Int. J. Environ. Sci. Technol. (2014) 11:597–604
DOI 10.1007/s13762-013-0373-2

ORIGINAL PAPER

Improving microbial oil production with standard and native oleaginous yeasts by using Taguchi design

M. Enshaeieh · I. Nahvi · M. Madani

Received: 7 October 2012 / Revised: 30 April 2013 / Accepted: 28 August 2013 / Published online: 25 September 2013

Abstract Production of microbial oil has attracted a great attention in recent years. The potential of lipid production by the yeast strains is the reason for using microorganisms for biodiesel production. Microbial lipid has high similarity to the oil obtained from plants and animals in type and composition. Production of oil from yeasts must be economical, so optimization of the cultivation condition to reach higher production must be done. Native oleaginous yeast, *Cryptococcus albidus*, was isolated from soil by the nitrogen-limited medium and screened by Nile red staining. *Yarrowia lipolytica* DSM 8218 was used for lipid production as a standard strain. *C. albidus* was an excellent oleaginous yeast, and the lipid quantity, dry biomass and lipid productivity of this strain were 11.81 g/l, 19.65 g/l and 60.1 %, respectively, in shaking flask cultivation at 150 rpm and 25 °C in nitrogen-limited medium containing per liter 75 g glucose, 1 g $(NH_4)_2SO_4$, 1 g yeast extract, 3 g KH_2PO_4, 1.5 g $MgSO_4.7H_2O$, 0.15 g $CaCl_2$, 0.06 g $MnSO_4.H_2O$, 0.02 g $ZnSO_4.7H_2O$ and 0.15 g $FeCl_3.6H_2O$ with pH adjusted to 6.5. Fourier transform infrared spectroscopy was used for analyzing and confirming the production of microbial oil in this study.

Keywords Biodiesel · *Cryptococcus albidus* · Microbial oil · Oleaginous yeast

M. Enshaeieh (✉) · M. Madani
Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran
e-mail: m_enshaeieh@yahoo.com

I. Nahvi
Department of Biology, Esfahan University, Esfahan, Iran

Introduction

Single cell oil (SCO) is a kind of lipid that produced oleaginous microorganisms as the supplementary source of conventional oil and fat. The economic values of these bioprocesses have become favorable when zero or negative value waste substrates are utilized as carbon or nitrogen sources (Kyle et al. 1992; Ratledge 1993; Boswell et al. 1996; Pan et al. 2009; Kraisintu et al. 2010). Glucose is the most common substrate used in lipid production process, because most oleaginous microorganisms are capable of assimilating glucose for producing SCO. Also, it is the most suitable substrate for SCO production and the best choice for the evaluation of lipid production in oleaginous microorganisms (Fakas et al. 2008).

The widespread use of fossil fuels such as petroleum, coal and natural gas, which is related to the high energy demand of today's industrialized world, raises the pollution problem; so it is necessary to develop renewable energy sources such as biodiesel (Dai et al. 2007; Karatay and Donmez 2010). Biodiesels are fatty acid methyl and ethyl esters derived from renewable lipid sources, and these fuels are good alternatives for fossil fuel because of being renewable, biodegradable and nontoxic (Zlatanov et al. 2001; Meng et al. 2009; Karatay and Donmez 2010; Liu et al. 2010; Kosa and Ragauskas 2011; Chatzifragkou et al. 2011). The high cost of biodiesel is the major obstacle for its commercialization. Use of waste oil and other cheap raw materials can reduce the cost of the process. Microbial oil has potential for application in biodiesel production, because it has great similarity in structure and fatty acid composition to the vegetable oil (Karatay and Donmez 2010; Fei et al. 2010). The advantages of microbial lipid are short life cycle of microorganisms, less labor required, less affection by

season and climate, and easier to scale-up (Li et al. 2008; Amaretti et al. 2010). If vegetable oil or animal fat is used for biodiesel production, the cost of the substrate will be 70–85 % of the total cost.

