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Optimization of parameters growth conditions of yeast biomass during single cell protein production by using simplex method

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The aim of this study is to find optima of operating conditions, such as temperature and pH, during yeast's cultivation by using simplex method. The idea behind applying simplex method was the interaction between these two variables. Yeast (*Saccaromyces cerevisiae)* was cultivated at different pH and temperatures ranges in complex medium of limiting glucose. Other parameters such as air flow rate and stirring speed were held constant as they have their own effect. Air flow rate was held constant at 1.5 L/min. while stirring speed was 600 rpm within bioreactor of 750 mL working volume. *Saccaromyces cerevisiae* was cultivated in a batch mode.

Optima of temperature of (27.7 ^{0}C) and pH of (4.0) was obtained within only 28 runs by using simplex method. The range of pH was (3.0-7.0) and the range of temperature was (26.4-32 ^{0}C) with an interval increment of 0.2 for both variables. By applying fully factorial design one needs to run as much 540 experiments within a bioreactor in order to cover the range of pH and temperature mentioned above. These results showed the effective of simplex method in optimization of parameters. Also the study shows a big economic aspect by reducing number of required experiments to obtain optima of pH and temperature. This means reducing the cost of energy, medium and time as well.

1. Introduction

Many of earlier researches were done in order to find optima by using different approaches such as one variable-at-a time or factorial methods. Temperature and pH were subjected to be optimized by using these methods. Many results were published with concerning that these two parameters are independent of each other.

But if there is an interaction between variables, one variable-at-a time doesn't work while factorial method needs numerous of runs. Simplex method has two major advantages over classical factorial. First, the number of experiments in the initial simplex design increases arithmetically, whereas the number of experiments in the initial factorial design increases geometrically. The second major advantage is that the simplex requires only one new experiment to move into an adjacent region of factor space whereas the factorial design requires one-half the number of experiments in the

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factorial design. Also simplex method can handle many variables at the same time [(Rao S., 2009), (Nelder J. et al, 1965), (Carpenter B. et al, 1965), (Umeda T. et al, 1971)]. The aim of this study is to apply simplex method over other methods in order to find the optima of operating conditions, such as temperature and pH. This optimization process was executed in order to get high biomass of yeast, *Saccaromyces cerevisiae*, during single cell protein production. The reason of applying simplex method was the interaction between these two variables (i.e. Temperature and pH) which made applying of other methods like factorial difficult.

2. Material and Methods

2.1 Growth medium and cultivation

Saccaromyces cerevisiae (DSM No 70451), which was obtained from German bank cells, was cultivated in complex medium of limiting glucose. The constituents of this medium were 0.25g glucose, 2.5g $(NH_4)_2SO_4$, 0.5g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.02 g $CaCL_2$.6H2O, 4 mg $FeSO_4.6H_2O$, 2 mg $ZnSO_4.7H_2O$, 0.3 mg $CuSO_4.5H_2O$, 1 mg Pyridoxine, 4.4 mg Thiamine, 0.5 mg Calcium pantothenate, 20 mg *myo*-Inositol, 0.03 mg D-Biotin, 0.75 g L-Glutamine , 0.25 g Hefeextrakt (Difco). Medium was prepared by dissolving these materials in 1L bidest water. Medium was sterilized by filtration within sterile cellulose acetate filter of (0.2 µm) within a safety cabinet. A 1L sterile bioreactor (New Brunswick) was used. The working volume was 750 mL. The bioreactor was controlled automatically to regulate pH and temperature during cultivation and to maintain other parameters such as stirring speed and air flow rate constant. In order to maintain a constant temperature, a cooling water system and a heat exchanger jacket were used. The cooling water was running through heat exchanger loop inside the fermenter for cooling while the heat exchanger jacket heated up the fermenter according to the indicated temperature. By using 1M NaOH and 8.5 % phosphoric acid, the pH was regulated automatically.

A 1 mL of thawed cell suspension was added to 35 mL of complex growth medium within sterile Erlenmeyer flask. The flask was closed with cotton wool plugs and put in the incubator .The temperature and the shaking speed was regulated at constant values of 30 ^{0}C and 120 rpm respectively. An adaptation step at each 24 hours of cultivation *Saccharomyces cerevisiae* in complex growth medium for three days was done. Pre-culture was held constant with an initial absorption unit of 0.15 each time. 30 mL of this *Saccharomyces cerevisiae* adapted pre-culture was added to the growth medium in fermenter. The initial biomass (in term of absorption) was kept constant of (0.15 absorption unit).The operating conditions such as temperature, pH and stirring speed were determined according to each experiment. These parameters were controlled automatically while air flow rate was regulated within a rotameter manually.

In order to follow the growth curve, samples from growth culture were withdrawn and the absorptions of samples were measured. The net obtained absorption was calculated by subtracting initial absorption value from maximum obtained absorption at stationary phase. The net obtained absorption was used because the absorption can be measured much easier than biomass and in order to make good comparison between results. However absorption can be converted in biomass by using a calibration curve.

