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OPTIMIZATION OF SINGLE-CELL-BIOMASS PRODUCTION BY *YARROWIA LIPOLYTICA* USING RESPONSE SURFACE METHODOLOGY AND PULSE METHOD

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ABSTRACT

The medium for fodder yeast production from crude rapeseed oil by the strain *Yarrowia lipolytica* A-101 was optimized using both the response surface methodology and the pulse method. The 2^4 central composite experimental design was used to study the combined effect of the medium components, such as crude rapeseed oil, ammonium sulphate, magnesium sulphate and yeast extract, on the biomass concentration and yield. To maximize the concentration of the biomass the optimal values of the medium components were determined with the two methods for the independent variables. Both methods yielded similar results. Thus the optimized concentrations of the medium components amounted to 25.7; 4.2; 0.12; 0.28 and 2.1 gL^{-1} for crude rapeseed oil, ammonium sulphate, magnesium sulphate, potassium dihydrogen phosphate and yeast extract, respectively.

Key words: medium optimization, rapeseed oil, *Yarrowia lipolytica*

INTRODUCTION

In the past decade plant oil sources and waste oils have become attractive starting materials for Single-Cell-Biomass (SCB) production. Lipids can be used as an energy and carbon source by the yeast of the genera *Yarrowia*, which take up the fatty acids by either facilitated or simple diffusion [3, 4]. The substrate must therefore be well dispersed in the aqueous phase to provide a maximal contact with the yeast surface. Non-conventional substrates such as palm oil, rapeseed oil and waste fatty acids are good substrates for microbial protein production [5, 12, 14, 22]. *Y. lipolytica* turned out to be suitable for SCB production on lipid substrates. Additionally, the content of essential amino acids in this yeast complied with the FAO standards for the fodder yeast [7]. SCB production on lipid culture media at pH of about 3.5 is preferable for industrial applications. In some instances, the acidification of the production medium may cause the dispersal of fatty balls, thus minimizing the risk of bacterial contamination [3]. The medium optimization stage is one of the most important stages when yeasts are used in SCB production on a commercial scale [1, 9, 11, 17]. The optimization of the medium plays a fundamental role in preventing metabolic deviations that directly affected the yield. The response surface methodology (RSM) and pulse method (PM) are amongst the most common approaches to the optimization of the growth medium giving a maximum biomass concentration, maximum growth rate and yield. The chemostat method (PM) has been used as a powerful technique for media optimization [6, 8, 19, 23]. Compared to the batch culture techniques, a major advantage of the chemostat method is that the effect of changing one culture parameter at a time can be studied when the other ones are maintained constant. Moreover, a steady-state chemostat culture eliminates the influence of cell age, pH and variable growth rates, thus enabling the concentration of different nutrients to be kept at a level optimal for the growth of the yeast cells. This method was used to optimize the culture medium for *Pseudomonas sp.* in culture on methanol [8]. San Martin et al. [19] used this technique to develop a synthetic medium for the continuous cultivation of a *Bacillus stearothermophilus* strain under anaerobic conditions. At present, the RSM is the most widely used statistical technique for the optimization of enzymes biosynthesis [15, 16, 21] and organic acid production [2, 10, 18, 20] by various strains of bacteria, yeast and fungi.

In this study, use was made of both RSM and PM approaches to the determination of the optimal medium in order to maximize biomass production and biomass yield by the yeast strain *Y. lipolytica* A-101. The variables chosen for the purpose of the study were the concentrations of crude rapeseed oil, ammonium sulphate, potassium phosphate, magnesium sulphate and yeast extract (YE). The results obtained with the two optimization techniques were compared.

MATERIALS AND METHODS

Microorganisms. The experiments were carried out with the strain *Yarrowia lipolytica* A-101 from the Department of Biotechnology and Food Microbiology, Agricultural University of Wroclaw (Poland). The A-101 strain was selected to produce biomass from rapeseed oil and waste fatty acids at low pH (3.5), the strain was maintained on yeast-malt-agar slants. In all cultures, crude rapeseed oil was used as the carbon source.

Chemostat culture

Inoculum preparation. The growth medium contained 20 gL^{-1} of crude rapeseed oil, 6 gL^{-1} of ammonium chloride, 1 gL^{-1} of potassium dihydrogen phosphate, 1 gL^{-1} of magnesium sulphate and 1 gL^{-1} of YE in tap water. Cultivation was carried out on a shaker in 250 mL flask containing 30 mL of growth medium at 30°C and 160 rpm for 62 h. The pH was stabilized between 4.5 and 5.5 with 10 gL^{-1} of calcium carbonate. The chemostat culture was inoculated with 100 mL of inoculum. The pumping of the medium was started after sufficient growth was observed.