Lipid accumulation in oleaginous microorganisms occurs with starvation of their cells from nitrogen or other nutrients such as zinc, iron, phosphorus and magnesium (Meester et al. 1996; Fakas et al. 2008). Cell response to the exhaustion of a key nutrient such as nitrogen, is entering into a lipid storage phase in which excess carbon is converted into storage lipid. If the cells return to a situation where nitrogen is available, the oil reserves could be mobilized and changed to cellular materials (Ratledge and Wynn 2002; Ratledge 2005; Daum et al. 2009). Formation of lipid particles starts during the late exponential phase and continues during the stationary phase, and this process continues until carbon source in the medium starts to diminish (Mullner and Daum 2004; Raschke and Knorr 2009). Lipid production in a medium with excess amount of carbon occurs in two stages. In the first stage, cell growth occurs and this stage finishes by exhausting the nutrient except carbon. During the second phase, the excess amount of carbon transforms into lipid reserves. Because of nitrogen limitation, cells can no longer grow and multiply. A key enzyme in citric acid cycle is isocitrate dehydrogenase that becomes inactive in the absence of nitrogen. This leads to isocitrate not being metabolized, and consequently, isocitrate and citric acid accumulate in mitochondria and immediately transport to the cytoplasm of the oleaginous yeasts (Melickova et al. 2004; Wynn and Ratledge 2005).

The major component of oleaginous yeast and fungi is triacylglycerol (TAG) composed of C_{16} and C_{18}, which is similar to rapeseed and soybean oil (Vijayakumar et al. 2010). The effective parameters that affect lipid accumulation in microorganism are carbon, nitrogen, C/N ratio, agitation rate, pH, temperature and time of incubation. In this study, Taguchi design was used to achieve the highest lipid production by optimizing the medium condition. Fourier transform infrared (FTIR) spectroscopy was used to confirm the composition of lipid production in this study. The base of this method is creating peaks in a special spectrum based on cm^{-1} unit, so each chemical group has a specific peak at a certain point in a determined spectrum.

The purpose of this study was to evaluate the lipid production in native and standard oleaginous yeasts. Also, optimization of cultivation condition was done by Taguchi design and the effect of this method on optimization process was evaluated. The extracted lipid was analyzed by FTIR spectroscopy to show its potential for application in biodiesel production. This study was done in 2012 at Flavarjan University.

Materials and methods

Yeast strains

The yeast strains that were used in this study were *Yarrowia lipolytica* DSM 8218 as a standard strain and the *C. albidus* as an isolated strain.

Preparation of inoculum

The oleaginous yeast colonies were first streaked on the YPD plates and then grown for 2 days. After that they were transferred to 250-ml erlenmeyer flask that contains 50 ml of inoculation medium containing (g per liter): glucose 15, $(NH_4)_2SO_4$ 5, KH_2PO_4 1, $MgSO_4.7H_2O$ 0.5 and yeast extract 0.5 that grown at 28 °C in a shaker incubator at 180 rpm for 2 days(Pan et al. 2009).

Preparation of flask culture

An inoculum of 5 ml was transferred to 45 ml of nitrogen-limited fermentation medium containing (g per liter): glucose 40, $(NH_4)_2SO_4$ 1, KH_2PO_4 7, NaH_2PO_4 2, $MgSO_4.7H_2O$ 1.5, yeast extract 1, $CaCl_2$ 0.15, $MnSO_4.H_2O$ 0.06, $ZnSO_4.7H_2O$ 0.02 and $FeCl_3.6H_2O$ 0.15 in 250-ml erlenmeyer flask, and incubated in a rotary shaker at 180 rpm and 28 °C for 3 days (Papanikolaou et al. 2001; Pan et al. 2009; Kraisintu et al. 2010).

Analysis of yeasts by Nile red staining

Fermented nitrogen-limited medium of 40 µl was mixed with 10 µl of Nile red solution (10 µg of Nile red in 1L of ethanol). After 5 min, yeast cells were observed under fluorescence microscope, which has gold emission caused by Nile red-stained lipid bodies (Kraisintu et al. 2010).

Determination of yeast dry mass

Portions of 5 ml of cultures were harvested by centrifugation at 6,000 rpm for 20 min. Harvested biomass was washed twice with 5 ml of distilled water and then dried at 80 °C to constant mass. The biomass was determined gravimetrically (Vijayakumar et al. 2010).

