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Factor Analysis in Itaconic Acid Fermentation using Filtered POME by *Aspergillus terreus* IMI 282743

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ABSTRACT

The production of itaconic acid by Aspergillus terreus IMI 282743 from filtered Palm Oil Mill Effluent (POME) as an added supplement was investigated. The factor analysis method was applied to screen the most influential parameters for the production of itaconic acid. Through this analysis, the filtered POME was proven to be a significant parameter in the fermentation. In addition, the parameter could be considered as an inducer to the production of itaconic acid since the addition of filtered POME into the media was able to increase the itaconic acid concentration. The production of itaconic acid was achieved up to 5.76 g/L in shake flask fermentation from production medium consisted of 56 mL of filtered POME containing 51% of filtered POME. It is also believed that if POME formulated media is used with the industrial strain, higher concentration of itaconic acid could be attained since according to some researches, it has been shown that industrial type strain is capable to produce more of the acid. Other parameters that were found to have significant influence on the itaconic acid production include percentage of glycerol, pH , temperature, concentration of NH_4NO_3 and agitation speed.

Keywords: Itaconic acid fermentation, Aspergillus terreus, factor analysis, Palm Oil Mill Effluent

ABSTRAK

Penghasilan asid itakonik oleh kulat Aspergillus terreus IMI 282743 menggunakan air buangan kilang sawit (POME) sebagai tambahan pemakanan telah dikaji. Kaedah analisis faktor telah digunakan untuk menyaring parameter yang memberi kesan kepada penghasilan asid itakonik. Dari analisis ini, POME yang dituras telah dibuktikan merupakan parameter berkesan dalam fermentasi. Seterusnya, parameter ini boleh dianggap sebagai penggalak kepada penghasilan asid itakonik kerana pertambahannya dalam medium pertumbuhan telah membolehkan kepekatan asid itakonik meningkat. Penghasilan asid itakonik mencapai 5.76 g/L dalam fermentasi kelalang goncang menggunakan medium yang mengandungi 56 mL POME terturas yang mengandungi kira-kira 51% dari kepekatan POME terturas. Adalah diramalkan sekiranya formulasi media POME ini digunakan bersama baka jenis industri, maka penghasilan asid itakonik akan meningkat

memandangkan baka ini telah terbukti berpotensi menghasilkan kepekatan asid itakonik yang lebih tinggi. Faktor-faktor lain yang mempengaruhi penghasilan asid itakonik dari analisis ini adalah peratus glicerol, pH, suhu, kepekatan NH_4NO_3, dan kelajuan penggoncang.

Katakunci: Fermentasi asid itakonik, Aspergillus terreus, analisis faktor, air buangan kilang sawit

INTRODUCTION

Itaconic acid ($C_5H_6O_4$) is an unsaturated dicarboxylic organic acid. It can be easily incorporated into polymers and may serve as a substitute for petrochemical-based acrylic or methacrylic acid (Willke & Vorlop 2001). Itaconic acid is produced on an industrial scale by the cultivation of *Aspergillus terreus* or *A. itaconicus* using sugar molasses or glucose (Yahiro et al. 1995). Other carbon sources like wood (Kobayashi 1978), hydrolysed starch (Cros & Schneider 1993; Petruccioli et al. 1999; Yahiro et al. 1997a), glycerol and mixtures of sucrose and glycerol (Jarry & Seraudie 1997), organic acids and glycine (Petuchow et al. 1980) and even market refuse fruits (Reddy & Singh 2002) were also tested. It is important to note that synthesis of itaconic acid has proven to be uneconomical because of high substrate costs and/or relatively low yields (Reddy & Singh 2002).

In this study, attempts were made to utilize abundantly available substrate as an extra supplement in the production medium, namely, palm oil mill effluent (POME), to produce itaconic acid by *A. terreus* IMI 282743. Factor analysis was applied in the study to screen some parameters that give significant effects to the itaconic acid production. The parameters that have been selected were agitation speed (rpm), temperature (°C), volume of filtered POME (mL), percentage of glycerol (% v/v), concentration of NH_4NO_3 (g/L), concentration of $MgSO_4$ (g/L) and pH. Two simple models were generated at the end of this study in order to relate the significant experimental variables to the biomass of mycelium and production of the itaconic acid in the shake flask fermentation.