Culture conditions. Continuous cultures were conducted in a 3.5-L jar bioreactor BIOFLO III (New Brunswick) with a working volume of 1 L at 30°C; pH was controlled at 3.5 by addition of 5N KOH. The agitation rate was 700 rpm and aeration rate was fixed at 2 vvm. The dilution rate was 0.1 h^{-1}. The compositions of the media used in the chemostat cultures are shown in Figures 1 and 2.

Figure 1. Optimization of growth medium by the PM approach. *Y. lipolytica* A-101 cells were grown in a chemostat on a starting medium containing (gL^{-1}): 1.7 $(NH_4)_2SO_4$; 0.128 KH_2PO_4 ; 0.1 $MgSO_4 \cdot 7H_2O$ and 1.0 YE. Dilution rate, stirring rate and aeration rate was 0.1 h^{-1}, 700 rpm and 2 vvm, respectively. Numbers indicate the compounds added (gL^{-1}) to the growth vessel: 1 – $(NH_4)_2SO_4$, 3.4; 2 – $(NH_4)_2SO_4$, 6.8; 3 – $(NH_4)_2SO_4$, 13.6; 4 – KH_2PO_4, 0.256; 5 – KH_2PO_4, 0.512; 6 – KH_2PO_4, 1.024; 7 – $MgSO_4 \cdot 7H_2O$, 0.2; 8 – $MgSO_4 \cdot 7H_2O$, 0.4; 9 – YE, 2.0; 10 –YE, 4.0; 11 – YE, 8.0.

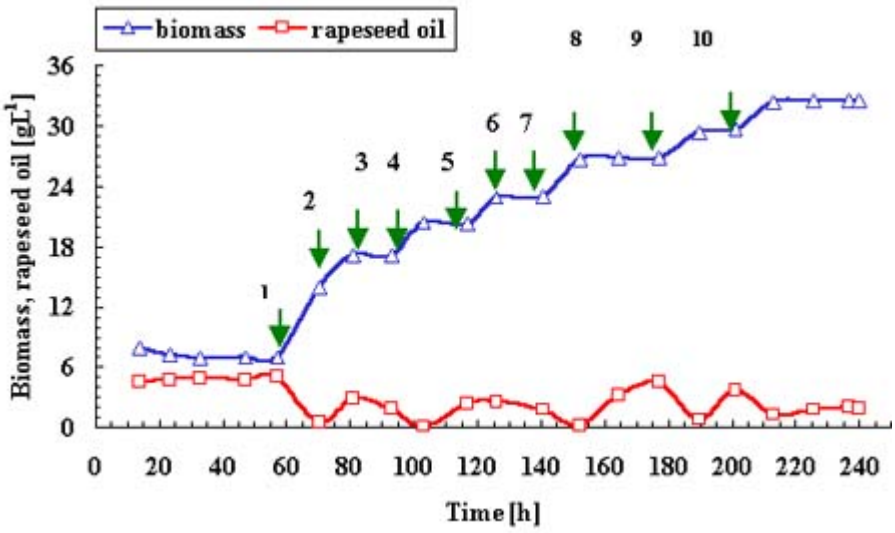
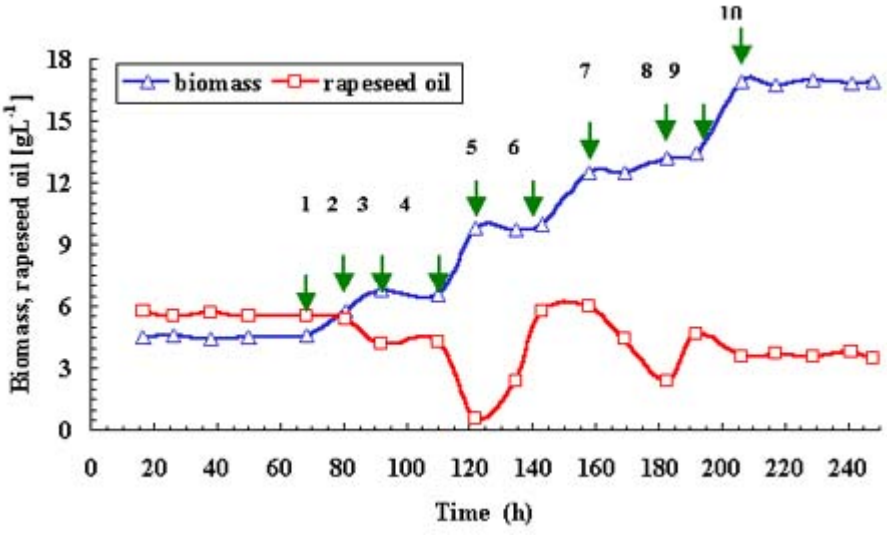