Analysis of supernatant for glucose and nitrogen concentration

The supernatant was analyzed for glucose consumption by dinitrosalicylic acid (DNS) solution. Nitrogen concentration was also analyzed by Kjeldahl method. The growth yield efficiency, SCO yield efficiency and SCO productivity were calculated according to the following equations:

$$\text{growth yield efficiency} = \frac{\text{cell dry weight } \left(\frac{g}{L}\right)}{\text{Sugar consumed } \left(\frac{g}{L}\right)} \times 100$$

$$\text{SCO yield efficiency} = \frac{\text{SCO weight } \left(\frac{g}{L}\right)}{\text{Sugar consumed } \left(\frac{g}{L}\right)} \times 100$$

$$\text{SCO productivity} = \frac{\text{SCO weight } \left(\frac{g}{L}\right)}{\text{cell dry weight } \left(\frac{g}{L}\right)} \times 100$$

Single cell oil extraction

Extraction of lipid was carried out according to Bligh and Dyer method with some modification (Pan et al. 2009); 40 ml of sample was centrifuged at 6,000 rpm for 10 min. After that the yeasts were washed with 40 ml of distilled water. The latter process was repeated and then 8 ml of 4 M HCl was added and incubated at 60 °C for 2 h. Then, acid-hydrolyzed mass was stirred with 16 ml chloroform/methanol mixture (1:1) at room temperature for 2–3 h. At the end, centrifugation was done at 5,000 rpm for 5 min at room temperature to separate the aqueous upper phase and organic lower phases. Then, the lower phase containing lipid was recovered with Pasteur pipette and evaporated in the vacuum. After that the dry lipid was weighed.

Qualification analysis of microbial lipid by thin-layer chromatography

Silica gel plates and lipid standards (triolein as reference substance for triacylglycerol) were used. The solvent was n-hexane–diethyl ether–acetic acid (90:10:2). The bands were observed after staining the TLC plate by iodine vapor. So, the qualitative and semiquantitative analyses of intracellular lipid were carried out by thin-layer chromatography (Alvarez et al. 2008).

Single cell oil production by FTIR spectroscopy

Lipid production in oleaginous yeast was confirmed by Sudan black staining at first, and then further confirmation of certain oil compounds was determined by FTIR spectroscopy using JASCO FT/IR-6300, Japan device. The range of spectrum analyzed by the device was set from 400 to 4,000 cm^{-1}. Triolein (bought from Sigma-Aldrich) was used as control sample for comparison with produced SCO.

Medium optimization by Taguchi design

Design of experiment (DOE) was done by Taguchi method for evaluating the effects of different physical and chemical parameters on lipid production. These parameters were glucose and nitrogen concentrations, pH, agitation rate, time and temperature of incubation.

Table 1 Setting of different factors and their level for lipid production by Taguchi design

Number	Variables	Level 1	Level 2	Level 3	Level 4
1	Glucose	55	75	95	115
2	$(NH_4)_2SO_4$	0.5	1	1.5	–
3	Time	24	48	72	96
4	Temperature	25	35	–	–
5	pH	5	5.5	6	6.5
6	rpm	150	200	–	–

Table 2 L16 array of Taguchi design

Number of trials	Nitrogen	Glucose	Temperature	Time	pH	rpm
1	0.5	55	25	24	5	150
2	0.5	75	25	48	5.5	200
3	0.5	95	35	72	6	150
4	0.5	115	35	96	6.5	200
5	1	55	25	96	6	200
6	1	75	25	72	6.5	150
7	1	95	35	48	5	200
8	1	115	35	24	5.5	150
9	1.5	55	35	48	6.5	150
10	1.5	75	35	24	6	200
11	1.5	95	25	96	5.5	150
12	1.5	115	25	72	5	200
13	0.5	55	35	72	5.5	200
14	0.5	75	35	96	5	150
15	0.5	95	25	24	6.5	200
16	0.5	115	25	48	6	150

Glucose, time and pH had 4 levels, nitrogen had 3 levels, and time and temperature had two levels (Table 1). Qualitek-4 software designed an experimental plan (L16) that contains 16 experiments for each strain (Table 2). Statistical analysis was done by ANOVA (one-way analysis of variance) and Taguchi design. The best optimum condition for the highest lipid production for each strain was achieved by Taguchi method.

Results and discussion

Lipid determination in nitrogen-limited medium

Between the amounts of lipid that were obtained from these strains, the extracted lipid from *C. albidus* was higher. Table 3 shows the lipid production, dry biomass and lipid productivity for these strains.

