THE ANNALS OF "DUNAREA DE JOS" UNIVERSITY OF GALATI
FASCICLE III, 2000 ISSN 1221-454X
ELECTROTECHNICS, ELECTRONICS, AUTOMATIC CONTROL, INFORMATICS

MODELING OF THE BIODEGRADATION IN MULTIPHASE STIRRED TANK REACTOR

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Abstract: The surface response methodology is used to verify predicted models and to adapt them on particular conditions of growth. The effect of solvent addition during the linear cell growth on the enhancement of biodegradation is presented and used to study the enhancement of mass transfer.

Keywords: biotechnology, bioreactor design, mass transfer, surface response methodology.

1. INTRODUCTION

Biological techniques are based on the ability of many microorganisms (generally bacteria) to degrade a broad variety of organic and some inorganic compounds.

Under aerobic conditions these microorganisms can oxidize the compounds present in the waste gases into mineral end-products (e.g. H_2O, CO_2), part of the organic compound being transformed into new cell material (Ottengraf, 1987; Ottengraf, *et al.*, 1993).

When the compounds are poorly water soluble none of the conventional techniques, namely biofilters, bioscrubbers or trickling filters is efficient for those compunds removal from the gas phase toward the water phase.

In that situation the mass transfer of the compound poorly water soluble from the gas to the aqueous phase is the limiting factor.

Some authors studied the enhancement of oxygen transfer rate in aerobic growth. They showed that oxygen solubility in water being very low, the oxygen transfer rates could be enhanced by using oxygen-vectors having a higher oxygen solubility than water (Rols and Goma, 1991).
To improve the absorption rate of poorly water soluble compounds a liquid where the pollutant shows a high affinity may be used. In many cases this liquid is an apolar water immiscible organic solvent. Consequently the compound is removed from the gas to the solvent and finally it has to be transferred to the water phase where it will be degraded by microbial activity.

Organic fluids applied as carrier vectors were fluorocarbons (Damiano and Wang, 1985; Hamamoto, *et al.*, 1987; Ju, *et al.*, 1991; Mattioason and Aldercreutz, 1987), soybean oil (Pols and Goma, 1991), phtalates and silicone fluids (El Aalam, *et al.*, 1993; Leonhardt, *et al.*, 1985; Poncelet, *et al.*, 1993).

The solvent used as compound-vector in mass transfer enhancement of the compound to be degraded should meet some criteria: very low water solubility, low volatility, low viscosity, non biodegradable, non toxic for microorganisms, not corrosive and not expensive.

A step forward was to a better understanding of the physical processes occuring in bioreactors which has to fill the gap between knowledge and real

This paper was recomended for publication by E. Ceanga

problems. In this area, a large number of papers on the design of bioreactors is now available (Diks *et al.*, 1991*a*, 1991*b*; van't Riet *et al.*, 1991; Baltzis *et al.*, 1992; Kawase *et al.*, 1992; Fernandes *et al.*, 1993; Dudley, 1995; Hodge *et al.*, 1995; Ibrahim *et al.*, 1995; Hodge *et al.*, 1997; Baltzis *et al.*, 1997; Barton *et al.*, 1998; Alonso *et al.*, 1999).

The study of the mass transfer is generally based on the knowledge of the specific surface area and the mass transfer coefficient of the compound to be transferred.

Unfortunately, these parameters are not measurable due to the continuous aeration and the non-homogenous growth of the microorganisms in the liquid phase.

A bioreactor featuring high specific areas for mass transfer between the solvent and the water should be used. Therefore it was chosen as bioreactor a stirred tank reactor in which the mechanical stirring increases the contact between the water phase with microorganisms and the solvent with pollutant.

The aim of the work was to study the enhancement of mass transfer from the gas phase to the aqueous phase by using an apolar organic solvent, namely silicone oil, which acts as ethylene vector. This solvent in nonbiodegradable, nontoxic for bacteria, insoluble in water, and shows high affinity for ethylene, his partition coefficient in ethylene being seven times higher than in aqueous medium.