Figure 2. Optimization of growth medium by the PM approach. *Y. lipolytica* A-101 cells were grown in a chemostat on a starting medium containing (gL^{-1}): 0.85 $(NH_4)_2SO_4$; 0.064 KH_2PO_4 ; 0.05 $MgSO_4 \cdot 7H_2O$ and 0.5 YE. Dilution rate, stirring rate and aeration rate was 0.1 h^{-1}, 700 rpm and 2 vvm, respectively. Numbers indicate the compounds added (gL^{-1}) to the growth vessel: 1 – $(NH_4)_2SO_4$, 1.7; 2 – $(NH_4)_2SO_4$, 3.4; 3 – $(NH_4)_2SO_4$, 6.8; 4 – KH_2PO_4, 0.128; 5 – KH_2PO_4, 0.256; 6 – KH_2PO_4, 0.512; 7 – $MgSO_4 \cdot 7H_2O$, 0.1; 8 – $MgSO_4 \cdot 7H_2O$, 0.2; 9 – YE, 1.0; 10 –YE, 2.0; 11 –YE, 4.0.



Response surface methodology

Inoculum preparation. For inoculum preparation, the yeast was grown in 250 mL flasks containing 30 mL of the yeast-malt medium on a shaker at 30°C for 2 days. One mL of cell suspension ($3 \cdot 10^7$ cells mL^{-1}) was transferred into the production medium.

Production medium. The production medium varied, containing 2-25 gL^{-1} of crude rapeseed oil, 0.1-0.8 gL^{-1} of ammonium sulphate, 0.4-2 gL^{-1} of magnesium sulphate and 0.2-1.5 gL^{-1} of YE in 0.3 M phosphate buffer at pH 5.0 in various combinations according to the experimental design, as explained in Table 2.

Table 1. Optimal medium composition determined by the MP for biomass production by the yeast strain *Y. lipolytica* A-101 from rapeseed oil

Growth-limiting agent	Medium component	Culture I[a] max. biomass concentration x = 32.5 gL^{-1}	Culture II[b] max. biomass concentration x = 17.0 gL^{-1}	Optimal concentration of medium components per 10 g dry wt. yeast L^{-1}
N	$(NH_4)_2SO_4$	6.8	3.4	2.1
P	KH_2PO_4	0.512	0.256	0.15
Mg	$MgSO_4 \cdot 7H_2O$	0.2	0.1	0.06
YE	YE	4.0	2.0	1.2

[a] See Fig.1. [b] See Fig.2.

Table 2. Central composite rotatible design for the optimization of four nutrients (each of five levels) for biomass production by *Y.lipolytica* A-101, as well as experimental values of biomass concentration and biomass yield

