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Full Length Research Paper

Energetics of binary mixed culture of *Pseudomonas aeruginosa* and *Pseudomonas fluorescence* growth on phenol in aerobic chemostat culture

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Bioenergetic analysis of the growth of the binary mixed culture *(Pseudomonas aeruginosa* and *Pseudomonas fluorescence)* on phenol chemostat culture was carried out. The data were checked for consistency using carbon and available electron balances. When more than the minimum number of variables are measured, and measurement errors are taken into account, the results of parameter estimation depend on which of the measured variables are chosen for this purpose. Similar parameter estimates were obtained using Pirt's model based on the Monod equation approach and a modified model based on substrate consumption being rate limiting. Coupled with the covariate adjustment estimation technique, the best estimates were the maximum likelihood estimates (MLE) based on when all the measured data were used. For the aerobic growth of the mixed culture on phenol, $\eta_{\max} = 0.396$ and $m_e = -0.020$ h^{-1}. From the 95% confidence intervals, a maximum of about 38 – 41.3% of the energy contained in phenol is incorporated into the mixed culture biomass. The balance (58.7 – 62%) is evolved as heat with little or no energy needed for the maintenance of organisms.

Key words: Binary mixed culture, biomass energetic yield, chemostat culture, energetic analysis, maintenance coefficient, Pirt's model.

INTRODUCTION

Proper design and operation of biological systems have the potential of being the most cost effective ways to treat toxic and hazardous chemicals because almost complete oxidation may be accomplished. The toxicity of phenol and the need to find ways of removing it from the environment have made the compound 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 (Pawlowsky et al., 1973, Solomon et al., 1994; Ruiz-ordaz et al., 2001; Paller et al., 1995; Hill and Robinson,1975; Nikakhari and Hill, 2006). Several studies have been carried out on the kinetics of phenol degradation by various microorganisms and on its inhibitory effects (Hill and Robinson, 1975; Yang and Humphrey, 1975; Schroeder et al., 1997; Folsom et al., 1990). The concept of material and energy balance in biotechnology has been identified and widely used in the analysis of experimental data concerning product formation, biomass formation and substrate consumption (Erickson, 1980; Layokun et al., 1985; Solomon et al., 1985, 1994). Also, the role of statistical techniques in data analysis and parameter estimation in biological system is widely gaining recognition (Yang et al., 1984; Solomon et al., 1984, 1985; Layokun et al., 1985. This is because measurement errors make

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accurate estimation of yield and maintenance parameters a difficult task (Layokun et al., 1985). Erickson and other researchers (Erickson, 1980; Solomon et al., 1984; Yang et al., 1984) have identified some multivariate statistical procedures which can be used to improve the quality of estimated parameters by making use of all the measured variables. Most data in the literature on phenol biodegradation by mixed culture do not lend themselves to energetic analysis using the concept of carbon and available electron balances which have been widely used for data analysis (Erickson, 1980; Ferrer and Erickson, 1979; Solomon et al., 1984, 1985, 1994; Oner et al., 1986). The reason for this is that the data are incomplete as many variables required are either not measured or reported. In spite of the extensive use of phenol biodegradation processes, surprisingly, no work has been published on energetic analysis of phenol microbial degradation using a mixed culture and an influent phenol concentration of 100 mg/L, a level that is lower than what has been investigated by previous studies. Therefore, the main objective of this study was to carry out the energetic analysis of complete data obtained on the aerobic degradation of phenol (with a concentration of 100 mg/L) by binary mixed culture of *Pseudomonas aeruginosa* and *Pseudomonas fluorescence* in chemostat culture. The data of Agarry (2009) was 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. In this work, the multivariate statistical technique which has been referred to as the covariate adjustment technique (CAT) (Solomon et al., 1983; Solomon et al., 1994) was employed in the estimation of true biomass energetic yield (η_{max}) and maintenance coefficient (m_e) associated with the growth of mixed culture of *P. aeruginosa* and *P. fluorescence* on phenol. The consistency of the data obtained was examined using the concept of material and energy balance. 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 (Pirt, 1965), which assumes that substrate uptake is a consequence of growth. The second model is a modified form of Pirt's model (Solomon et al., 1994) which assumes that growth is a consequence of substrate uptake.

