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ORIGINAL PAPER

K. Kiviharju · M. Leisola · T. Eerikäinen

Optimization of *Streptomyces peucetius* var. *caesius* N47 cultivation and ε-rhodomycinone production using experimental designs and response surface methods

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Abstract *Streptomyces peucetius* var. *caesius* is an aerobic bacterium that produces doxorubicin as a secondary metabolite. A mixture design was applied for the screening of suitable complex medium components in the cultivation of *S. peucetius* var. *caesius* N47, which is an ε-rhodomycinone-accumulating mutant strain. ε-Rhodomycinone is a non-glycosylated precursor of doxorubicin. Best growth results were obtained with soy peptone and beef extract. A central composite face-centered (CCF) experimental design was constructed for the investigation of pH, temperature and dissolved oxygen (DO) effects on the cultivation growth phase. Another CCF was applied to the production phase to investigate the effects of aeration, pH, temperature and stirring rate on ε-rhodomycinone production. An increase in cultivation temperature increased both cell growth and glucose consumption rate. Best ε-rhodomycinone productivities were obtained in temperatures around 30°C. DO control increased all growth phase responses, but aeration in the production phase coupled with pH decrease resulted in rapid ε-rhodomycinone decay in the medium. In non-aerated production phases a pH change resulted in better productivity than in experiments without pH change. A pH increase with a temperature decrease seemed most beneficial for productivity. This implies that dynamic control strategies in batch production of ε-rhodomycinone could increase the overall process productivity.

Keywords Central composite face-centered design · ε-Rhodomycinone · Mixture design · Optimization *Streptomyces peucetius* var. *caesius*

K. Kiviharju (✉) · M. Leisola · T. Eerikäinen
Laboratory of Bioprocess Engineering,
Helsinki University of Technology,
PL 6100, 02015 Hut, Finland
E-mail: kristiina.kiviharju@hut.fi
Tel.: +358-9-4512856
Fax: +358-9-462373

Introduction

The aerobic bacterium *Streptomyces peucetius* var. *caesius* is a producer of doxorubicin [1], which is used as an antitumor agent. Its biosynthetic route has been studied largely from the precursors malonyl-CoA and propionyl-CoA [2, 12], to aklanonic acid, aklaviketone, ε-rhodomycinone, carminomycin and doxorubicin [3, 7, 8, 11, 17]. Streptomyces antibiotic regulatory proteins have also been identified and studied [10, 16, 21]. The most important regulator proteins DnrO, DnrN and DnrI, located in two gene loci, $dnrR_1$ and $dnrR_2$, are inducers of the antibiotic biosynthesis proteins.

Central composite designs have been successfully used with other actinomycetes and other products. Glutamic acid and phosphate concentrations were optimized using a central composite circumscribed (CCC) experimental design for the production of a hybrid antibiotic with *Streptomyces lividans* TK21 [14]. Transglutaminase production with *Streptoverticillium cinnamoneum* was optimized with respect to casein and glycerol concentrations in the production medium using CCC experimental design [6]. The effects of pH and temperature on cellulase-free xylanase production of *Streptomyces* sp. Ab106 were studied using a Box-Wilson central composite design [18]. Aeration and agitation rates were optimized for the process mentioned above using a central composite face-centered (CCF) design [20]. The use of mixture designs in actinomycete process optimizations has been less popular. The effects of five different nutrient components on the cellulase-free xylanase production were studied using a mixture design [19]. Growth effects were not evaluated in this experiment.

The aim of this study was to find suitable complex medium components for the cultivation of *S. peucetius* var. *caesius* N47 and to optimize environmental conditions for an efficient ε-rhodomycinone production process. A mixture design was used in this attempt to find a production medium that contained no particulate matter

and was thus suitable for processes with on-line biomass measurements by traditional methods. Two CCF designs were constructed to investigate the effects of cultivation environment and its changes on growth, substrate consumption and ε-rhodomycinone production. The design factors for the growth phase were pH, temperature and dissolved oxygen (DO). The design factors for the production phase were aeration, pH, temperature and stirring rate. Different experimental designs were constructed in parallel to simultaneously determine the optimal conditions and possible limitations concerning a dynamic control strategy.