Factor Analysis

Interactions between variables become more recognizable in complex process such as biological process (Harman ,1967, Mulaik,1972). Method of Factor Analysis can be applied to translate experimental variables to mutually orthogonal factors which the interactions between variables are not taken into account This method has been shown successfully in screening of important experimental variables in particular type of batch fermentation (Moresi et al. 1979). Mutually orthogonal factors are uncorrelated factor that derived from various experimental variables. These factors are uncorrelated to each other which have the same mean and the same variance as the standardised form of the experimental variables. This type of factor is important as such factors are used to construct linear models. The significance of each factor in its effect related to the yield is determined by removing the particular factor from the model containing all factors (full factor) model. The mean square difference between the actual data and the prediction data, with the mean square difference between the actual data and the predictions of model involving all factors is then compared using the statistical F-test. If an experimental variable contributes only to factors which do not affect the yield significantly then it can be concluded that it is not relevant to the yield and can be dropped from subsequent experiment. If an experiment factor contributes to one or more factors which have significant effects on the yield then the experimental variables is relevant to the yield and should be retained for further investigation and optimization.

MATERIALS AND METHODS

Microorganism, media and culture conditions

A strain of *A. terreus* IMI 282743 was obtained from Microbiology Laboratory, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor. The culture was grown and maintained on Potato Dextrose Agar (PDA) medium at 37°C for 4-5 days. Following this process, about 10% (v/v) of the spore suspension of the culture was inoculated into 100 mL of the growth medium containing 60 g/L glucose; 4.0 g/L ammonium nitrate, 0.95 g/L magnesium sulfate, 0.088 g/L potassium dihydrogen phosphate, 0.004 g/L copper sulfate, 1.0 g/L itaconic acid. The growth medium was incubated in a 250 mL

Erlenmeyer flask with agitation speed of 150 rpm for 3 days at 30°C. After the incubation period, 10 mL of the culture was taken and inoculated into 100 mL of production medium. The value of media compositions in the production stage were randomly varied within the range of 50 - 100 mL of filtered POME, 0 - 44 % (v/v) of glycerol, 0 - 4.0 g/L of ammonium nitrate and 0 - 3.0 g/L of magnesium sulfate. The surrounding parameters such as pH between 3.0 to 9.0, temperature between 30 to 40°C and rotation speed from 0 to 200 rpm were also va-ried. The incubation time of each run was main-tained at 5 days. The different combinations of experimental design variables were shown in Table 1. The pH of the medium was adjusted either by adding 1 mol/L hydrochloric acid or 1 mol/L sodium hydroxide at the beginning of fermentation to the desired pH.

Pretreatment of POME

Raw POME was obtained from Seri Ulu Langat Palm Oil Mill, Dengkil, Selangor. It usually contains excessive high amounts of total solids (40,500 mg /L) and suspended solids (18,000 mg /L) (Ahmad et al. 2003) that might not be suitable for the production of itaconic acid if it is used without any treatment. Therefore, the raw POME needs to be filtered prior to any fermentation. In this study, the POME treatment was subjected to different types of Whatman filter papers No. 1, 2 and 40. The final filtrate was referred as filtered POME.

Analytical methods
Biomass determination

Biomass was determined by measuring the dry cell weight of the culture. 110 mL of the samples were filtered through dried, pre-weighted filter paper (Whatman No. 2), followed by two times washing with distilled water and then dried at 80°C to constant weight.

Itaconic acid concentration

Itaconic acid was measured by using a high-pressure liquid chromatography (HPLC) on a Zorbax SB-C18 column of 5 μm particle size, 250 mm length and 4.6 mm inner diameter. The mobile phase of the column was 5 mM phosphoric acid at the rate of 1 mL/min. The column was maintained at 30°C, attached to HPLC Agilent 1100 system (Agilent Technology, Germany), equipped with a variable wavelength ultraviolet-visible detector and a Agilent autosampler model 100 (Agilent Technology, Germany). In this study, itaconic acid was detected at 220 nm.