2.2 Simplex reflection move

Worst point in the simplex is found first at each iteration. Then, a new simplex is formed from the old simplex. The obtained new point replaces the worst point in the simplex and the algorithm continues with the new simplex. Movement through simplex method starts by rejection the worst response vertex. A new vertex will be created on the opposite side of simplex by projecting it through the average of the remaining vertexes as can be shown in Figure 1. The new simplex is not a simple mirror image of the old simplex. In effect, the point-through-point reflection has turned the old simplex inside out to make the new simplex. This new vertex corresponds to a new set of experimental conditions that can then be evaluated (Frederick, 1991), (Deb, 2005).

3. Results and discussion

Simplex method process was started by making a k+1 geometric figure in a k-dimensional space. That means running one more experiment than the number of variables to be optimized. With two variables the first simplex design is based on three trials. A random start points were chosen to cover a boundary of different range of pH and temperature. The start points can be shown in Figure 2 while the obtained net absorption of these points can be shown in Table 1.

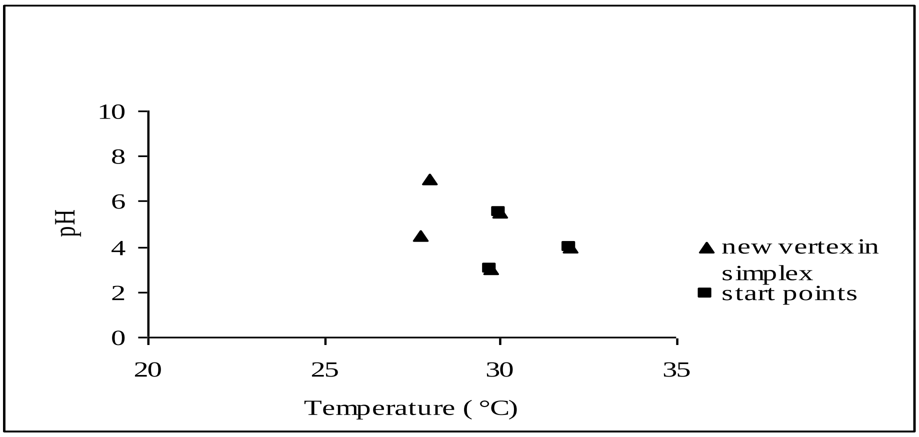

Figure 2: First starting points within simplex path way reflection

Table 1 Net obtained absorption for the first starting points

T (^{0}C)	pH	Net obtained absorption
32	4	0.2378
30	5.5	0.33
29.7	3	0.2588
27.7	4.5	0.371
28	7	0.162

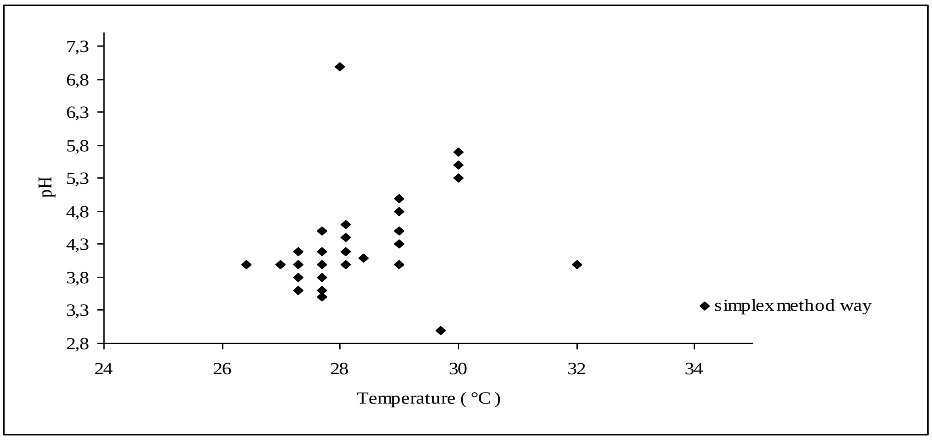

Figure 3: Simplex method path way of optimization temperature and pH parameters

The results show that net obtained absorption at (T= 32 ^{0}C and pH of 4) was the worse. Therefore, new point of (T= 27.7 ^{0}C and pH of 4.5) was tested. This point was chosen according to simplex reflection move. A simplex path way of this work can be shown in Figure3.

The results indicated optima of temperature of (27.7^{0}C) and pH of (4.0) as shown in Figures (4 and 5). These optima were found within only 28 runs by using simplex method. Some of these experiments were done in order to ensure the optima. The obtained results for these runs can be found in Table 2. Figures which are represented the individual results of Table 2 are not shown.