Materials and methods

Bacterial strain and inoculum preparation

The bacterial strain used in this study was *S. peucetius* var. *caesius* N47 (Galilaeus, Finland). Doxorubicin biosynthesis in this genetically modified strain is blocked at the glycosylation stage and thus ε-rhodomycinone accumulates. The strain was stored at −80°C in 20% glycerol. For inoculum preparation, 50 ml EP1 cultivation medium was inoculated with 100 µl frozen stock. This was incubated for 4 days in a 250 ml shake flask at 30°C with 330 rpm rotary shaking. The EP1 medium (pH 7.6) composition in g l^{-1} was: glucose 20, starch 20, Pharmamedia (Trader's Protein, Memphis, Tenn.) 5, yeast extract (LAB M, International Diagnostics Group, Bury, Lancashire, UK) 2.5, NaCl 3, KH_2PO_4 1, $CaCO_3$ 3 and $MgSO_4$ 0.49. The following trace elements were also added, in mg l^{-1}: $FeSO_4$ 1.1, $MnCl_2$ 2.2, $CuCl_2$ 0.8 and $ZnSO_4$ 3.9.

Complex medium: mixture design

A mixture design was constructed for ten complex medium components containing no particulate matter: bacto peptone (Difco, Detroit, Mich.), beef extract (Pronadisa, Hispanlab, Madrid, Spain), cotton seed extract (Sigma, St. Louis, Mo.), Pharmamedia extract (Trader's Protein), malt extract (Difco), nutrient broth (Difco), soy peptone (LAB M), tryptone (Pronadisa), yeast extract (LAB M) and YM broth (Difco.). The experimental design and evaluation of the results were carried out using Modde 4.0 (Umetri, Ume, Sweden). The design is shown in Table 1. Cultivations were performed in a Bioscreen C analyzer (Labsystems, Helsinki, Finland) using a cultivation volume of 400 µl. All experiments contained a total of 10 g l^{-1} complex medium components. Other components in g l^{-1} were: glucose 20, starch 20, NaCl 3, KH_2PO_4 1, $CaCl_2$ 3 and $MgSO_4$ 0.49. The following trace elements were also added in mg l^{-1}: $FeSO_4$ 1.1, $MnCl_2$ 2.2, $CuCl_2$ 0.8 and $ZnSO_4$ 3.9. The Bioscreen C analyzer measured culture optical densities (OD, $\lambda = 600$ nm) at 30 min intervals. Lag-phase length (Lag), maximum specific growth rate (μ) and absorbance change during growth phase (dExp) were determined and calculated from the Bioscreen results.

Environmental conditions: CCF design

A CCF experimental design was constructed to investigate the effects of temperature, pH and DO on cell growth, glucose consumption and ε-rhodomycinone

Table 1 Mixture design for complex medium optimization of *Streptomyces peucetius* var. *caesius* N47 cultivation. *SP* Soy peptone, *TR* tryptone, *CE* corn extract, *YE* yeast extract, *NB* nutrient broth, *BE* beef extract, *ME* malt extract, *BP* bacto peptone, *YM* YM medium, *FM* Pharmamedia, *Lag* lag-phase length, µ maximum specific growth rate, *dExp* absorbance change during growth phase