C.No [a]	Coded values and real values [b]				Experimental values	
	Factor X_1	Factor X_2	Factor X_3	Factor X_4	Biomass [gL^{-1}]	Biomass yield [gg^{-1} lipids consumed]
1	-1(12.5)	-1(2.25)	-1(0.85)	-1(0.07)	11.5	0.94
2	+1(27.5)	-1(2.25)	-1(0.85)	-1(0.07)	14.7	0.74
3	-1(12.5)	+1(5.75)	-1(0.85)	-1(0.07)	12.85	1.05
4	+1(27.5)	+1(5.75)	-1(0.85)	-1(0.07)	17.3	0.87
5	-1(12.5)	-1(2.25)	+1(2.15)	-1(0.07)	12.6	0.04
6	+1(27.5)	-1(2.25)	+1(2.15)	-1(0.07)	17.9	0.83
7	-1(12.5)	+1(5.75)	+1(2.15)	-1(0.07)	12.1	0.99
8	+1(27.5)	+1(5.75)	+1(2.15)	-1(0.07)	16.8	0.84
9	-1(12.5)	-1(2.25)	-1(0.85)	+1(0.17)	12.05	1.02
10	+1(27.5)	-1(2.25)	-1(0.85)	+1(0.17)	14.2	0.72
11	-1(12.5)	+1(5.75)	-1(0.85)	+1(0.17)	12.4	1.03
12	+1(27.5)	+1(5.75)	-1(0.85)	+1(0.17)	17.2	0.93
13	-1(12.5)	-1(2.25)	+1(2.15)	+1(0.17)	13.0	1.08
14	+1(27.5)	-1(2.25)	+1(2.15)	+1(0.17)	17.45	0.91
15	-1(12.5)	+1(5.75)	+1(2.15)	+1(0.17)	12.6	1.04
16	+1(27.5)	+1(5.75)	+1(2.15)	+1(0.17)	16.85	0.94
17	+2(35.0)	0(4.00)	0(1.50)	0(0.12)	16.1	0.69
18	-2(5.0)	0(4.00)	0(1.50)	0(0.12)	5.6	1.15
19	0(20.0)	+2(7.50	0(1.50)	0(0.12)	17.1	0.93
20	0(20.0)	-2(0.50)	0(1.50)	0(0.12)	8.2	0.55
21	0(20.0)	0(4.00)	+2(2.80)	0(0.12)	14.5	0.82
22	0(20.0)	0(4.00)	-2(0.20)	0(0.12)	19.0	0.99
23	0(20.0)	0(4.00)	0(1.50)	+2(0.22)	16.0	0.88
24	0(20.0)	0(4.00)	0(1.50)	-2(0.02)	12.9	0.75
25	0(20.0)	0(4.00)	0(1.50)	0(0.12)	15.8	0.90
26	0(20.0)	0(4.00)	0(1.50)	0(0.12)	17.4	0.99
27	0(20.0)	0(4.00)	0(1.50)	0(0.12)	17.3	0.96
28	0(20.0)	0(4.00)	0(1.50)	0(0.12)	16.5	0.92
29	0(20.0)	0(4.00)	0(1.50)	0(0.12)	17.2	0.92
30	0(20.0)	0(4.00)	0(1.50)	0(0.12)	16.7	0.91
31	0(20.0)	0(4.00)	0(1.50)	0(0.12)	16.3	0.91

[a] Combination number; [b] real values (given in parentheses) are in gL^{-1} ; Factors X_1, X_2, X_3 and X_4 are rapeseed oil, ammonium sulphate, YE and magnesium sulphate, respectively.

Shake-flask experiments. Batch shake-flask cultivations were carried out in a 300 mL Erlenmayer flask with 30 mL of the production medium at 30°C for 72 h, using a rotary shaker (G10 New Brunswick) with agitation of 160 rpm.

Experimental design. The central composite rotatible experimental design was used to optimize the composition of the medium for the maximum biomass production, as well as for the maximum biomass yield. Such experimental design was chosen because rotatibility ensures a spherical distribution of variance. Additionally a nearly constant variance was required for the sphere with the radius of 1. The second-order polynomial was used to predict the response. With these assumptions for four medium components the 2^4 factorial experimental design with eight star points (a = 2) and seven replicates at the central point leading to the total number of thirty one experiments should be used. Rapeseed oil (X_1, gL^{-1}), $(NH_4)_2SO_4$ (X_2 , gL^{-1}), yeast extract (X_3, gL^{-1}), and $MgSO_4 \cdot 7H_2O$ (X_4, gL^{-1}) concentrations were chosen for the independent variables in a series of batch cultures (Table 1). For statistical calculations, the variables X_i were coded as x_i according to Equation 1:

$$x_i = (X_i - X_{ci})/\Delta X_i, i = 1, 2, 3, 4 \quad (1)$$

where x_i = dimensionless value of an independent variable, X_i = real value of an independent variable, X_{ci} = real value of an independent variable at the central point and ΔX_i = step change.

To estimate the response of the polynomial parameters, use was made of the Microsoft® Excel 2000 software. The same software was used to obtain the final form of the polynomial, which was achieved by the rejection method, using the Student-t test at the significance level alfa = 0.1.

For biomass production and biomass yield, the maximum value of the above-mentioned polynomial and the optimum concentrations of the medium components were searched, using the MATLAB software (version 4. 2c.1), in the sphere with the radius of 2, because all points of the experimental design applied are included in this area.