METHODS OF DATA ANALYSIS

Consistency tests of experimental data

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:

$$CH_mO_l + aNH_3 + bO_2 = y_c\ CH_pO_nN_q + cH_2O + d\ CO_2 \quad (1)$$

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 (Solomon et al., 1994):

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

and

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

respectively.

For chemo stat operation, where $D = \mu$,

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

$$d = 12 Q_{CO_2} \Big/ [\{\sigma_s M_{CO_2} (S_0 - S_1)\mu\}] \quad (5)$$

$$\eta = \sigma_b \gamma_b X \Big/ \{\sigma_s \gamma_s (S_0 - S_1)\} \quad (6)$$

$$\varepsilon = 48 Q_{O_2} \Big/ [\{\sigma_b \gamma_b M_{O_2} \{\sigma_s \gamma_s (S_0 - S_1)\mu\}] \quad (7)$$

Equations (2) – (3) may be used to check the consistency of data as has been reported earlier (Solomon et al., 1984, 1985, 1994; Layokun et al., 1985).

Estimation of true yield (η_{max} and Y^{max}) and maintenance coefficient (m_e)

Pirt's model (Pirt, 1965) for growth processes has been written in the following forms:

$$r_s = \frac{\mu}{Y_{x/s}^{\max}} + m_s \quad (8a)$$

$$r_{O_2} = \frac{\mu}{Y_{x/O_2}^{\max}} + m_{O_2} \quad (9a)$$

$$r_{CO_2} = \frac{\mu}{Y_{x/CO_2}^{\max}} + m_{CO_2} \quad (10a)$$

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 (Solomon et al., 1994) to:

$$X_{1i} = \frac{\mu_i}{\eta_i} = \frac{\mu_i}{\eta_{\max}} + m_e + e_{1i} \quad (8b)$$

$$X_{2i} = \frac{\mu_i(\eta_i + \varepsilon_i)}{\eta_i} = \frac{\mu_i}{\eta_{\max}} + m_e + e_{2i} \quad (9b)$$

$$X_{3i} = \frac{\mu_i}{\eta_i}(y_{ci} + d_i) = \frac{\mu_i}{\eta_{\max}} + m_e + e_{3i} \quad (10b)$$

$$(i = 1,2.....n)$$

Where μ, is the specific growth rate, y_c and d are fractions of substrate carbon incorporated into biomass and that which is evolved as carbon dioxide, respectively. η and ε are fractions of substrate energy utilized in biomass formation and heat evolution, respectively. $e_{k1}(k = 1,2,3)$ are correlated error terms with mean 0 and variance-covariance matrix φ and n is the number of observations.

The estimates of $\eta_{\max}$ and m_e are the averages of individual estimates from equations (8b – 10b). However, combined estimates of the true biomass energetic yield, $\eta_{\max}$ and maintenance co-efficient, m_e can be obtained using equation (11) as given below:

$$\bar{X} = \frac{1}{3}\sum_{k=1}^{3} X_{ki} = \frac{\mu_i}{\eta_{\max}} + m_e + error \quad (11)$$

$$(i = 1,2,.....,n)$$

Nonetheless, the information contained in X_{1i}, X_{2i} and X_{3i} may not be efficiently utilized (Yang et al., 1984, Layokun et al., 1985). Thus, by application of the covariate adjustment technique (Solomon *et al.*, 1985, 1994) in which appropriate chosen set of covariates Z_{1i} and Z_{2i} which have expected values of zero and are linear function of X_{1i}, X_{2i} and X_{3i} are included in the above equation (11), thereby, a better estimate may be obtained. Hence, the model (Yang et al., 1984; Layokun et al., 1985):

$$\bar{X} = \frac{\mu_i}{\eta_{\max}} + m_e + \sum_{i=0}^{c} \alpha_j z_{ji} + error \quad (12)$$

$(0 \le c \le 2)$ is preferred. In this work, we assume the full set of linearly independent covariates that have zero means as:

$$Z_i = \begin{bmatrix} z_{1i} \\ z_{2i} \end{bmatrix} = \begin{bmatrix} 1 & -2 & 1 \\ 1 & 0 & -1 \end{bmatrix} \begin{bmatrix} X_{1i} \\ X_{2i} \\ X_{3i} \end{bmatrix}$$