Factors										Responses		
SP	TR	CE	YE	NB	BE	ME	BP	YM	FM	Lag (h)	μ (h^{-1})	dExp
1	0	0	0	0	0	0	0	0	0	7.0 ± 1.7	0.0114 ± 0.0013	0.43 ± 0.06
0	1	0	0	0	0	0	0	0	0	10.4 ± 0.9	0.0091 ± 0.0019	0.45 ± 0.05
0	0	1	0	0	0	0	0	0	0	NG[a]	NG	NG
0	0	0	1	0	0	0	0	0	0	13.8 ± 2.8	0.0085 ± 0.0029	0.27 ± 0.08
0	0	0	0	1	0	0	0	0	0	7.4 ± 0.7	0.0082 ± 0.0017	0.34 ± 0.07
0	0	0	0	0	1	0	0	0	0	7.1 ± 0.9	0.0085 ± 0.0019	0.44 ± 0.08
0	0	0	0	0	0	1	0	0	0	NG	NG	NG
0	0	0	0	0	0	0	1	0	0	8.5 ± 0.8	0.0101 ± 0.0013	0.43 ± 0.05
0	0	0	0	0	0	0	0	1	0	9.1 ± 1.1	0.0087 ± 0.0034	0.26 ± 0.08
0	0	0	0	0	0	0	0	0	1	9.4 ± 2.7	0.0053 ± 0.0041	0.14 ± 0.06
0.14	0	0.14	0.14	0.02	0.14	0.14	0.14	0	0.14	8.4 ± 0.8	0.0077 ± 0.0011	0.26 ± 0.03
0.14	0.14	0	0.14	0.02	0.14	0.14	0.14	0	0.14	9.0 ± 0.5	0.0089 ± 0.0011	0.33 ± 0.03
0.14	0.14	0.14	0	0	0.14	0.14	0.14	0.02	0.14	8.1 ± 1.9	0.0112 ± 0.0067	0.40 ± 0.19
0.14	0.14	0.14	0.14	0.02	0.14	0.14	0	0	0.14	9.0 ± 2.1	0.0106 ± 0.0025	0.40 ± 0.08
0	0.14	0.14	0.14	0	0.14	0.14	0.14	0.02	0.14	9.4 ± 0.8	0.0096 ± 0.0023	0.29 ± 0.05
0.14	0.14	0.14	0.14	0	0	0.14	0.14	0.02	0.14	10.0 ± 1.9	0.0097 ± 0.0025	0.34 ± 0.12
0.14	0.14	0.14	0.14	0	0.14	0	0.14	0.02	0.14	8.1 ± 0.8	0.0095 ± 0.0010	0.40 ± 0.04
0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	8.6 ± 2.0	0.0098 ± 0.0028	0.36 ± 0.11
0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	8.9 ± 1.9	0.0099 ± 0.0042	0.39 ± 0.14
0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	8.6 ± 1.1	0.0100 ± 0.0018	0.32 ± 0.04

[a]No growth detected

formation during the growth phase. The design is shown in Table 2. Another CCF design was constructed to investigate the effects temperature, pH and stirring rate on ε-rhodomycinone production after the growth phase. This design is shown in Table 3. Both designs were constructed and their results evaluated with Modde 4.0. A temperature transformation to K^{-1} was used in both models to obtain linearity concerning temperature effects. The cultivation medium in all experiments was EP1. The growth phase experiments were carried out in Biostat MD reactors (Braun Biotech, Melsungen, Germany) with 1.0 l working volumes. After a 40–80 h growth phase the medium was transferred to two production phase reactors. The production phase experiments were performed in two parallel Biostat Q reactors with 400 ml working volumes, one with and another without aeration. Each production phase was continued for 88 h. Samples were taken every 8 h from both growth and production phases.

Sample analysis

Viability and glucose concentration were analyzed from the growth phase samples. Cell viabilities were analyzed from diluted samples as colony forming units (cfu) on nutrient agar plates incubated at 30°C for 7 days. Maximum specific growth rate (μ) was determined from the logarithmic values and used as a model response. Glucose concentrations were analyzed from cell-free samples using a YSI 2700 D Select (Yellow Springs Instrument, Yellow Springs, Ohio) enzymatic membrane analyzer. Maximum glucose consumption rate (r_G) was determined from the measurement results. The natural logarithm of this was used as a model response.