Experimental variables screening and model construction

In this work, experimental value of each variable was varied randomly and a wide range of the value was needed to achieved maximum knowledge of performance in the studied system. As mentioned earlier, the first step in factor analysis is to standardised the experimental data in matrix form and thus a matrix correlation could be formed from the data. The matrix correlation is then required to find the Eigen values and Eigen vectors, where in the later process it is used to determine the orthogonal factor. The significant variables for each factor can also be identified at this stage. Then, the linear regression model or the non-linear regression was performed to examine if the response surface possess a maximum. The estimations of regression coefficients of all models were done using SPSS ver 12.0 (Apache Software Foundation, USA) . The evaluation of significance of the linear or non-linear model is made by statistical F-test to find for the best model that representing the variables that relevant to the yields as describes in the earlier section. The yields in this study are referred to the biomass formation and the production of itaconic acid.

RESULTS AND DISCUSSIONS

Fourty runs of the fermentation experiments were conducted with appropriate random value of variables. The assumption due to the experimental error for normal biological selection experiments falls between 30 - 40 % (Brock and Madigan,1991). The studied variables are agitation speed (rpm), temperature (°C), volume of filtered POME (ml), percentage of glycerol (% v/v), concentration of NH_4NO_3 (g/L), concentration of $MgSO_4$ (g/L) and pH. Each run of the experiment was performed in duplicate and thus the results of biomass of mycelium or Y (g/L) as well as the itaconic acid production or Z (g/L) were the averages of two experiments.

Table 1. Different combinations of experimental variables and the respective results

No.	Agitation speed (rpm)	Temperature (°C)	Filtered POME (ml)	Glycerol (%)	NH_4NO_3 (g/L) (ml)	$MgSO_4$	PH	Yields	
								Biomass formation, (g/L)	Itaconic acid production, (g/L)
1	100	35	56	44	0	0.00	6.0	11.60	0.6740
2	100	35	100	0	2	0.00	6.0	12.76	2.5811
3	150	40	56	44	0	0.00	6.0	13.96	0.0000
4	100	35	63	38	1	0.00	7.0	9.78	2.2935
5	150	30	56	44	1	0.00	5.8	9.62	5.7586
6	180	35	69	31	1	0.20	4.5	10.81	0.2802
7	100	35	81	19	4	0.95	6.6	24.58	2.2104
8	150	30	81	19	0	0.00	7.6	21.40	0.5059
9	190	39	81	19	3	0.00	8.3	7.32	0.7797
10	100	35	88	13	0	0.00	5.0	14.49	0.9710
11	100	35	94	6	4	0.00	7.3	19.44	1.3810
12	180	35	100	0	2	2.00	5.2	8.61	0.3707
13	180	35	69	31	0	2.00	4.3	13.36	0.2970
14	150	40	63	38	4	2.00	5.5	14.33	0.3889
15	150	30	63	38	2	2.00	7.4	9.58	1.1023
16	180	35	63	38	3	2.00	5.5	12.16	0.2950
17	190	39	100	0	0	2.00	4.6	5.39	0.2989
18	150	40	50	50	0	0.00	4.9	13.22	0.3496
19	150	40	50	50	4	0.90	8.2	14.70	1.3171
20	150	30	56	44	2	0.00	7.0	7.75	0.7675
21	190	39	56	44	2	1.00	5.0	10.86	0.4258
22	0	40	100	0	0	0.00	4.9	6.51	0.3884
23	0	35	56	44	1	0.30	4.5	11.32	0.3265
24	150	40	63	38	0	0.00	4.1	13.94	0.4720
25	150	30	56	44	0	0.00	9.0	11.60	1.9572
26	150	30	62	38	0	0.00	9.9	22.25	0.7070
27	190	39	63	38	4	0.00	4.8	8.64	0.4813
28	180	35	69	31	4	0.00	9.7	10.59	1.3623
29	150	30	56	44	4	0.10	6.4	12.29	0.8471
30	150	40	56	44	4	1.00	3.6	20.38	5.1790
31	150	30	56	44	2	1.00	8.9	10.26	0.5698
32	100	35	75	25	3	0.95	7.5	28.22	3.0054
33	100	35	69	31	3	0.00	8.0	20.15	0.8525
34	190	39	69	31	0	3.00	4.9	7.98	0.3894
35	190	39	69	31	2	0.00	8.9	7.00	0.8043
36	180	35	56	44	0	0.40	8.9	11.54	0.8432
37	180	35	63	38	0	0.00	3.7	9.88	0.2817
38	190	39	88	13	0	0.00	4.7	7.24	0.3594
39	190	39	100	0	0	0.00	8.5	14.12	0.9002
40	150	40	56	44	1	0.00	4.2	16.22	3.5069