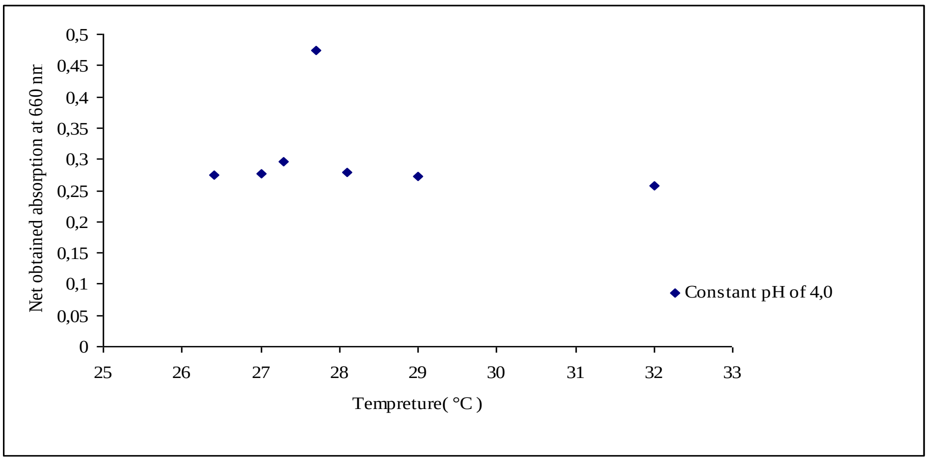

Figure 4: Optima of temperature at constant pH of 4.0. Values were taken from Table 2.

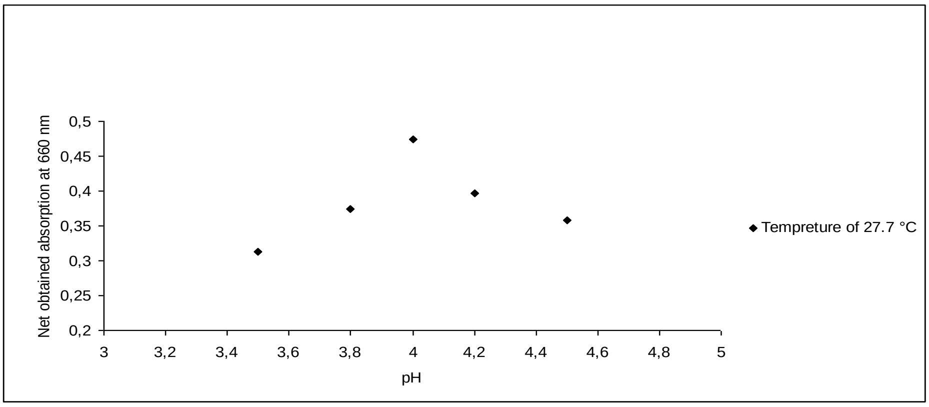

Figure 5: Optima of pH at constant temperature 27.7 ^{0}C. Extracted values from Tab 2.

The net obtained absorption for many experiments at different pH and temperature were nearly the same, had slightly difference as can be shown clearly through the runs in Table 2 such as{(2,10,11,24) , (4, 9, 12,16) , (7,14,21,19) , (3,17) , (5, 6, 26) , (13,18, 22 ,28) , (15,25) or (20,26) }. These results will certain the idea of temperature effect on pH of cells. That leads to prove the idea of the existence of interaction between temperature and pH effects on growth of yeast. As self-evident, pH of any solution changes when its temperature changes. That can happen for pH inside cells too. This pH change will stress the cells sometimes causing consuming more energy leading to lower biomass. Therefore, temperature and pH has a combine effect on cell growth.

Table 2 Results of simplex methods runs of Sacharomyces cerevisiae cultivation at constant air flow rate of 1.5 L/L.min and constant stirring speed of 600 rpm within 750 mL working volume bioreactor

Run No.	Temperature ^{0}C	pH	Net Absorption
1	27.7	4	0.4747
2	27.7	4.5	0.3716
3	29	4.5	0.3578
4	26.4	4	0.2738
5	27.7	3.5	0.3136
6	29	5	0.3180
7	29	4.3	0.2923
8	28.4	4.1	0.3032
9	29	4	0.2722
10	29	4.8	0.3700
11	27.7	3.8	0.3740
12	27	4	0.2770
13	27.3	4.2	0.2540
14	27.3	4	0.2960
15	27.7	4.2	0.3960

Table 2 (Continued)

Run No.	Temperature ^{0}C	pH	Net Absorption
16	28.1	4	0.2800
17	30	5.5	0.3460
18	30	5.7	0.2600
19	30	5.3	0.3050
20	27.3	3.8	0.3117
21	27.3	3.6	0.2980
22	29.7	3	0.2588
23	28	7	0.1620
24	28.1	4.2	0.3760
25	28.1	4.4	0.3900
26	28.1	4.6	0.3100
27	27.7	3.6	0.3200
28	32	4	0.2584

The range of pH was (3.0-7.0) and the range of temperature was (26.4-32 ^{0}C) with an interval increment of 0.2 for both variables. The number of intervals of pH range was 20 while for temperature range were 27 as the interval increment was 0.2 for both parameters. Theoretically, number of required experiments will be 540 runs by applying fully factorial design. This work shows the effective of simplex method as the number of experiments was reduced to 28 runs instead of 540. These numbers of runs were sufficient to determine optima of such interaction parameters i.e. (temperature and pH) at wide ranges. An economic aspect can be considered for this study. The cost of operating of runs was lowered dramatically to 5 % of the cost when a factorial method is applied. The lowering in cost was due to lowering in the required runs number. This cost of operating includes cost of energy, medium and time as well.

Acknowledgements

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