Production phase samples were extracted with dichloromethane (DCM). A 500 µl sample was thoroughly mixed with 200 µl phosphate buffer (pH 7) and 200 µl methanol. ε-Rhodomycinone was extracted twice from this solution by addition, mixing and separation of 500 µl DCM. The DCM was evaporated at room temperature from the samples, which were then dissolved in 1 ml 3.8% trifluoroacetic acid (TFA) and 24% acetonitrile (ACN) and analyzed with high performance liquid chromatography (HPLC; Waters, Milford, Mass.). An XTerra RP_{18} column (Waters) was used at 30°C with a UV detector ($\lambda = 254$ nm). A 16 min linear elution gradient was applied from 76% to 1% 0.05% TFA and from 24% to 99% ACN. Average ε-rhodomycinone productivity (r_e) was calculated from the growth phase and its natural logarithm used as a growth phase model response. Average ε-rhodomycinone productivities were also calculated from the production phase in the aerated (p_+) and non-aerated (p_-) bioreactor. These were used as production phase model responses.

Table 2 Central composite face-centered (CCF) design for the optimization of *S. peucetius* var. *caesius* growth phase. The design factors are pH, temperature (*T*) and dissolved oxygen (*DO*). The design responses are maximum specific growth rate, μ, natural logarithms of maximum glucose consumption rate, $\ln(r_G)$, and average product formation rate, ($\ln r_e$)

Factors			Responses		
pH	T(°C)	DO (%)	μ (h^{-1})	$\ln(r_G)$ ($g\ l^{-1}\ h^{-1}$)	$\ln(r_e)$ ($g\ l^{-1}\ h^{-1}$)
6	25	0	0.046	−1.17	−2.10
8	25	0	0.030	−1.66	−1.84
6	35	0	0.058	−0.53	−3.42
8	35	0	0.101	−0.25	−1.24
6	25	30	0.023	−0.73	−0.97
8	25	30	0.031	−0.60	−0.15
6	35	30	0.123	−0.67	−1.91
8	35	30	0.079	−0.01	−0.64
6	30	15	0.038	−0.78	0.42
8	30	15	0.102	−0.40	−0.51
7	25	15	0.033	−1.24	−1.77
7	35	15	0.100	−0.17	−0.93
7	30	0	0.055	−0.84	0.13
7	30	0	0.052	−0.64	−0.69
7	30	15	0.051	−0.58	−0.44
7	30	15	0.099	−0.60	−2.01
7	30	15	0.063	−0.89	0.25

Table 3 CCF design for the optimization of *S. peucetius* var. *caesius* production phase. The design factors are pH, temperature (*T*) and stirring rate (*Stir*). The design responses are productivities in non-aerated (p_-) and aerated cultivation (p_+)

Factors			Responses	
pH	T (°C)	Stir (rpm)	p_-($mg\ l^{-1}\ h^{-1}$)	p_+ ($mg\ l^{-1}\ h^{-1}$)
6	25	100	−0.346	−0.341
8	25	100	−0.034	−0.051
6	35	100	0.012	−0.245
8	35	100	0.093	−0.024
6	25	400	0.023	−0.869
8	25	400	0.122	−0.238
6	35	400		−0.635
8	35	400	−0.052	−0.149
6	30	250	0.329	0.181
8	30	250	0.011	−0.139
8	25	250	0.012	−0.006
7	35	250	0.034	−0.082
7	30	100	−0.162	−0.156
7	30	400	0.184	−0.305
7	30	250	−0.07	−0.122
7	30	250	−0.073	−0.099
7	30	250	0.003	−0.013

Results

Mixture design

Ten replicates of each Bioscreen cultivation experiment were carried out. The cultivation responses Lag, μ, and dExp, with standard deviations from the repeated experiments are shown in Table 1. The models for each response are shown in Table 4. Model statistics are shown in Table 5. The coefficient of determination (R^2) and probability to 0-hypothesis (*P*) were satisfactory for Lag and dExp. Figure 1 shows the normalized effects of