Analytical methods. Biomass (determined gravimetrically) was harvested by centrifugation (5000 g/5 min), washed with petroleum ether (in order to remove extracellular fat from the cell surface) and dried to a constant weight at 105°C. Unconsumed lipids were extracted from the culture medium twice, using petroleum ether as solvent. The organic phase was dried to a constant weight at 50°C.

RESULTS AND DISCUSSION

Pulse method

To use the PM in order to determine the optimal medium composition for biomass production by the yeast strain *Y. lipolytica* A-101, two chemostat cultures differing in the initial composition of the medium were grown. In one of these (culture I) the initial medium contained N, P, Mg and YE concentrations which were twice as high as in the other one (culture II). As in a previous study (unpublished data), both chemostat processes were carried out at D=0.1 h^{-1}. This value complies with the PM requirement which says that the dilution rate, D, should range between 40% and 60% of the maximal specific growth rate value for the strain under study [8]. In the PM, a necessary condition is that an excess rapeseed oil substrate be maintained throughout to make sure that it will not become a yeast growth limiting factor.

The course of culture I is depicted in Fig. 1. After 54 h of continuous cultivation, when a steady state was achieved, the addition of concentrated medium components was started. The arrows in Fig. 1 indicate the sequence and time of introducing particular components. If the addition of a component brought about an increase in the biomass level and a reduction in the lipid substrate, the substance was classified as a growth limiting agent. In the consecutive doses, the concentration of the component was increased twofold, until the biomass stopped to increase. Thus, following the addition of the first dose of the nitrogen source (1N), biomass concentration increased from 7 to 14 gL^{-1}. The next $(NH_4)_2SO_4$ dose (2N), which was increased in the medium up to 3.4 g/l, raised the biomass concentration to 17.5 g/l. The successive (and the highest) ammonium salt dose (3N) did not affect biomass growth at all. Hence, the optimal $(NH_4)_2SO_4$ concentration amounted to 6.8 gL^{-1}. The optimal concentra-tions of the medium components, which totalled 0.512 gL^{-1}, 0.2 gL^{-1} and 4.0 gL^{-1} for KH_2PO_4, $MgSO_4 \cdot 7H_2O$ and YE, respectively, were determined in the same way. Final biomass concentration in the medium with optimal concentrations of the components amounted to 32.5 gL^{-1}.

Culture II was grown with the same method in a medium where the concentrations of the components were half those for culture I. Figure 2 illustrates the course of the process. Taking into account the biomass concentration variations after successive doses of the medium components (from 1N to 11YE), the following optimal medium composition was established: $(NH_4)_2SO_4$, 3.4 gL^{-1}; KH_2PO_4, 0.256 gL^{-1}; $MgSO_4 \cdot 7H_2O$, 0.1 gL^{-1}, and YE, 2.0 gL^{-1}. With these concentrations of the medium components, the biomass concentration rose from 5 gL^{-1} at the beginning of the continuous process to 17.0 gL^{-1} by the end of cultivation.

Making use of the results obtained with the two continuous cultures, the optimal medium composition for achieving a biomass concentration of 10 g per litre was calculated (Table 1). The PM approach did not allow the determination of the optimal substrate dose because it was necessary to maintain an excess carbon source throughout the chemostat culture. The optimal concentration of this component in the production medium was determined using the experimental design method.

Experimental design method

In the experimental design method, the optimal concentrations of the carbon (rapeseed oil), nitrogen ($(NH_4)_2SO_4$) and magnesium ($MgSO_4 \cdot 7H_2O$) source, as well as those of the yeast extract were determined using the results obtained with the shake-flask cultures. The concentration ranges for the medium components under study were established on the basis of the data reported in the literature [4], where the concentrations of the particular components in the biomass produced by a variety of yeast can be found. In order to optimize the composition of the production medium by the experimental design method, 31 shake-flask cultures differing in the composition of the medium were grown (Table 2). To provide a constant pH, the medium was produced in a 0.3 M phosphate buffer. Combinations of concentrations for particular medium components were established according to the plan of a rotatible experiment for four independent variables. The concentrations and yields of the biomass for these combinations are summarized in Table 2. The final form of the polynomials approximating the experimental results, which has been derived by the rejection method, is described by Equation 2 and Equation 3 for biomass concentration and biomass yield, respectively:

$$X=A_{11}*X_1^2+A_{22}*X_2^2+A_{44}*X_4^2+A_1*X_1+A_2*X_2+A_3*X_3+A_0; \quad (2)$$

$$Y_{x/s}=A_{13}*X_1*X_3+ A_{34}*X_3*X_4+ A_2*X_2+ A_4*X_4+A_0; \quad (3)$$

where X is biomass concentration, $Y_{X/S}$ denotes biomass yield, X_1 is rapeseed oil concentration, X_2 stands for $(NH_4)_2SO_4$ concentration, X_3 indicates YE concentration and X_4 is $MgSO_4 \cdot 7H_2O$ concentration.