Not every work on modeling has to be focused on developing new models but it can be done to verify predicted models and to adapt them to particular conditions of biodegradation: bacteria, compound, growth conditions. A relativ simple way to do this is to use surface response methodology which penetrate recently in the biotechnological field (Smith, 1977; Lah, 1980).

2. RESULTS AND DISCUSSION

2.1 Experimental matrix

To estimate the functional depdendence between independent and dependent variables it has to perform a number of experiments, in such order, one very used way to organise the experiments being the factorial programme.

In this study the steps of the factorial experiment were:

1. Selection of model dimensions:
- Independent variables: biomass concentration at the moment of solvent addition (x) and solvent concentration (C_s), than $n = 2$;
- Dependent variables (responses): linear cell growth rate (r_x), ethylene transfer rate (*ETR*), biomass yield ($Y_{x/s}$) and volumetric overall mass transfer coefficient ($k_{el} \cdot a$).

2. Establish of variation interval of independent variables:
- Biomass concentration at the moment of solvent addition x = 2 – 3.5 g/l (2.1 g/l; 2.45 g/l and 3.25 g/l);
- Solvent concentration in the bioreactor after the solvent adddition: C_s = 2.5 %; 5 % and 10 % (v/v) silicone oil;

3. Codification of variables (table 1).

Table 1 Indepdendent variables – natural and codificate values

Indep. variable	Symbol Natural	Symbol Codif.	Level Natural	Level Codif.
Biomass conc., g/l	x	y_1	2.1	-1
			2.45	0
			3.25	+1
Solvent conc., %(v/v)	C_s	y_2	2.5	-1
			5	0
			10	+1

To organise a complete factorial experiment one needs to perform 3^2 = 9 experiments in order to obtain all the possible combinations of independent variables. The combinations and the values obtained for responses are presented in table 2 (all the responses are analysed as adimensional values obtained dividing the response value after the solvent addition by the value before the addition).

Table 2 Experimental matrix

Indep. Variabiles y_1	y_2	Responses $\frac{r_{x2}}{r_{x1}}$	$\frac{ETR_2}{ETR_1}$	$\frac{Y_{x/s,2}}{Y_{x/s,1}}$	$\frac{(k_{e,l} \cdot a)_2}{(k_{e,l} \cdot a)_1}$
-1	-1	1.49	1.45	1.036	1.12
-1	0	1.68	1.55	1.055	1.17
-1	+1	1.69	1.56	1.056	1.17
0	-1	1.50	1.42	1.035	1.13
0	0	1.64	1.53	1.053	1.18
0	+1	1.65	1.54	1.054	1.18
+1	-1	1.49	1.43	1.038	1.11
+1	0	1.62	1.53	1.056	1.17
+1	+1	1.63	1.53	1.055	1.17

2.2 Proposed models

The choice of a linear correlation model between the dependent variables (r_x, *VTE*, $Y_{x/s}$ and $k_{el} \cdot a$) and the independent ones (% of siliconic oil and x) is not recommended because it simplifies the analyse, and certainly does not take into account all the aspects. It is also possible to loose some reciprocal influences. Therefore, it must be chosen a nonlinear correlation, and the responses has to be analysed on a two variables function form:

$$\frac{r_{x2}}{r_{x1}} = f\left(x, C_s\right) \quad (1)$$

$$\frac{ETR_2}{ETR_1} = f\left(x, C_s\right) \quad (2)$$

$$\frac{Y_{x/s,\ 2}}{Y_{x/s,\ 1}} = f\left(x, C_s\right) \quad (3)$$

$$\frac{(k_{e,l} \cdot a)_2}{(k_{e,l} \cdot a)_1} = f\left(x, C_s\right) \quad (4)$$

In most of the cases one use, in practice, the polynomial of second order due to the fact that non-linear functions are or can be transformed in this form (Resa, *et al.*, 1984).