Table 4 Mixture design model for Lag, μ and dExp

	Lag	μ	dExp
ME	5.15	−0.007	−0.303
CE	5.01	−0.007	−0.292
TR	0.087	2.8E-03	0.209
BE	−3.75	1.9E-03	0.185
SP	−3.77	0.005	0.182
BP	−2.08	3.5E-03	0.165
FM	−1.24	−1.4E-03	−0.140
NB	−2.56	3.4E-04	0.045
YM	−0.695	1.0E-03	−0.041
YE	3.84	1.6E-03	−0.009

Table 5 Analysis of variance (ANOVA) for the mixture design, growth and production phase CCF design models. Mixture design responses are Lag, μ, and dExp. Growth phase CCF responses are μ, $\ln(r_G)$, and $\ln(r_e)$. Production phase CCF responses are the production phase productivities in the non-aerated (p_-) and aerated reactor (p_+) as well as the modified model productivities in the non-aerated (p'_-) and aerated reactor (p'_+). The parameters shown are coefficient of determination (R^2), coefficient of model prediction (Q^2), F and P values for model (F, P) and lack of fit (F_L, P_L)

	Response	R^2	Q^2	F	P	F_L	P_L
Mixture design	Lag	0.846	–	6.11	0.0046	67.2	0.015
	μ	0.713	–	2.76	0.0650	–	–
	dExp	0.851	–	6.36	0.0039	4.71	0.187
Growth phase CCF	μ	0.657	0.374	3.7	0.0330	1.18	0.493
	$\ln(r_G)$	0.914	0.709	17.76	0.0001	0.73	0.673
	$\ln(r_e)$	0.559	0.196	2.78	0.0730	0.53	0.788
Production phase CCF	p_+	0.673	0.308	3.43	0.0420	11.88	0.080
	p_-	0.575	0.221	2.03	0.1640	10.45	0.090
	p'_+	0.635	0.055	4.18	0.0200	8.12	0.115
	p'_-	0.822	0.212	5.26	0.0160	0.27	0.913

the complex medium components on the responses. The effects lengthening Lag the most were corn extract, malt extract and yeast extract. The biggest stimulating effects on Lag were found with soy peptone and beef extract. Strongest positive effects on μ were obtained with soy peptone, bacto peptone and tryptone. Significant negative effects were obtained with corn extract and malt extract. The dExp effects were best with soy peptone, tryptone, beef extract and bacto peptone. Significant negative effects were obtained with corn extract and malt extract.

CCF designs

Representative glucose consumption graphs in the CCF experiments at temperatures 25, 30 and 35°C are shown in Fig. 2. The growth phase responses μ, $\ln(r_G)$ and $\ln(r_e)$ are shown in Table 2. Model statistics are shown in Table 5. The coefficient of model prediction (Q^2), R^2 and P suggest that the model for $\ln(r_G)$ was very significant and almost significant for μ. For $\ln(r_e)$ the model was more significant than the lack of fit. The model for μ was

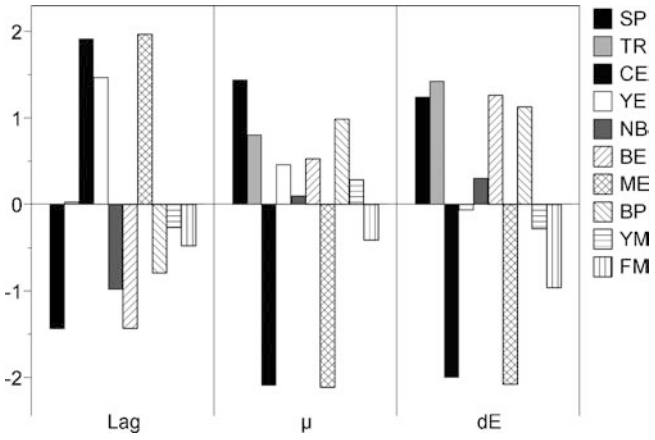

Fig. 1 Normalized effects on lag-phase length (*Lag*), maximum specific growth rate (μ) and absorbance change during growth phase (*dE*) in the mixture design experiments with *Streptomyces peucetius* var. *caesius*. The factors are soy peptone (*SP*), tryptone (*TR*), corn extract (*CE*), yeast extract (*YE*), nutrient broth (*NB*), beef extract (*BE*), malt extract (*ME*), bacto peptone (*BP*), YM broth (*YM*) and Pharmamedia extract (*FM*)