Figure 3 relates the biomass concentration and biomass yield to the concentrations of two production medium components; the remaining two components taking optimal concentrations values. Considering that for both optimization criteria it is possible to establish as many as 12 relations, we selected – for the needs of graphical representation – three relations for each criterion. The decoded optimal concentration values obtained with the experimental design method for the components of the production medium are gathered in Table 3.

Figure 3. Contour plots depicting the response surface (A-C) of biomass concentration (gL^{-1}) correlated to the levels of the following variables: ammonium sulphate concentration (gL^{-1}), yeast extract concentration (gL^{-1}) and rapeseed oil concentration (gL^{-1}) and (D-F) of biomass yield (gg^{-1}) correlated to the levels of the following variables: ammonium sulphate concentration (gL^{-1}), magnesium sulphate concentration (gL^{-1}) and yeast extract concentration (gL^{-1}). Dotted lines indicate the contours of the range of independent variables.

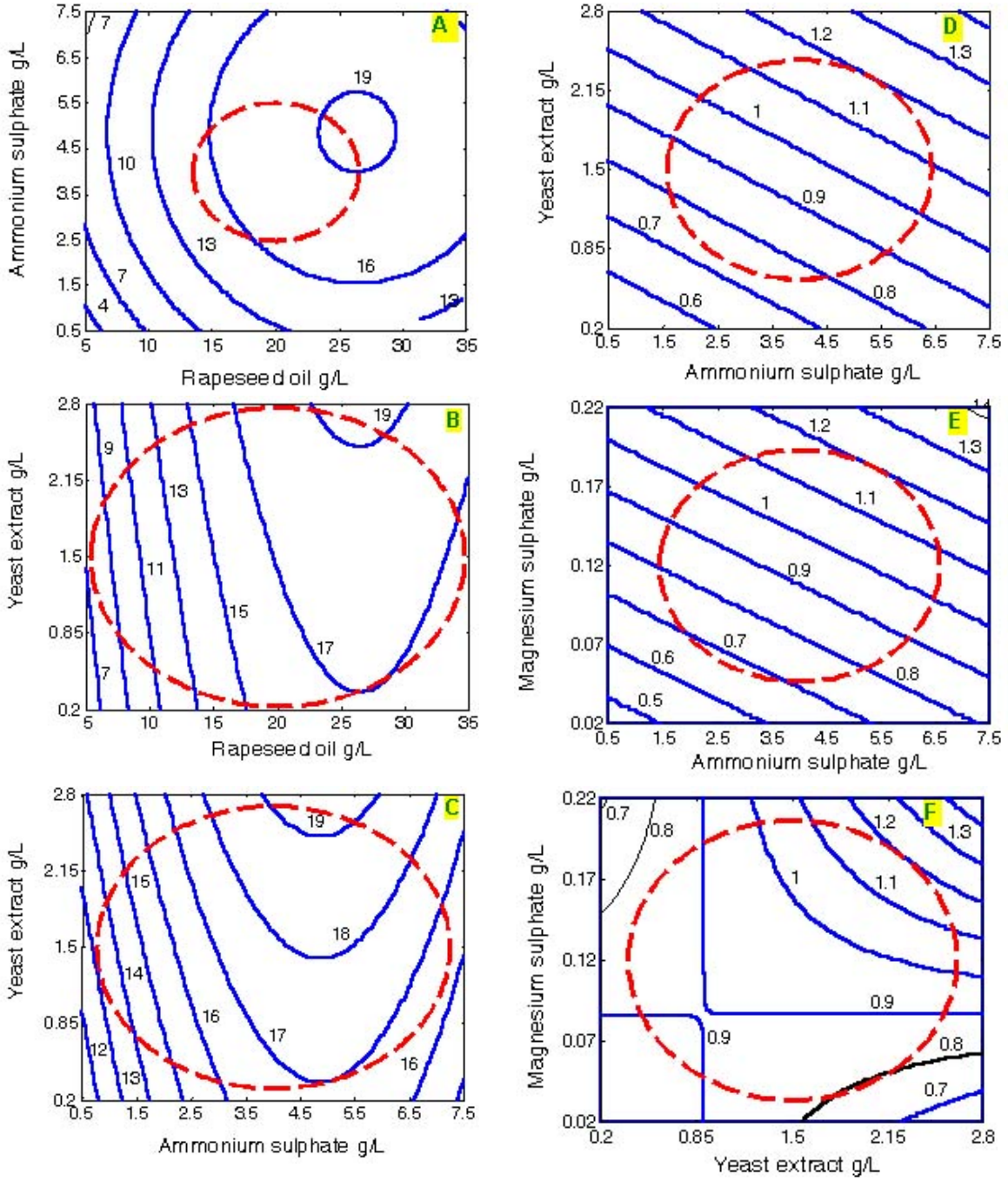


Table 3. Optimal medium composition determined by the experimental design method for biomass production by *Y. lipolytica* A-101 from rapeseed oil in terms of maximum biomass concentration (A) and biomass yield (B)

Medium component (gL^{-1})	A	B
Rapeseed oil	25.7	25.2
$(NH_4)_2SO_4$	4.73	5.30
YE	2.67	2.26
$MgSO_4 \cdot 7H_2O$	0.12	0.18

The optimal concentrations of particular medium components were similar, irrespective of whether they were established by the PM or the experimental design method (Table 4). Both methods are supplementary – only one of these (RSM) made it possible to determine the optimal concentration of the carbon source, and only one (PM) enabled the determination of the optimal concentration for the phosphorus source. The final optimal concentrations of the production medium components – calculated as averages from the two methods – for obtaining the biomass concentration of 10 gL^{-1} are included in Table 4. When calculating these averages, we decided to take into account also the results achieved from the optimization of the medium with respect to the maximization of the biomass yield. In this way, we wanted to include into the proposed optimization of the medium composition the problem of how to economize the optimized substrate.