According to the results, the experiments were set as L16 design of Taguchi method. The advantage of Taguchi design is that a lot of experiment can be evaluated without doing all of them. The software evaluates all of the parameters based on the results of 16 experiments. Tables 4 and 5 show the results of Taguchi experiments for *Y. lipolytica* DSM 8218 and *C. albidus*.

The results of glucose concentrations are shown in Tables 4 and 5 as growth yield efficiency and SCO yield efficiency.

Table 3 Lipid production, dry biomass and lipid productivity in nitrogen-limited medium before optimization

Number	Strain	Lipid (g/L)	Dry biomass (g/L)	Lipid productivity (%)
1	*Yarrowia lipolytica* DSM 8218	4.21	13.28	31.7
2	*Cryptococcus Albidus*	5.83	16.4	35.54

Table 4 Results of Taguchi design, dry biomass, lipid productivity, growth yield efficiency, SCO yield efficiency and nitrogen concentration for *Yarrowia lipolytica* DSM 8218

Number	Lipid production (g/l)	Dry biomass (g/l)	Lipid productivity (%)	Growth yield efficiency	SCO yield efficiency	Nitrogen concentration
1	3.78	12.60	30.00	29.64	8.89	0.08
2	4.22	13.18	32.00	26.84	8.59	0.006
3	6.71	15.97	42.00	20.16	8.47	0.11
4	2.68	9.50	28.20	27.53	7.73	0.09
5	2.17	7.80	27.80	28.15	7.83	0.15
6	3.81	12.57	30.30	27.77	8.41	0.08
7	4.01	12.61	31.80	27.32	8.68	0.11
8	2.01	7.65	26.25	32.86	8.63	0.20
9	1.98	7.87	25.15	34.01	8.55	0.29
10	3.18	10.81	29.40	29.92	8.80	0.26
11	3.65	11.06	33.00	26.65	8.79	0.25
12	3.10	10.64	29.13	30.18	8.79	0.10
13	4.45	13.73	32.40	25.90	8.39	0.09
14	4.01	12.93	31.00	28.63	8.88	0.09
15	4.52	13.90	32.50	26.47	8.60	0.01
16	3.25	11.01	29.50	28.97	8.55	0.08

Table 5 Results of Taguchi design, dry biomass, lipid productivity, growth yield efficiency, SCO yield efficiency and nitrogen concentration for *Cryptococcus albidus*

Number	Lipid production (g/l)	Dry biomass (g/l)	Lipid productivity (%)	Growth yield efficiency	SCO yield efficiency	Nitrogen concentration
1	4.32	12.93	33.40	47.13	15.74	0.10
2	6.16	16.60	37.10	41.50	15.40	0.10
3	5.05	13.87	36.40	43.20	15.73	0.12
4	3.63	10.62	34.40	46.07	15.74	0.09
5	5.72	15.54	34.15	42/75	15.73	0.19
6	11.81	19.65	36.80	26.55	15.95	0.10
7	3.98	11.81	60.10	46.86	15.79	0.12
8	4.95	13.67	33.68	43.39	15.71	0.19
9	2.82	10.09	28.14	51.11	14.28	0.14
10	3.77	11.52	32.70	47.80	15.64	0.30
11	6.41	16.35	39.20	39.87	15.63	0.30
12	6.17	15.82	39.00	39.35	15.34	0.14
13	3.80	11.74	32.35	46.96	15.20	0.10
14	5.84	16.15	36.15	43.06	15.57	0.05
15	5.26	14.85	35.40	43.67	15.47	0.09
16	4.65	13.60	34.18	44.59	15.24	0.10

Table 6 Analysis of variance (ANOVA) of Taguchi results for *Cryptococcus albidus*

Factors	DOF	Sum of squares (S)	Variance (V)	F-ratio (F)	Pure sum (S′)	Percentage P (%)
Nitrogen	2	9.616	4.808	343.038	9.588	15.389
Glucose	3	16.144	5.381	383.915	16.102	25.842
Temperature	1	17.305	17.305	1,234.607	17.291	27.751
Time	3	13.24	4.413	314.874	13.198	21.183
pH	3	2.568	0.856	61.079	2.526	4.054
rpm	1	3.404	3.404	242.848	3.39	5.44
Other/error	2	0.027	0.013	–	–	0.341
Total	15	62.307	–	–	–	100.00