$$\mu = 0.064 + 2.4 \times 10^{-3}\mathrm{pH} - 0.023\mathrm{T}^{-1} + 2.6 \times 10^{-3}\mathrm{DO} - 1.8 \times 10^{-3}\mathrm{pH} \cdot \mathrm{DO} - 0.005\mathrm{T}^{-1} \cdot \mathrm{DO}$$

The most significant interacting factors on μ were temperature and DO. The response surfaces with constant pH values are shown in Fig. 3. At all pH values both high temperature and DO values yielded the biggest μ values. The model for $\ln(r_G)$ was

$$\ln(r_G) = -0.671 + 0.097\mathrm{pH} + 0.377\mathrm{T}^{-1} + 0.173\mathrm{DO} + 0.162\mathrm{pH} \cdot \mathrm{T}^{-1} + 0.126\mathrm{pH} \cdot \mathrm{DO} + 0.176\mathrm{T}^{-1} \cdot \mathrm{DO}$$

The most significant interactions on $\ln(r_G)$ were temperature and pH, and temperature and DO. The response surfaces for the latter with constant DO and pH values are shown in Fig. 4. All response surfaces indicate that a high value for all factors yields the biggest $\ln(r_G)$ values. The model for $\ln(r_e)$ was

$$\ln(r_e) = -0.350 + 0.316\mathrm{pH} + 0.107\mathrm{T}^{-1} + 0.331\mathrm{DO} - 0.233\mathrm{pH} \cdot \mathrm{T}^{-1} - 0.741\mathrm{T}^{-2}$$

The most significant interacting factors on $\ln(r_e)$ were temperature and pH. The response surfaces for this with constant DO values are shown in Fig. 5. The best values were obtained with DO 30% and temperatures around 30°C.

The production phase responses are shown in Table 3. The p_+ showed a product decay rate rather than a product formation rate. Model statistics for p_+ and p_- are shown in Table 5. The R^2, Q^2 and P values for the model and lack of fit imply that the model is a bad representation of the data. The results were also evaluated using a modified CCF design with pH and temperature changes and stirring rate as design factors. The statistics for the modified model are also shown in

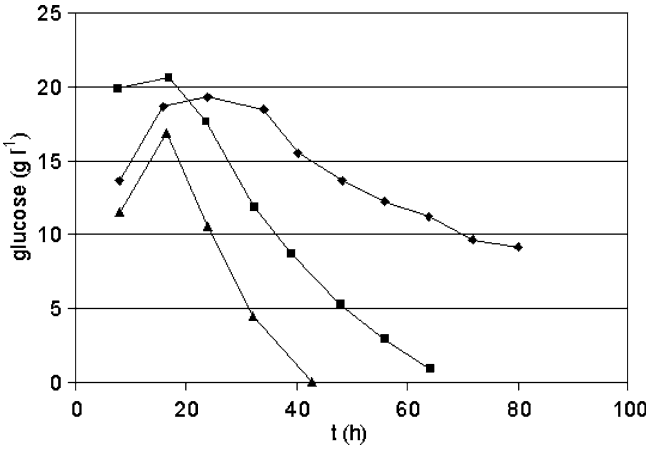

Fig. 2 Glucose concentrations in cultures of *S. peucetius* var. *caesius* N47 grown at 25 (*diamonds*), 30 (*squares*) and 35°C (*triangles*)