Results of factor analysis

Table 1 represents the value of each experimental variable and the fermentation results. The results of biomass formation are ranging from 5.39 g/L to 28.22 g/L while for itaconic acid, the production falls between 0 g/L to 5.76 g/L. The use of too large variation of experimental variable value such as no agitation and no food supplement except POME in run no. 22 give low value in both biomass and itaconic acid production. The results with higher itaconic acid value does not indicate that higher biomass has also been produced as shown in run no. 30 and no. 40. Besides, results showing higher in biomass for example in run 32, only produced half of the maximum value of itaconic acid. Therefore, it can be concluded that biosynthesis of itaconic acid is very dependent on composition of the culture medium and is inflenced by chemical, physicochemical and biological factor (Larsen 1957, Rychtera and Wase 1981). Results for correlation matrix and Eigen values of correlation matrix are showed in

Table 2. Eigen value of the Correlation Matrix

	Agitation speed	Temperature	Filtered POME	Glycerol	NH_4NO_3	$MgSO_4$	pH
Agitation speed	1.0000	0.1029	-0.1123	0.1149	0.0027	0.2602	0.0725
Temperature	0.1029	1.0000	0.1463	-0.1435	0.0162	0.0906	-0.4204
FilteredPOME	-0.1123	0.1463	1.0000	-0.9996	-0.0841	0.0574	-0.0410
Glycerol	0.1149	-0.1435	-0.9996	1.0000	0.0838	-0.0552	0.0327
NH_4NO_3	0.0027	0.0162	-0.0841	0.0838	1.0000	0.1326	0.1875
$MgSO_4$	0.2602	0.0906	0.0574	-0.0552	0.1326	1.0000	-0.2273
pH	0.0725	-0.4204	-0.0410	0.0327	0.1875	-0.2273	1.0000

Table 3. Eigen value of the Correlation Matrix

No.	Eigen value	% Contribution
1	2.1022	30.03
2	1.5127	51.64
3	1.2147	67.00
4	0.9716	82.87
5	0.8016	94.33
6	0.3969	99.99
7	0.0003	100

Table 4. Eigen Vector coefficient and characterization factor

Agitation speed	Temperature	Filtered POME	Glycerol	NH_4NO_3	$MgSO_4$	pH	Significant parameters
-0.1064	0.2382	0.6632	-0.6622	-0.1181	0.085	-0.1798	filtered POME and glycerol
0.3437	0.5201	-0.1716	0.177	-0.0088	0.4775	-0.5678	pH and temperature
-0.4812	0.1727	-0.1453	0.1469	-0.5418	-0.4678	-0.4284	NH_4NO_3 and Agitation speed
0.5720	-0.2658	0.0405	-0.0405	-0.7611	0.0495	0.1312	NH_4NO_3
0.4293	0.5871	0.0152	-0.0162	0.1245	-0.6207	0.264	temperature and $MgSO_4$
-0.357	0.4771	-0.0894	0.08	-0.3125	0.3977	0.6121	pH
0.0027	-0.0013	-0.7069	-0.7073	0.0013	-0.0008	-0.007	filtered POME

Table 2 and Table 3 respectively. Table 4 shows Eigen vector that is associated with eigen values of correlation matrix. The last column in Table 4 shows the characterized variables that are significant for each factor (F_1 to F_7). F_1 is characterized by the amount of filtered POME and initial concentration of glycerol. F_2 is the environmental factor and being characterized by pH and temperature of the fermentation. NH_4NO_3 is representing factor F_3 and F_4 and factor F_3 is also characterized by agitation speed. Values of the factor are shown in Table 5.

Model Evaluation

Initially a linear model had been examined to fit the orthogonal factors and yields of biomass and itaconic acid production. However it was found that the linear model might not be sufficient enough to represent a good response to the maximum area. Therefore, non-linear model was later applied. This exercise was done to account the curvature in the area as if it had contained the maximum. Table 6 and Table 7 show the evaluation of non-linear regression models for biomass