$(i = 1,2,.....,n)$, although due to measurement errors (which lead to data inconsistencies), the values of Z_{1i} and Z_{2i} are not usually zero. By using this full set of covariates, the least square estimates of $\eta_{\max}$ and m_e based on model (12), then becomes the maximum likelihood estimates (that is estimate with minimum variance), based on the combined models (8b), (9b) and (10b). However, maximum likelihood estimates may not the best estimate as in cases where covariates which are uncorrelated with $\overline{X_i}$ are excluded. In such cases, the residual variance of model (12) is not decreased but that of the degree of freedom for fitting the model, because the covariates included contain no information about X_1 (Layokun et al., 1985). Therefore, a measure of "goodness" of a set of covariates that should be included in model (12) is J = $\sigma^2 / (n - r - c - 1)$ where σ^2 is the mean square error for fitting the model, r is the number of parameters of interest, c is the number of covariates included in the model, and n is the number of observations. For this study, r = 2 and $0 \le c \le 2$ and selection of the "best" estimate is based on the value of $\sigma^2 / (n - 3 - c)$ which is a measure of the "goodness" of the set of covariates that are included in model (12). The lowest value of "J" was considered the best for fitting the model for the range when no covariate is included to when both covariates are included. Further details of this statistical method are found in Solomon et al. (1984) and Yang et al. (1984).

Nonetheless, the above equations are based on Monod kinetics, which is,

$$\mu = \frac{\mu_{\max} S}{K_s + S} \quad (13)$$

that requires a well defined substrate consumption rate. However, in many cases, growth of microbes is a consequence of substrate consumption and not vice versa (Sonnleitner and Kappeli, 1986; Solomon et al., 1994). It has been shown that in this approach as S tends to 0, μ = 0 and yet a finite quantity of substrate consumption, m_e, is required that is due to maintenance (Solomon et al., 1994). 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 (13), a model of the form:

$$r_s = \frac{r_s^{\max} S}{K_s + S} \quad (14)$$

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

$$\mu = Y_{x/s}^{\max} r_s - \mu_{ms} \quad (15a)$$

$$\mu = Y_{x/O_2}^{\max} r_{O_2} - \mu_{mO_2} \quad (16a)$$

$$\mu = Y_{x/CO_2}^{\max} r_{CO_2} - \mu_{mCO_2} \quad (17a)$$

The equations (15a) to (17a) have been reparametrized in energetic terms and are shown to be correspondingly equivalent (Solomon et al., 1994) to:

Table 1. Calculated oxygen and carbon dioxide transfer rates for the continuous degradation of phenol by binary mixed culture of *P. aeruginosa* and *P. fluorescence*.

D (h^{-1})	Q_{O_2} ($mgL^{-1}h^{-1}$)	Q_{CO_2} ($mgL^{-1}h^{-1}$)	r_s $mgmg^{-1}h^{-1}$	r_{O_2} $gg^{-1}h^{-1}$	r_{CO_2} $gg^{-1}h^{-1}$
0.01	1.286	1.503	0.011	0.014	0.017
0.02	2.571	3.005	0.025	0.033	0.038
0.03	3.857	4.596	0.039	0.050	0.060
0.04	5.143	6.188	0.054	0.070	0.084
0.05	7.071	8.397	0.069	0.097	0.115
0.06	8.357	9.723	0.082	0.114	0.133
0.10	14.143	16.745	0.143	0.202	0.240
0.14	19.929	23.866	0.206	0.293	0.351
0.15	21.857	25.634	0.0224	0.326	0.383
0.17	24.429	29.170	0.263	0.382	0.456
0.18	26.357	30.938	0.291	0.432	0.507
0.19	27.643	32.705	0.312	0.461	0.545
0.20	28.929	34.473	0.322	0.474	0.565

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

$$\mu = \frac{\mu(\eta+\varepsilon)\eta_{\max}}{\eta} + m' \quad (16b)$$

$$\mu = \frac{\mu(y_c+d)\eta_{\max}}{\eta} + m' \quad (17b)$$

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 (Solomon et al., 1994). Equations (15b) to (17b) were also used to estimate $\eta_{\max}$ and m_e.