Therefore, the form of the suggested model for each of the responses corresponds with the general relation:

$$F_{m,K} = a_{k0} + \sum_1^n a_{ki} \cdot y_i + \sum_1^n a_{kii} \cdot y_i^2 + \sum_1^n \sum_1^m a_{kij} \cdot y_i \cdot y_j \quad (5)$$

Having $n = 2$ (two independent variables) the equation (5) can be written particularly as follows:

$$F_{m,K} = a_{k0} + a_{k1} \cdot y_1 + a_{k2} \cdot y_{2,1} + a_{k11} \cdot y_1^2 + a_{k22} \cdot y_{2,1}^2 + a_{k12} \cdot y_1 \cdot y_{2,1} \quad (6)$$

In this form, second order terms give information about non-liniarity, and the last term is used to recognise the interactions between indepdendent variables (binary interactions).

2.3 Results

The coefficients form of the equation (6) were determined by non-linear regression analysis, their values for all the responses (linear cell growth rate, r_x, ethylene transfer rate, *ETR*, biomass yield, $Y_{x/s}$ and volumetric overall mass transfer coefficient, $k_{el}{\cdot}a$ being presented below (table 3 – table 6).

Table 3 Non-linear regression coefficients estimation and confidence limits of biomass linear growth rate (r_{x2}/r_{x1})

Indep. variable	Coefficients Symbol	Coefficients Value	Standard Error	t - value	Confidence Limits 95% min.	Confidence Limits 95% max.	P>|t|	Imp. Coeff.
x, C_s	a_{k0}	1.5287	0.3136	4.8740	0.5379	2.5194	0.016	✓
	a_{k1}	-0.2177	0.2348	-0.9274	-0.9595	0.5239	0.422	
	a_{k2}	0.1341	0.0157	8.5114	0.0843	0.1839	0.003	✓
	a_{k11}	0.0398	0.0431	0.9233	-0.0964	0.1762	0.423	
	a_{k22}	-0.0079	0.0009	-8.2370	-0.0109	-0.0048	0.003	✓
	a_{k12}	-0.0051	0.0037	-1.3988	-0.0168	0.0065	0.256	

Table 4 Non-linear regression coefficients estimation and confidence limits of ethene transfer rate (ETR_2/ETR_1)

Indep. variable	Coefficients Symbol	Coefficients Value	Standard Error	t - value	Confidence Limits 95% min.	Confidence Limits 95% max.	P>|t|	Imp. Coeff.
x, C_s	a_{k0}	1.7353	0.0713	24.3062	1.5098	1.9609	0.0001	✓
	a_{k1}	-0.3454	0.0534	-6.4631	-0.5143	-0.1766	0.0075	✓
	a_{k2}	0.0793	0.0035	22.1073	0.0679	0.0906	0.0002	✓
	a_{k11}	0.0615	0.0098	6.2685	0.0305	0.0926	0.0082	✓
	a_{k22}	-0.0049	0.0002	-22,768	-0.0056	-0.0042	0.0001	✓
	a_{k12}	-0.0002	0.0008	0.2926	-0.0029	0.0024	0.788	

Table 5 Non-linear regression coefficients estimation and confidence limits of biomass yield ($Y_{x/s2}/Y_{s/x1}$)

Indep. variable	Coefficients Symbol	Coefficients Value	Standard Error	t - value	Confidence Limits 95% min.	Confidence Limits 95% max.	P>|t|	Imp. Coeff.
x,	a_{k0}	1.0261	0.0135	75.9962	0.9834	1.0689	0.1e-04	✓
	a_{k1}	-0.0203	0.0102	-1.9866	-0.0527	0.0120	0.14	

C_s	a_{k2}	0.0171	0.0007	22.1077	0.0146	0.0195	0.0002	✓
	a_{k11}	0.0038	0.0018	2.0267	-0.0021	0.0098	0.135	
	a_{k22}	-0.0014	8.2e-05	-17.7593	-0.0017	-0.0012	0.0003	✓
	a_{k12}	0.0004	0.0002	1.6848	-0.0003	0.0011	0.19	

Table 6 Non-linear regression coefficients estimation and confidence limits of volumetric overall mass transfer coefficient $(k_{e,l}\times a)_2 / (k_{e,l}\times a)_1$