Table 5. Values of the Factors

No	F_1	F_2	F_3	F_4	F_5	F_6	F_7
1	0.0750	-0.3353	0.7653	0.4263	0.3366	-0.1930	0.0012
2	0.2158	-0.4042	0.2813	-0.1665	0.4415	-0.4595	0.0020
3	0.1649	-0.1254	0.8211	0.3365	0.5746	-0.0161	0.0008
4	0.0076	-0.5215	0.4106	0.1650	0.4678	-0.1332	-0.0033
5	-0.0561	-0.4979	0.4971	0.2313	0.1523	-0.5486	0.0027
6	0.1513	0.0539	0.5774	0.1025	0.1039	-0.5020	0.0048
7	-0.1294	0.1658	-0.8482	-0.6880	-0.2638	-0.0441	0.0008
8	0.0257	-0.8420	0.4481	0.6152	0.2511	-0.0969	-0.0018
9	0.0103	-0.6042	-0.1803	-0.4425	0.8748	-0.0133	-0.0026
10	0.2982	-0.2060	0.8592	0.3965	0.2599	-0.3926	0.0004
11	0.0181	-0.6284	-0.3141	-0.7235	0.6450	-0.4632	0.0003
12	0.0106	1.1253	-0.9817	-0.0343	-1.3978	0.5249	0.0015
13	-0.0114	1.3321	-0.3951	0.5266	-1.5737	0.6180	0.0026
14	-0.2065	1.3159	-1.3352	-0.7529	-1.0572	0.5365	-0.0013
15	-0.4016	0.5894	-1.2859	0.1368	-1.4589	0.7709	-0.0054
16	-0.2534	1.1224	-1.1929	-0.3381	-1.3287	0.4709	-0.0012
17	0.2112	1.3995	-0.4195	0.4689	-1.3613	0.8084	0.0014
18	0.1933	0.0740	0.9722	0.2904	0.4850	-0.2196	0.0032
19	-0.2868	0.1071	-0.9317	-0.7273	0.1441	0.4156	-0.0029
20	-0.1691	-0.7097	0.1239	-0.0242	0.2988	-0.4487	0.0007
21	-0.0174	0.6961	-0.1774	-0.2080	-0.3398	0.0775	0.0030
22	0.4679	-0.0204	0.9471	0.2666	0.4612	-0.2142	0.0027
23	0.0785	0.1121	0.5657	0.0572	-0.0235	-0.4094	0.0045
24	0.3041	0.1953	1.0623	0.2624	0.4221	-0.3744	0.0020
25	-0.1849	-1.0498	0.2942	0.6632	0.3609	0.1748	-0.0046
26	-0.2023	-1.2145	0.1693	0.7012	0.4343	0.3409	-0.0066
27	0.0567	0.0258	0.0837	-0.8925	0.6396	-0.7857	0.0027
28	-0.2513	-0.9969	-0.6170	-0.6140	0.8516	-0.0177	-0.0046
29	-0.2426	-0.5437	-0.2973	-0.6483	0.2602	-0.7529	0.0029
30	-0.0142	0.9660	-0.4039	-0.9027	-0.3159	-0.3900	0.0068
31	-0.3962	-0.3506	-0.7998	0.1234	-0.4433	0.4806	-0.0045
32	-0.1638	0.0215	-0.7429	-0.3502	-0.2416	0.2513	-0.0016
33	-0.1068	-0.7112	-0.1602	-0.4000	0.6487	-0.1978	-0.0016
34	-0.0935	2.0799	-1.0971	0.5422	-2.2364	1.4504	-0.0002
35	-0.0397	-0.6880	-0.0286	-0.1188	0.8720	0.2284	-0.0043
36	-0.1374	-0.5502	0.0976	0.5926	0.2286	0.5658	-0.0053
37	0.2321	0.0676	1.0409	0.3576	0.1679	-0.6394	0.0034
38	0.3842	0.0220	0.9318	0.3260	0.4361	-0.3156	0.0007
39	0.2421	-0.6551	0.4195	0.4825	0.7443	0.3906	-0.0046
40	0.2168	0.1835	0.8407	-0.0393	0.4790	-0.4776	0.0052

Table 6. Evaluation of non-linear model for biomass of mycelium

No.	Model represents factor(s)	Mean square error	Degree of freedom	S_2^2/S_1^2	$F_{0.55}$	$F_{0.7}$	$F_{0.8}$
1	1234567	17.79	31	1.0000			
2	123456	17.85	32	1.0034			
3	12345	18.54	33	1.0418	/		
4	1234	19.98	34	1.1227	/		
5	123	23.75	35	1.3345	/	/	
6	12	24.08	36	1.3527	/	/	/
7	1	24.95	37	1.4022	/	/	/