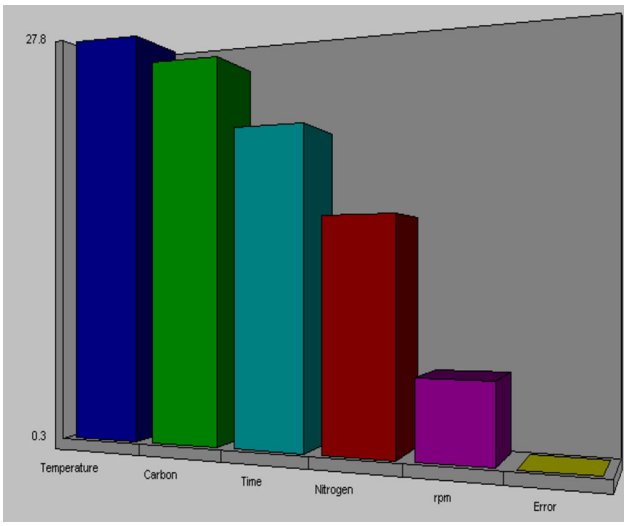

Fig. 1 Percentage contribution for each factor in lipid production by *Cryptococcus albidus* that was obtained from Qualitek-4 software. The *last column* shows error of the experiment which was the least. Temperature has the most effect on lipid production in this strain (27.8 %)

The best experiment for *Y. lipolytica* DSM 8218 and *C. albidus* was arrays 3 and 6, respectively. Table 6 shows the ANOVA results for the better lipid-producing strain. The last column of the table shows the influence percentage for each factor. The analysis shows that temperature, glucose concentration, time of incubation and nitrogen concentration had significant effect on lipid production, respectively. pH and rpm had the less effect on lipid production for this strain. Other error is the error of the experiment which was the least one, about zero (Figs. 1, 2).

FTIR spectroscopy results

Microbial lipid graphs are shown in Fig. 3. Comparison of two graphs shows significant similarity between extracted oil from oleaginous yeast and the standard (Triolein). Significant peaks were created between 1,670 and 1,820 cm^{-1}, confirmed presentation of carbonyl groups. Peaks between 2,850 and 2,929 cm^{-1} show methyl group presentation. All of the peaks in mentioned points confirmed that produced oil can be converted into biodiesel

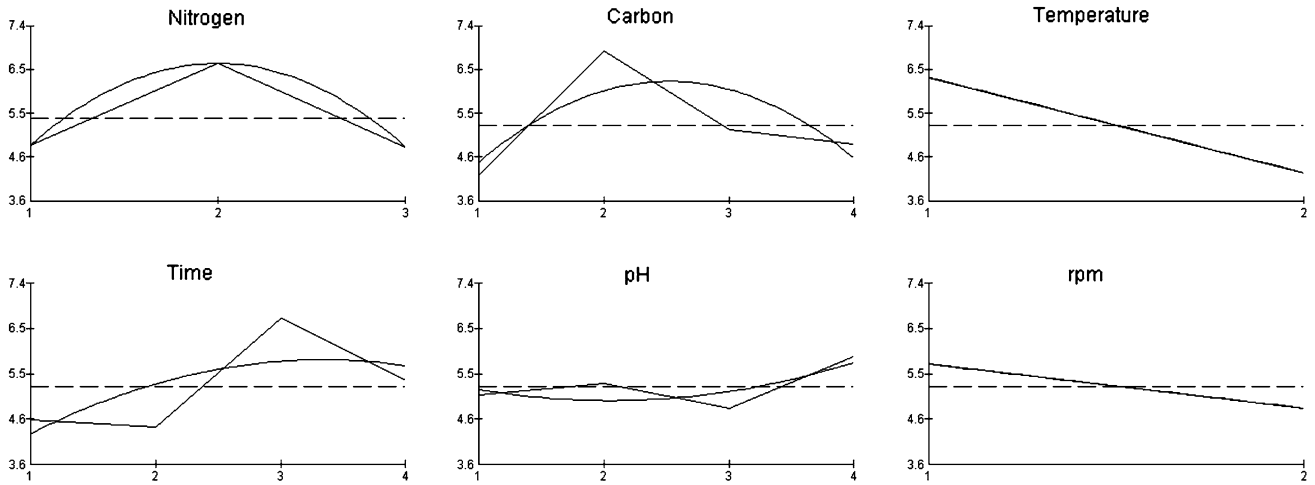

Fig. 2 Plot obtained from Qualitek-4 software that shows the effect of each factor on lipid production by *Cryptococcus albidus*. *Y-axis* of the *plot* shows the average lipid production in each level of different factors. *X-axis* shows the number of levels for different factors