Indep. variable	Coefficients		Standard Error	t - value	Confidence Limits 95%		P>|t|	Imp. Coeff.
	Symbol	Value			min.	max.		
x, C_s	a_{k0}	0.7976	0.0549	14.5062	0.6239	0.9713	0.0007	✓
	a_{k1}	0.1906	0.0411	4.6309	0.0606	0.3206	0.0189	
	a_{k2}	0.0392	0.0027	14.2148	0.0305	0.0480	0.0007	✓
	a_{k11}	-0.0373	0.0075	-4.9313	-0.0612	-0.0134	0.0159	
	a_{k22}	-0.0027	0.0001	-16.259	-0.0032	-0.0022	0.0005	✓
	a_{k12}	0.0009	0.0006	1.5355	-0.0010	0.0030	0.2222	

The evolution of linear cell growth rate after the solvent addition divided by the one before the addition was represented in 3-D graph (fig. 1).

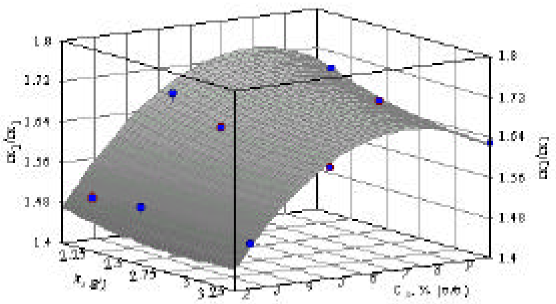

Fig. 1. The response surface form of linear biomass growth rate (r_{x2}/r_{x1}).

In figure 2 is presented the variation of ethylene transfer rate against the cell concentration and solvent concentration after the addition. The values used for ethylene transfer rate were adimensional.

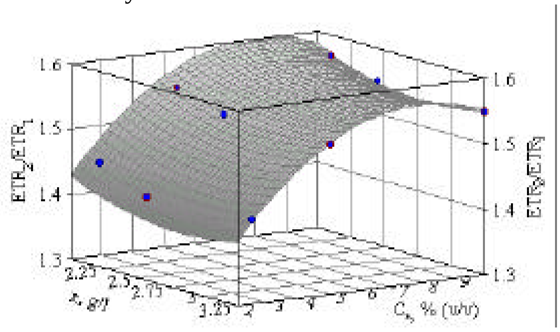

Fig. 2. The response surface form of ethylene transfer rate (ETR_2/ETR_1).

They were obtained dividing the values of ethylene transfer rate during the linear cell growth after the silicone oil addition by the values of ethylene transfer rate during the linear cell growth before the addition.

The evolution of biomass yield after the solvent addition during the linear cell growth divided by the value before the solvent addition in 3-D graph is presented in figure 3.

Finally, in figure 4 is presented the variation of volumetric overall mass transfer coefficient against the biomass concentration in the moment of solvent addition and solvent concentration after the addition of a certain volume of silicone oil into the bioreactor medium during the linear cell growth.

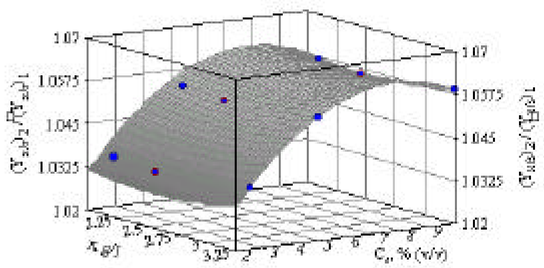

Fig. 3. The response surface form of bimass yield ($Y_{x/s2}/Y_{s/x1}$).

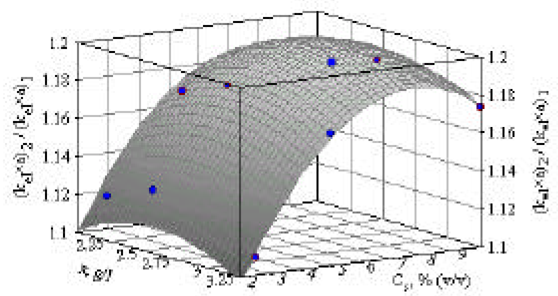

Fig. 4. The response surface form of volumetric overall mass transfer coefficient $(k_{e,l}\times a)_2$ / $(k_{e,l}\times a)_1$

2.4 Discussion

The analysis of non-linear regression leads to some interesting aspects. Thus the results obtained demonstrate a good proximity of functional dependecies of variables and polynomial functions.