/ indicates significance

Table 7. Linear Evaluation of non-linear model for Itaconic Acid Production

No.	Model	Mean square error	Degree of freedom	S_2^2/S_1^2	$F_{0.50}$
1	1234567	162.571	31	1.0000	
2	123456	162.625	32	1.0003	
3	12345	162.399	33	0.9989	
4	1234	162.774	34	1.0012	
5	123	163.431	35	1.0052	/
6	12	163.316	36	1.0045	/
7	1	163.710	37	1.0070	/

/ indicates significance

Table 8. F distribution

N1:N2	$F_{0.5}$	$F_{0.55}$	$F_{0.6}$	$F_{0.7}$
31-31	1.0000	1.0146	1.0961	1.1498
31-32	1.0007	1.0146	1.0961	1.1494
31-33	1.0013	1.0147	1.0960	1.1489
31-34	1.0019	1.0147	1.0960	1.1485
31-35	1.0025	1.0147	1.0960	1.1482
31-36	1.0030	1.0148	1.0959	1.1478
31-37	1.0035	1.0148	1.0959	1.1474

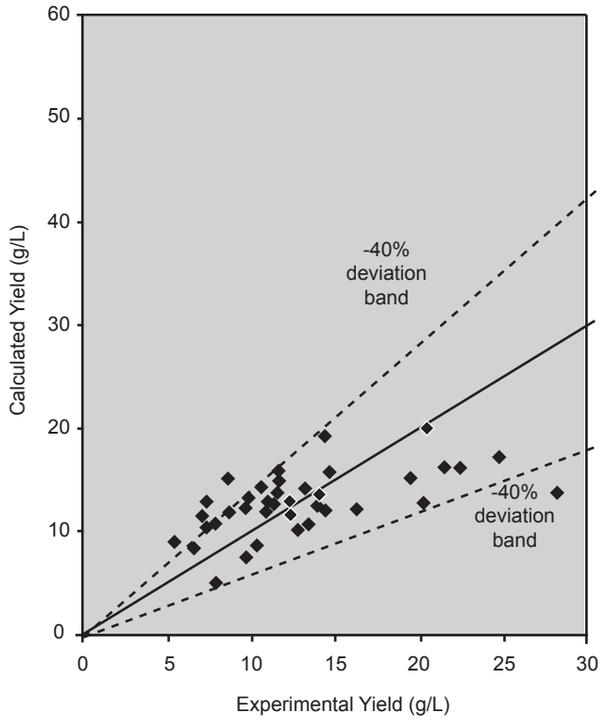

Figure 1. Performance of non-linear model involving all factors for biomass of mycelium

and itaconic acid production, respectively. In Table 6 for biomass production, by removing all factors except F_1 and F_2 does not incur error in the quadratic model at 80% confidence level compared to the model involving all factors. This indicates the model involving F_1 and F_2 have significant effect on biomass production. The variables associated with the F_1 and F_2 are filtered POME, glycerol, pH and temperature. On the other hand for the results of itaconic acid production shown in Table 7, factors involving F_1, F_2 and F_3 have significant effects on the yields since they do not incur error at 50% of confidence level. The variables associated with the F_1, F_2 and F_3 are filtered POME, glycerol, pH, temperature and NH_4NH_3. Therefore it can be suggested that the all variables except $MgSO_4$ are important and will be considered later in the optimization experiment. F- distribution and degree of freedom at various confidence levels are shown in Table 8.

Evaluation of the non-linear involving full factors model

The performance of non-linear model involving all the factors for biomass is shown in Figure 1. It is found that 86% of the data points lie within the 40% deviation. Nevertheless, only less than 50% of the data points fall within the 50% deviation band for itaconic acid production as shown in Figure 2. The performance of non-linear model in this case also involves all factors. Thus, the model for biomass is more accurate compared to the itaconic acid production. The biomass weights can be determined accurately because the harvesting time is fixed within the stationary phase in the fermentation for the whole run. While for the product formation, although the itaconic acid is known as secondary metabolite product, the exact time of the itaconic acid being secreted during the stationary phase is not known.