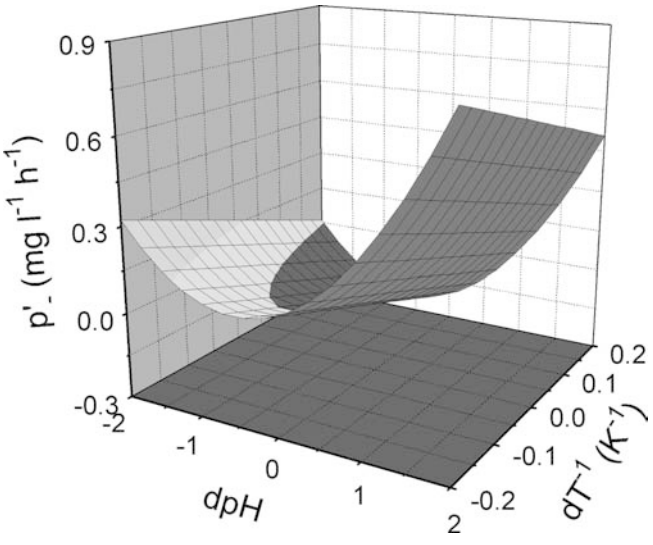

Fig. 6 Response surface for p'_- with stirring rate 400 rpm. The factors are pH change (*dpH*) and temperature change (dT^{-1}) between growth and production phase

Table 5. The R^2 and Q^2 values are better for p'_- although still not good. The *P*-value, on the other hand, shows that the model for both responses is more significant than its lack of fit. The model for p'_- was

$$p'_- = -0.12 + 0.079\mathrm{dpH} + 0.014\mathrm{dT}^{-1} + 0.131\mathrm{stir} + 0.169\mathrm{dpH}\cdot\mathrm{stir} - 0.17\mathrm{dT}^{-1}\cdot\mathrm{stir} + 0.461\mathrm{dpH}^2 - 0.058\mathrm{stir}^2$$

and the model for p'_+

$$p'_+ = -0.113 + 0.194\mathrm{dpH} + 0.047\mathrm{dT}^{-1} - 0.106\mathrm{stir} - 0.159\mathrm{dpH}\cdot\mathrm{dT}^{-1} - 0.193\mathrm{stir}^2$$

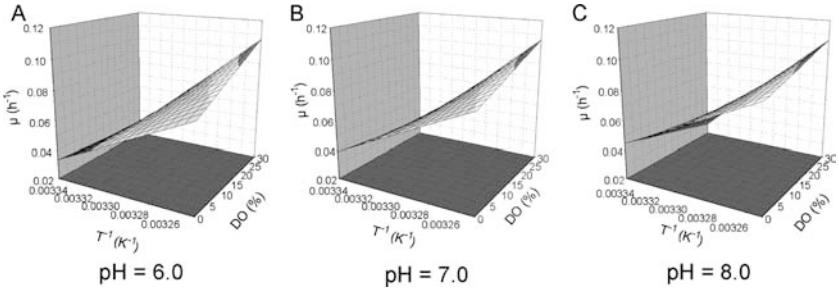

Fig. 3 Response surfaces for μ in the central composite face-centered (CCF) experiments. The surfaces show temperature (T^{-1}) and dissolved oxygen (*DO*) effects at three constant pH values

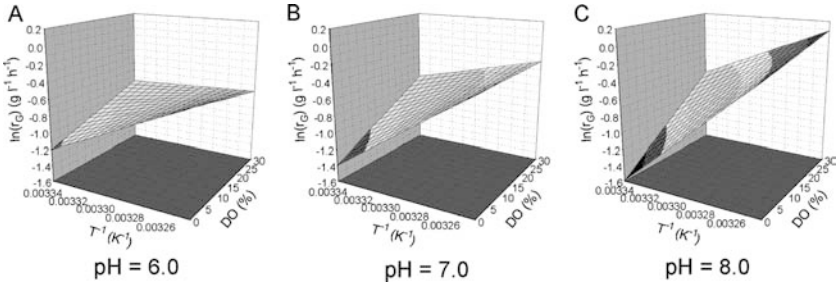

Fig. 4 Response surfaces for $\ln(r_G)$ in the CCF experiments. The surfaces show temperature (T^{-1}) and DO effects at three constant pH values