EXPERIMENTAL

The organisms, fermentor, fermentation medium, inoculum preparation, experimental design for the study and the analytical methods have been described elsewhere (Agarry et al., 2008).

RESULTS AND DISCUSSION

The calculated values of phenol consumption rates (Q_s), oxygen uptake rate (Q_{O_2}) and carbon dioxide production rate (Q_{CO_2}) (Table1) were used for the estimation of the biomass energetic yield (η) and carbon yield (y_c) for the binary mixed culture of *P. aeruginosa* and *P. fluorescence* using the carbon and available electron balances as given in equations (4) – (7). For the estimation, the average values of σ_b = 0.490 and γ_b = 4.793 which have been calculated from the measured composition of *Pseudomonas* species obtained by Layokun (1982) were used. The instantaneous available electron and carbon balances results obtained are presented in Table 2. From Table 2, it could be seen that the biomass energetic yield (η) and carbon yield (y_c) are low (that is, less than 1) which thus agree with the available electron and carbon balance equation. It could also be seen from the table that both the biomass energetic yield (η) and carbon yield (y_c) decreased as the dilution rate increased. Consistency tests (checks) were made using equations (2) – (3). It has been established (Solomon et al., 1981) 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 ≤ ($y_c + d$) ≤ 1.06 and 0.93 ≤ ($\eta + \varepsilon$) ≤ 1.07. The results of the data consistency tests are as shown in Table 2. Thus, it could be seen from Table 2 that the consistency equations are generally satisfied. Also, it could be seen from Table 2 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, the binary mixed culture of *P. aeruginosa* and *P. fluorescence* were 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) to (10a) was 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 (r_{O_2}) and

Table 2. Examination of data consistency using instantaneous available electron and carbon balances for the growth of binary mixed culture of *P. aeruginosa* and *P.fluorescence* in phenol-limited continuous culture (chemo stat operation).

$D = \mu$	y_c	d	$y_c + d$	η	ε	$\eta + \varepsilon$
0.01	0.576	0.535	1.111	0.591	0.540	1.133
0.02	0.505	0.535	1.040	0.519	0.539	1.058
0.03	0.493	0.546	1.039	0.506	0.539	1.045
0.04	0.473	0.550	1.023	0.486	0.539	1.025
0.05	0.467	0.598	1.065	0.479	0.593	1.072
0.06	0.467	0.577	1.044	0.479	0.584	1.063
0.10	0.448	0.598	1.043	0.460	0.593	1.053
0.14	0.435	0.607	1.043	0.447	0.597	1.044
0.15	0.429	0.608	1.037	0.440	0.611	1.051
0.17	0.414	0.618	1.032	0.425	0.610	1.035
0.18	0.396	0.621	1.017	0.406	0.623	1.029
0.19	0.390	0.622	1.012	0.400	0.620	1.020
0.20	0.397	0.624	1.021	0.408	0.618	1.026

Table 3. Estimates of true biomass growth yields and maintenance coefficient for the growth of mono and mixed culture of *Pseudomonas* species in phenol-limited continuous culture using Pirt's model (Equations 8a - 10a).

Organism	$Y_{x/s}^{\max}$ **gg^{-1}**	$Y_{x/O_2}^{\max}$ **gg^{-1}**	$Y_{x/CO_2}^{\max}$ **gg^{-1}**	m_s **gg^{-1}h^{-1}**	m_{O_2} **gg^{-1}h^{-1}**	m_{CO_2} **gg^{-1}h^{-1}**
Binary mixed culture	0.608	0.408	0.344	-0.0125	-0.0257	-0.0305

carbon dioxide production (r_{CO_2}) obtained for the binary mixed culture were plotted as a function of dilution rate (D) to obtain the true growth yield and maintenance coefficients, respectively. The estimated values are given in Table 3.