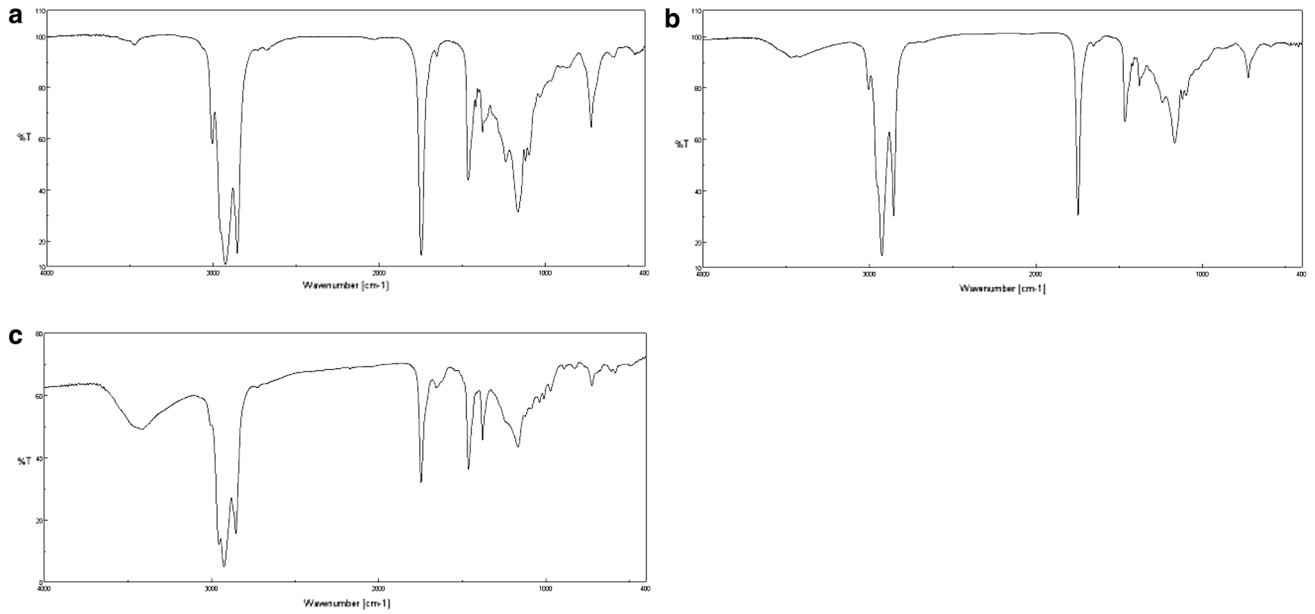

Fig. 3 **a** FTIR graph of triolein standard. **b** FTIR graph of produced SCO by yeast *Cryptococcus Albidus*. **c** FTIR graph of produced SCO by yeast *Yarrowia lipolytica* 8218

Table 7 Optimum condition predicted by Taguchi for the highest production by *Cryptococcus albidus*. Expected result at optimum condition was lipid production of 11.782 g/l

Factor	Optimized level	Level	Contribution
Nitrogen	1	2	1.342
Carbon	75	2	1.622
Temperature	25	1	1.039
Time	72	3	1.434
pH	6.5	4	0.612
rpm	150	1	0.461

(Lin-Vien et al. 1991; Elumalai et al. 2011; European Standard EN 14078). FTIR was used for analyzing and confirming biodiesel production from *Chloralla vulgaris* and *Senedesmis* sp (Elumalai et al. 2011).

According to the results, the native strain *C. albidus* was excellent in lipid production. Lipid productivity of this strain reaches 60 % with Taguchi method. The percentage contribution of different factors is shown in Fig. 1 for this strain.