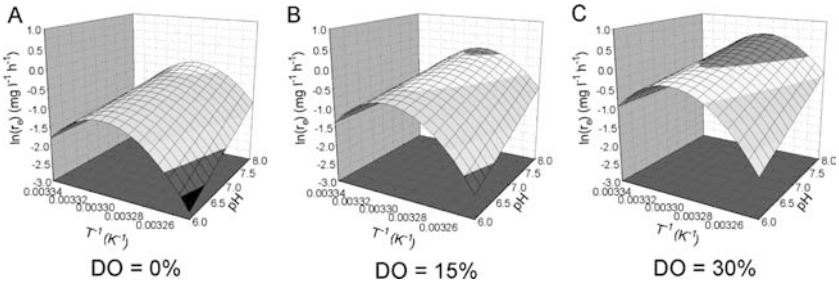

Fig. 5 Response surfaces for $\ln(r_e)$ in the CCF experiments. The surfaces show temperature (T^{-1}) and pH effects at three constant DO values

Better productivities were obtained in the non-aerated production phase and the response surface for pH and temperature change with stirring rate 400 rpm is shown in Fig. 6. Highest productivities were obtained with biggest positive changes in pH and decreasing temperatures.

Discussion

A clear complex medium is not always advantageous for the production of anthracyclines with actinomycetes [9]. It is, however, a requirement for efficient monitoring of cultivations with traditional methods e.g., optical density or cell dry weight measurements. The goal in the mixture design experiments was to rapidly determine the complex medium components for successful cultivation of the organism. ε-Rhodomycinone production with components beneficial for growth was not investigated. The best overall results in the mixture design were obtained with soy peptone and beef extract. Their effects on growth and ε-rhodomycinone production should be compared to the behavior with EP1 prior to application in further optimization or production cultivations.

The metabolic activities of cells are clearly temperature dependent. This was shown in the growth phase experiments, as an increase in temperature increased both μ and $\ln(r_G)$. Glucose repression of anthracycline formation in *S. peucetius* var. *caesius* has been reported [4, 15] as well as a carbon repression on nystatin production by *S. noursei* [5]. The cultivation experiments at temperatures below 30°C showed a glucose consumption behavior similar to the reported repression cultivations [4]; glucose consumption in the 25°C cultivations ceased at around 8 g l^{-1}, while in the higher temperature cultivations all glucose was consumed (Fig. 2). DO control increased all growth phase responses, μ, $\ln(r_G)$ and $\ln(r_e)$. It has been reported that DO control can have a positive effect on antibiotic production by streptomycetes [13], which is supported by our results in the growth phase experiments (Fig. 5); the average production level increased when the DO was increased.

The production phase results were rather complicated to interpret, as the start point differed in medium composition (substrate and metabolite amounts) and viable cell counts. Aerated production phase conditions seemed to destroy the ε-rhodomycinone faster than the non-aerated production conditions. Increasing the stirring rate increases the oxygen transfer to cultivation medium, and this can increase metabolic activities in the cells, aiding product decay. The modified model showed that aeration in the production phase coupled with a pH decrease resulted in a rapid ε-rhodomycinone decay in the medium. In non-aerated production phases a pH change resulted in better productivity than in experiments without pH change. A pH increase with a temperature decrease seemed most beneficial for p'_- (Fig. 6). This implies that dynamic control strategies in batch production of ε-rhodomycinone can increase the overall process productivity. A suitable control strategy could be an initial 40 h batch phase at pH 6.5, temperature 33°C and DO control at 30%, and a subsequent 20 h dynamic phase with pH increase to 7.5, temperature decrease to 30°C and an aeration decrease to zero. The production can be completed with a batch phase under these constant environmental conditions. The effects of environmental changes on the metabolism can be further studied using dynamic phase chemostat cultivations.

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