The Pirt's model was reparametrized to produce multi-response models with common parameters as given in equations (8b) to (10b) and application of covariate adjustment technique (Solomon et al., 1994) to these equations resulted in a unit variate linear model with covariates. These allow combined point and interval estimates of biomass energetic yield and maintenance coefficient to be obtained using standard multiple regression programs. Therefore, using equations (8b) to (10b), various estimates of the true biomass energetic yield and maintenance coefficients based on the data in Table 1 were obtained for the binary mixed culture and are presented in Table 4. The first three estimates in Table 4 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 equation (10b), respectively. These estimates are quite comparable but differ because of measurement errors.

When all the measured data were used (that is, Q_s, Q_{O_2}, Q_{CO_2}, μ) the best estimate was the maximum likelihood estimate (MLE) which corresponded to when one covariate (Z_2) was included. This was based on the lowest value of J = 3.030 x 10^{-5}. The respective combined point estimates for $\eta_{\max}$ and m_e were 0.396 and –0.020 h^{-1} with the corresponding 95% confidence intervals (0.380, 0.413) and (-0.033, -0.007) h^{-1}. When the carbon dioxide data were excluded (that is, Q_s, Q_{O_2}, μ were used), then the respective best point and interval estimates for $\eta_{\max}$ were 0.393 and (0.378, 0.410) and the m_e are –0.019 h^{-1} and (-0.032, 0.006) h^{-1}. With the oxygen data excluded (that is, Q_s, Q_{CO_2}, μ were used), $\eta_{\max}$ = 0.396 with interval (0.382, 0.412) and m_e = -0.018 h^{-1} with interval (-0.029, -0.006) h^{-1}. Lastly, when substrate measurements were excluded (that is, Q_{O_2}, Q_{CO_2}, μ were used), $\eta_{\max}$ = 0.392 with interval (0.376, 0.404) and m_e = -0.018 h^{-1} with interval (-0.029, -0.007) h^{-1}.

For the mixed culture of 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

Table 4. Estimates of true biomass energetic yields and maintenance coefficient for the growth of binary mixed culture of *P. aeruginosa* and *P. fluorescence* in phenol-limited continuous culture using Pirt's model (Equations 8b – 10b).

Data	Covariates Included	η_{max}		m_e		J
		Point	Interval	Point	Interval	
Q_s, μ	-	0.400	(0.383, 0.418)	-0.019	(-0.032, -0.006)	-
Q_{O_2}, μ	-	0.388	(0.372, 0.404)	-0.020	(-0.033, -0.006)	-
Q_{CO_2}, μ	-	0.392	(0.380, 0.406)	-0.016	(-0.027, -0.006)	-
$Q_s, Q_{O_2}, Q_{CO_2}, \mu$	-	0.393	(0.379., 0.408)	-0.019	(-0.031, -0.007)	3.548 x10^{-5}
	Z_1	0.399	(0.384, 0.415)	-0.018	(-0.030, -0.006)	4.141 x10^{-5}
	Z_2	0.396	(0.380, 0.413)	-0.020	(-0.033, -0.007)	3.030 x10^{-5}
	Z_1 Z_2	0.394	(0.380, 0.410)	-0.019	(-0.031, -0.007)	4.545 x10^{-5}
Q_s, Q_{O_2}, μ	-	0.393	(0.378, 0.410)	-0.019	(-0.032, -0.006)	3.511 x10^{-5}
	Z_1	0.396	(0.381, 0.413)	-0.018	(-0.030, -0.005)	3.838 x10^{-5}
Q_s, Q_{CO_2}, μ	-	0.396	(0.382, 0.412)	-0.018	(-0.029, -0.006)	3.197 x10^{-5}
	Z_1	0.382	(0.370, 0.399)	-0.010	(-0.017, -0.010)	3.535x10^{-5}
Q_{O_2}, Q_{CO_2}, μ	-	0.390	(0.376, 0.404)	-0.018	(-0.029, -0.007)	3.364 x10^{-5}
	Z_1	0.395	(0.383, 0.408)	-0.015	(-0.025, -0.005)	3.434 x10^{-5}

Table 5. Estimates of true biomass energetic yields and maintenance coefficient for the growth of binary mixed culture of *P. aeruginosa* and *P. fluorescence* in phenol-limited continuous culture using modified Pirt's model (Equations 15b – 17b).