Table 4. Optimal averaged medium composition , calculated by PM and RSM for biomass production by *Y. lipolytica* A-101 from rapeseed oil for 10 g dry wt. L^{-1}

Medium component	PM (gL^{-1})	RSM (with respect to X) (gL^{-1})	RSM (with respect to $Y_{x/s}$) (gL^{-1})	Average medium composition (gL^{-1})
Rapeseed oil	-	13.5	8.3	10.9
$(NH_4)_2SO_4$	2.1	2.5	1.75	2.1
KH_2PO_4	0.15	-	-	0.15
$MgSO_4 \cdot 7H_2O$	0.06	0.06	0.06	0.06
YE	1.2	1.4	0.74	1.1

Many investigators believe that the optimal composition of the cell production medium varies according to the optimization method applied [13, 23]. Goldberg and Er-el [8] found that the concentrations of all components of the culture medium for biomass production by the bacteria of the genus *Pseudomonas* and by the yeast of the species *Candida utilis* were noticeably higher when determined in batch cultures than when established by the PM. For example, nitrogen concentration was twice as high, amounting to 191.8 mg Ng^{-1} dry wt. yeast. Furthermore, the medium composition obtained with the PM correlated with the macroelement composition of the biomass produced by the yeast. In our own studies, N, P and Mg concentrations in the medium for biomass production by *Y. lipolytica* A-101 from rapeseed oil were markedly lower than those obtained for the yeast of the species *C. utilis*. These difference can be attributed, on one hand, to the type of the carbon source applied. In the investigations into *C. utilis*, use was made of fruit by-products containing sugars and organic acids. On the other hand, the YE used in the present study as a thiamine source is a complex component which contains also small amounts of N, P and Mg.

Summing up, it should be noted that with the MP and the experimental design method we were able to obtain an optimal medium for an effective production of fodder yeast *Y. lipolytica* from rapeseed oil. In addition, our results provide a useful tool for future investigations into the cultivation of high cell density of yeast on lipid media in semicontinuous processes.