Taguchi method limited the number of experiments, so it is a powerful tool for investigating the effect of all the parameters. Fig. 2 shows the effect of different parameters on lipid production for *C. albidus*. Another advantage of this software is suggesting the optimum condition. The optimum condition for *C. albidus* strain is shown in Table 7. This condition was provided for this strain and the amount of lipid was approximately the same as Taguchi predictions. Estimated interaction of severity index (SI) for different parameters is shown in Table 8. Fifteen interactions between two factors were calculated by Qualitek-4

Table 8 Estimated interactions of severity index (SI) for different parameters by Qualitek-4 software

No	Interacting factor pairs (order based on SI)	Columns	SI %	Col	Opt
1	Carbon × rpm	4 × 10	48.04	14	(2,1)
2	Nitrogen × rpm	1 × 10	43.64	11	(2,1)
3	Nitrogen × Temperature	1 × 5	34.63	4	(2,1)
4	Nitrogen × Carbon	1 × 4	23.13	5	(2,2)
5	Carbon × Temperature	4 × 5	21.88	1	(2,1)
6	Carbon × Time	4 × 6	21.57	2	(2,3)
7	Time × rpm	6 × 10	15.42	12	(3,1)
8	Temperature × pH	5 × 7	14.85	2	(1,4)
9	Temperature × Time	5 × 6	14.02	3	(1,3)
10	pH × rpm	7 × 10	12.06	13	(4,1)
11	Nitrogen × Time	1 × 6	8.81	7	(2,3)
12	Time × pH	6 × 7	8.62	1	(3,4)
13	Nitrogen × pH	1 × 7	5.95	6	(2,4)
14	Carbon × pH	4 × 7	4.67	3	(2,4)
15	Temperature × rpm	5 × 10	1.66	15	(1,1)

SI interaction severity index (100 % for 90 °angle between lines, 0 % for parallel lines)

Col column that should be reserved if this interaction effect were to be studied

Opt the factor level desirable for the optimum conditions

software. Although rpm did not influence lipid production a lot, it shows the highest SI percentage with carbon source (48.04 %), so Taguchi method helps to analyze different interactions of the parameters. According to the results, this native strain, *C. albidus,* is capable of producing high amount of lipid.

Conclusion

The results of this investigation showed that native strains such as *C. albidus* have high potential for industrial use and biodiesel production. This study revealed that designing experiments based on Taguchi method acts excellent in optimizing processes. It can make this process economical and give us exact results. The effect of a number of different factors was investigated simultaneously by using this method. Through this method, the optimization condition was gained and lipid production and lipid content reached 11.81 g/l and 60.1 %, respectively, for *C. albidus*. This extracted oil can be used for biodiesel production.

Acknowledgment The authors would like to thank Department of Biology of Falavarjan Islamic Azad University for their support for this research work.

References

Alvarez FA, Alvarez MH, Kalscheuer R, Waltermann M, Steinbuchel A (2008) Cloning and characterization of a gene involved in triacylglycerol biosynthesis and identification of additional homologous genes in the oleaginous bacterium Rhodococcus opacus PD630. Microbiology 154(Pt 8):2327–2335. doi:10.1099/mic.0.2008/016568-0

Amaretti A, Raimondi S, Sala M, Roncaglia L, Lucia DM, Leonardi A, Rossi M (2010) Single cell oil of cold adapted oleaginous yeast Rhodotorula glacialis DBVPG 4785. Microb Cell Fact 9:73. doi:10.1186/1475-2859-9-73

Boswell K, Koskelo EK, Carl L, Glaza S, Hensen DJ, Williams KD, Kyle DJ (1996) Preclinical evaluation of single- cell oils that are highly enriched with arachidinic acid and docosahexaenoic acid. Food Chem Toxicol, 34:585-593. http://dx.doi.org/10.1016/0278-6915(96)00019-1

Chatzifragkou A, Makri A, Belka A, Bellou S, Mavrou M, Mastoridou M, Mistorioti P, Onjaro G, Aggelis G, Papanikolaou S (2011) Biotechnological conversions of biodiesel derived waste glycerol by yeast and fungal species, Elsevier, 36:1097-1108. http://dx.doi.org/10.1016/j.energy.2010.11.040

Dai C, Tao J, Xie F, Dai YJ, Zhao M (2007) Biodiesel generation from oleaginous yeast Rhodotorula glutinis with xylose assimilating capacity. Afr J Biotechnol 6(18):2130–2134

Daum G, Wanger A, Czabany T, Athenstaedt K (2009) Dynamics of neutral lipid storage and mobilization in yeast. Biochimie 89:243–248