This aspect is illustrated by the correlation coefficients r^2 (mean squared standard deviation) which are very closed to 1 (table 7).

To verify the assumptions concerning the selected models and the coefficients, tests *t* and *F* are used. Both tests are calculated for a significance level a = 0,05 (table 7). This leads to the selection of certain coefficients from table 3 to 6, the ones marked "✓" which frame in the risque level and to the elimination of the variables and their interactions irrelevant for the process from the regression equation, obtaining models to describe better the process (table 7).

3. CONCLUSION

Solvent addition in the culture during the linear cell growth leads to the enhancement of the linear cell growth rate, the adimensional factor rx2/rx1 increasing from 1.49 for 2,5 % (v/v) silicone oil to 1.66 for 10 % (v/v) solvent.

Table 7. Correlation coefficients, F test and final models of the responses

Dep. Var. (response)	Indep. var	r^2	*F* - test	P > *F*	Model
r_x (r_{x2}/r_{x1})	x, C_s	0.984	38.31	0.00641	$r_{x2}/r_{x1} = 1.5287 + 0.1341 \cdot C_s - 0.0079 \cdot (C_s)^2$
ETR (ETR_2/ETR_1)	x, C_s	0.998	378.31	0.00021	$VTE_2/VTE_1 = 1.7353 - 0.3454 \cdot x + 0.0793 \cdot C_s + 0.0615 \cdot x^2 - 0.0049 \cdot (C_s)^2$
$Y_{x/s}$ $(Y_{x/s})_2/(Y_{x/s})_1$	x, C_s	0.999	888.12	0.000	$Y_{x/s,2}/Y_{x/s,1} = 1.0261 + 0.0171 \cdot C_s - 0.0014 \cdot (C_s)^2$
$k_{e,l} \cdot a$ $(k_{e,l} \cdot a)_2/(k_{e,l} \cdot a)_1$	x, C_s	0.997	150.68	0.00053	$(k_{e,l} \cdot a)_2/(k_{e,l} \cdot a)_1 = 0.7976 + 0.0392 \cdot (C_s) - 0.0027 \cdot (C_s)^2$

Ethylene transfer rate, biomass yield and volumetric overall mass transfer have the same variation: they improve vith higher amount of solvent added in the broth. Surface response form for all the responses shows an enhancement with higher amounts of solvent but the size of this enhancement is lower for higher amount of solvent, therefore an optimum of solvent concentration around 5 5 (v/v) should be pointed.

In the model equations (table 7) only the terms containing as independent variable solvent concentration appear. This means the biomass concentration value in the moment of solvent addition does not influence the mass transfer enhancement. Missing terms as product of indepdendent variables show the inexistence of binary interactions.

One can conclude that silicone oil is a good solvent being able to enhance the mass transfer of poorly degradable compounds and to increase their biodegradation. Also, the non-linearity of the functions represented is demonstrated by the presence of second order terms in the model equation.

List of symbols

a – mass transfer specific area, in m^2/m^3;
a_{ko}, a_{ki}, a_{kii}, a_{kij} – regression coefficients;
C_s – solvent concentration (v/v);
ETR – ethylene transfer rate, in mol/s;
$F_{m,k}$ – form of the proposed model;
$k_{el} \cdot a$ – volumetric overall mass transfer coefficient, in 1/s;
k_{el} – mass transfer coefficient, in m/s;
n – independent variables number;
r^2 – standard deviation coefficient;
r_x – linear growth rate of biomass, in $mol/(m^3 \cdot s)$;
x is the biomass concentration, in g/l;
y_i, y_j – indepdendent variables codified;
y_1 – codified symbol of biomass concentration;
y_2 – codified symbol of solvent concentration;
$Y_{x/s}$ – biomass yield, in g/mol;

Particular indexes:
$_1$ – index for the responses before solvent addition;
$_2$ – index for the responses after solvent addition.

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