REFERENCES

1. Adamsen A.K., Lindhagen J., Ahring B.K., 1995, Optimization of extracellular xylanase production by *Dictyoglomus* sp. B1 in continuous culture. Appl. Microbiol. Biotechnol. 44: 327-332.
2. Bohlmann J.T., Cameselle C., Nunez M.J., Lema J.M., 1998, Oxalic acid production by Aspergillus niger. Part II: Optimisation of fermentation with milk whey as carbon source. Bioprocess Eng. 19: 337-342.
3. Boze H., Moulin G., Galzy P., 1992, Production of food and fodder yeasts. Critical Rev Biotechnol. 12: 65-86.
4. Boze H., Moulin G., Galzy P., 1995, Production of microbial biomass. In Rehm H.J., Reed G., eds. Biotechnology, Vol.9, 2nd ed. Weinheim:VCH: 673-683.
5. Ercoli E., Ertola R., 1983, SCP production from olive beach water. Biotechnol. Lett. 5: 457-462.

6. Fabregas J., Dominguez A., Regueiro M., Maseda A., Otero A., 2000, Optimization of culture medium for continuous cultivation of the microalga *Haematococcus pluvialis*. Appl. Microbiol. Biotechnol. 53: 530-535.
7. FAO/WHO, 1989, Protein Quality Evaluation, Report of a Join FAO/WHO Expert Consultation, Rome, FAO.
8. Goldberg I., Er-el Z., 1981, The chemostat – an efficient technique for medium optimization. Process Biochem. 16: 2-8.
9. Hongpattarakere T., H-Kittikun A., 1995, Optimization of single-cell-protein production from cassava starch using *Schwanniomyces castellii*. World J. Microbiol. Biotechnol. 11: 607-609.
10. Kautola H., Rymowicz W., Linko Y.Y., Linko P., 1991, Itaconic acid production by immobilized *Aspergillus terreus* with varied metal additions. Appl. Microbiol Biotechnol. 35: 154-158.
11. Konlani S., Delgenes J.P., Moletta R., Traore A., Doh A., 1996, Optimisation of cell yield of *Candida krusei* SO1 and *Saccharomyces* sp. LK3G cultured in sorghum hydrolysate. Bioresource Technol. 57: 275-281.
12. Lee C., Yamakawa T., Komada T., 1993, Rapid growth of thermotolerant yeast on palm oil. World J. Microbiol. Biotechnol. 9: 187-190.
13. Milavec P., Podgornik A., Stravs R., Koloini T., 2002, Effect of experimental error on the efficiency of different optimization methods for bioprocess media optimization. Bioprocess Biosyst. Eng. 25: 69-78.
14. Montet D., Ratomahenina R., Ba A., Pina M., Graille J., Galzy P., 1983, Production of Single Cell Protein from vegetable oils. J. Ferment. Technol. 61: 417-420.
15. Murthy M.S.R.C., Swaminathan T., Rakshit S.K., Kosugi Y., 2000, Statistical optimization of lipase catalyzed hydrolisis of methyloleate by response surface methodology. Bioprocess Eng. 22: 35-39.
16. Rama Mohan Reddy P., Gopal Reddy, Seenayya G., 1999, Production of thermostable pullulanase by *Clostridium thermoslfurogenes* SV2 in solid-state fermentation: optimization of nutrients levels using response surface methodology. Bioprocess Eng. 21: 497-503.
17. Riaublanc A., Boze H., Demuynck M., Moulin G., Ratomahenina R., Graille P., 1992, Optimisation of biomass production from palm oil culture using *Candida rugosa*. Fat. Sci. Technol. 2: 41-46.
18. Rymowicz W., Kautola H., Linko Y.Y., Linko P., 1993, Studies on citric acid production with immobilized *Yarrowia lipolytica* in repeated batch and continuous air-lift bioreactors. Appl. Microbiol. Biotechnol. 39: 1-4.
19. San Martin R., Bushell D., Leak D.J., Hartley B.S., 1992, Development of a synthetic medium for continuous anaerobic growthand ethanol production with a lactate dehydrogenase mutant of *Bacillus stearothermophilus.* J. Gen. Microbiol. 138: 987-996.
20. Santoro R., Cameselle C., Rodriguez S., Sanroman A., 1999, Influence of milk whey, nitrogen and phosphorus concentration on oxalic acid production by *Aspergillus niger*. Bioprocess Eng. 20: 1-5.
21. Sunitha K., Lee J.K., Oh T.K., 1999, Optimization of medium components for phytase production by *E. coli* using response surface methodology. Bioprocess Eng. 21: 477-481.
22. Takata Y., 1992, Production of SCP from soybean oil and waste soybean oil. J. Jpn. Soc. Food Sci. Technol. 39: 253-256.
23. Zhang J., Greasham R., 1999, Chemically defined media for commercial fermentations. Appl. Microbiol.Biotechnol. 51: 407-421.

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