Data	η_{max}		m' (h^{-1})		m_e (h^{-1})	
	Point	Interval	Point	Interval	Point	Interval
Q_s, μ	0.398	(0.381, 0.415)	0.008	(0.003, 0.013)	-0.020	(-0.032, -0.008)
Q_{O_2}, μ	0.386	(0.370, 0.402)	0.008	(0.003, 0.013)	-0.021	(-0.032, -0.008)
Q_{CO_2}, μ	0.392	(0.379, 0.404)	0.007	(0.003, 0.011)	-0.017	(-0.026, -0.008)
Average	0.392	(0.377, 0.407)	0.008	(0.003, 0.012)	-0.019	(-0.030, -0.008)

obtained when J = 3.030 x 10^{-5} which was for the case when all the measured data were used and corresponded to the maximum likelihood estimate (MLE) value of η_{max} = 0.396 with 95% confidence intervals (0.380, 0.413) and m_e = -0.020 h^{-1} with interval (-0.033, 0.007) h^{-1}.

In earlier applications of this procedure (Solomon et al., 1981, 1983) the best combined estimates was always assumed to be obtained when all the measured data were used. The results obtained for binary mixed culture (*P. aeruginosa* and *P. fluorescence*) have shown that a combined estimate from all the measured data might in fact lead to a better estimate. This is in agreement with the observation of Solomon et al. (1994) when all the measured data was used.

The estimates of η_{max} and m_e using the modified Pirt model (equations15b – 17b) and 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. However, there was good agreement between the corresponding individual estimates for the two cases (Pirt's model and the modified Pirt's model) as shown in Tables 4 and 5, respectively. The most reliable estimate in Table 5 was the average which gave η_{max} = 0.392 and m_e = -0.019 h^{-1} with the respective 95% confidence interval (0.377, 0.407) and (-0.030, -0.008) h^{-1}. The estimates of m_e in

Table 6. Summary of true biomass growth yields and maintenance coefficient for the growth of binary mixed culture of *P. aeruginosa* and *P. fluorescence* in phenol-limited continuous culture.

Equations Used	η_{max} (-)	$Y_{x/s}^{max}$ gg^{-1}	Y_{x/O_2}^{max} ggmol^{-1}	Y_{x/CO_2}^{max} ggmol^{-1}	m_e h^{-1}	$m_{x/s}$ gg^{-1}h^{-1}	$m_{O_2/x}$ molg^{-1}h^{-1}	$m_{CO_2/x}$ molg^{-1}h^{-1}
Pirt's Model	0.396	0.608	0.408	0.344	-0.020	-0.013	-0.026	-0.031
Modified Model	0.392	0.606	0.406	0.342	-0.019	-0.013	-0.027	-0.032

Tables 4 and 5 are statistically significantly lower than zero and therefore negligible. Hill and Robinson (1975) 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 true biomass energetic and growth yield obtained for the binary mixed culture of *P. aeruginosa* and *P. fluorescence* was found to be higher than that obtained for the monoculture of *P. fluorescence* (η_{max} =0.315 and $Y_{x/s}^{max}$ = 0.463) and *P. aeruginosa* (η_{max} = 0.359 and $Y_{x/s}^{max}$ = 0.540) (Agarry , 2009).

Conclusions

The advantage of combined estimates using covariate adjustment technique has been demonstrated. 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 kinetics) which require well-defined substrate consumption as well as the modified approach which assumed that substrate consumption was rate limiting were similar and led to similar estimates However, the latter approach did not allowed the application of a multivariate statistical method for parameter estimation.

From this analysis, about 38 – 41.3% of the energy in phenol may be incorporated into binary mixed culture of *P. aeruginosa and P. fluorescence* biomass, while the balance, 58.7 – 62% is mostly evolved as heat with little or no use for maintenance of the cells. 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 fermentor.

NOMENCLATURE

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

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

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

d Moles of carbon dioxide per quantity of organic substrate containing 1 g 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).

m_{CO_2} 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_{O_2} 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_{CO_2} Rate of CO_2 production (mgL^{-1}hr^{-1}).

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

r_{CO_2} Specific rate of CO_2 production (g-mol g biomass^{-1} hr^{-1}).

r_{O_2} 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 carbon 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 biomass (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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