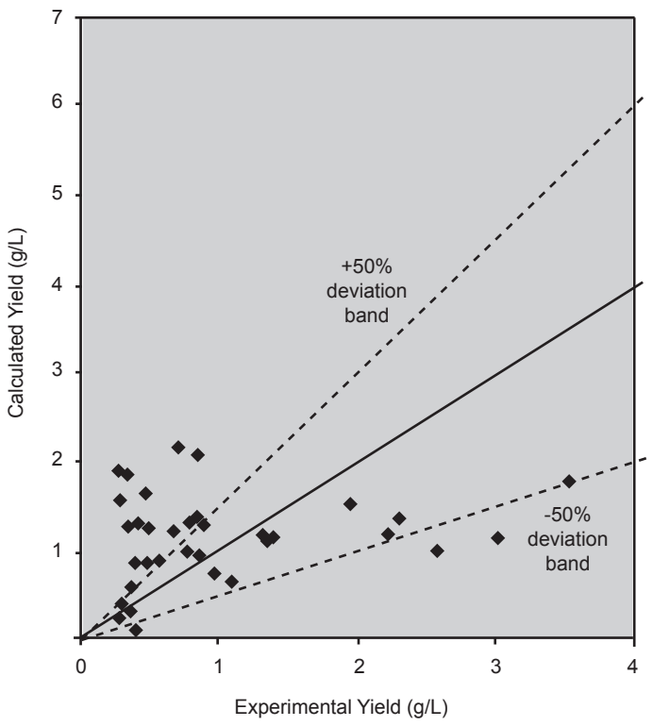

Figure 2. Performance of non-linear model involving all factors for itaconic acid production

Linear model for biomass

A simple non-linear equation is correlated for the best non-linear model of biomass formation, Y. The yield of biomass that found to be a function of amount of filtered POME and glycerol, the initial pH and temperature is given as:

$$Y = 14.78 - 28.19\ F_1^2 - 1.18\ F_2^2 \quad (1)$$

Linear model for itaconic acid production

On the other hand, model equation for the itaconic acid production, Z, as a function of the variables such as amount of filtered POME and glycerol, the initial pH, temperature, the initial amount of NH_4NH_3 and rotation speed is found to be:

$$Z = 1.628 - 4.71\ F_1^2 - 0.27\ F_2^2 - 0.23\ F_3^2 + 0.85\ F_4^2 \quad (2)$$

Itaconic acid production comparisons

It was shown from the experiments that POME was suitable to be used as an additional supplement in the fermentation medium of itaconic acid. The production of itaconic acid was greatly improved (up to 5.76 g/L) compared to the study that was done by Mutalib and Md. Jahim (2003) with the highest concentration attainable at 0.199 g/L, using the standard production medium and same strain of *A. terreus*. The advantages of utilizing POME in the fermentation as marvelous nutrient has also been proven by Hassan et al. (1997), Md. Jahim (1993), Somrutai et al. (1996), Suwandi (1991) and others. POME was even suitable to be used as culture medium for growing chironomid larvae (Habib et al. 1997).

A. terreus IMI 282743 produced smaller amounts of itaconic acid, compared to other industrial strains such as native strain of NRRL 1960 (which have produced up to 52 g/L) (Gyamerah 1995), strain RC4' (which produced up to 67 g/L) (Bonnarme et al. 1995), mutant strain TN-484 (which produced up to 51.5 g/L) (Yahiro et al. 1997b) and others. As according to Willke and Vorlop (2001), the product concentration of itaconic acid is as high as 80 g/L.

The low production of itaconic acid might be due to the application of a low production strain, which is exhibited by *A. terreus* IMI 282743. Similar finding was reported by Bonnarme et al. (1995) who tested two different strains (strain RC4' and CM85J) cultured in the same type of production medium and condition, in order to produce itaconic acid. The result showed that only the RC4'strain gave high concentration of itaconic acid (67 g/L) compared to the CM85J strain, which never produced more than 8 g/L.

CONCLUSIONS

This study had demonstrated that the use of factor analysis in identifying the significant parameters affecting the biomass and itaconic acid production. This methodology could therefore be extended to any process that might involve the effects and interactions of many possible experimental factors. The study has also shown that filtered POME as one of the good supplements that could enhance the production of itaconic acid. The utilization of filtered POME could contribute towards reducing the cost of raw material in itaconic acid production. Further investigations are recommended to be engaged in the future study to discover in more detail if the filtered POME could retain its superiority among other strains of *A. terreus*, especially on industrial strains with high yield of itaconic acid production.

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