evolved as heat (dimensionless)

η Fraction of substrate energy which is in bio mass (biomass energetic yield) (dimensionless).

η_{max} True biomass energetic yield (dimensionless).

μ Specific growth rate (hr^{-1}).

μ_{max} Maximum specific growth rate (hr^{-1}).

σ Mass fraction carbon.

σ^2 Mean square error.

Subscripts

l Atomic ratio of oxygen to carbon in organic substrate (dimensionless)

m Atomic ratio of hydrogen to carbon in organic substrate (dimensionless)

n Atomic ratio of oxygen to carbon in biomass (dimensionless)

p Atomic ratio of hydrogen to carbon in biomass (dimensionless)

q Atomic ratio of nitrogen to carbon in biomass (dimensionless)

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