Elumalai S, Sakthivel R, Ganesh Kumar S (2011) Ultra Structural and Analytical Studies of Biodiesel Producing Microalgae (Chlorella vulgaris and Senedesmis sp) Collected from Tamil Nadu. Current Botany 2(6):19–25

European Standard EN 14078: Liquid petroleum products—Determination of fatty acid methyl esters (FAME) in middle distillates—Infrared spectroscopy method

Fakas S, Papanikolaou S, Galiotou-Panayotou M, Komaitis M, Aggelis G (2008) Biochemistry and biotechnology of Single Cell Oil, university of Patras, Greece, 38–60

Fei Q, Chang H et al (2010) The effect of volatile fatty acid as a sole carbon source on lipid accumulation by *Cryptococcus albidus* for biodiesel production. Bioresour Technol 102:2695–2701

Karatay SE, Donmez G (2010) Improving the lipid accumulation properties of the yeast cells for biodiesel production using molasses. Bioresource Technology, 101(20): 7988–7990. http://dx.doi.org/10.1016/j.biortech.2010.05.054

Kosa M, Ragauskas AJ (2011) Lipids from heterotrophic microbes: advances in metabolism research. Trends Biotechnol, 29(2):53–61. http://dx.doi.org/10.1016/j.tibtech.2010.11.002

Kraisintu P, Yongmanitchai W, Limtong S (2010) Selection and optimization for lipid production of a newly isolated oleaginous yeast, Rdodosporidium toruloides DMKU3-TK16, Kasetsart university, Thailand, 44:436–445

Kyle DJ, Sicotte VJ, Singer JJ, Reeb SE (1992) Bioproduction of docosahexaenoic acid(DHA) by microalgae. In: Industrial Application of Single Cell Oil, American Oil Chemists Society, 287–300

Li Q, Du W, Liu D (2008) Perspective of microbial oils for biodiesel production. Appl Microbial Biotechnol 80:749–756

Lin-Vien D, Colthup NB, Fateley WG, Grasselil JG (1991) The handbook of infrared and raman characteristic frequencies of organic molecules. Academic Press, Inc, London 141

Liu GQ, Lin QL, Jin XC, Wang XL, Zhao Y (2010) Screening and fermentation optimization of microbial lipid-producing molds from forest soils. Afr J Microbiol Res 4(14):1462–1468

Meester PAEP, Huijberts GNM, Eggink G (1996) High-cell-density of the lipid accumulating yeast Cryptococcus curvatus using glycerol as a carbon source. Appl Microbial Biotechnol 45(5):575–579

Meng X, Yang J, Xu X, Zhang L, Nie Q, Xian M (2009) Biodiesel production from oleaginous microorganisms. Renew Energy, 34(1):1–5. http://dx.doi.org/10.1016/j.renene.2008.04.014

Mullner H, Daum G (2004) Dynamics of neutral lipid storage in yeast. Acta Biochim Pol 51:323–347

Pan LX, Yang DF, Shao L, Li W, Chen GG, Liang ZQ (2009) Isolation of oleaginous yeast from the soil and studies of their lipid-producing capacities, ISSN. Food Technol Biotechnol 47(2):215–220

Papanikolaou S, Chevalot I, Komaitis M, Marc I, Aggelis G (2001) Single cell oil production by *Yarrowia lipolytica* growing on an industrial derivative of animal fat in batch cultures. Appl Microbial Biotechnol 58:308–312

Raschke D, Knorr D (2009) Rapid monitoring of cell size, vitality and lipid droplet development in oleaginous yeast Waltomyces lipofer. J Microbiol Methods, 79(2):178–183. http://dx.doi.org/10.1016/j.mimet.2009.08.011

Ratledge C (1993) Single cell oils—have they a biotechnological future? Trends Biotechnol 11:278–284

Ratledge C (2005) Single cell oils, lipid research center. University of Hull, Department of biological sciences, Hull, pp 1–20

Ratledge C, Wynn JP (2002) The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. Adv Appl Microbial 51:1–44

Vijayakumar S, Kumutha K, Krishnan PS, Gopal H (2010) Effect of carbon sources on lipid and biomass production by oleaginous yeast cultures. Tamil Nadu Agriculture university, Coimbatore, pp 1–3

Wynn PJ, Ratledge C (2005) Oils from microorganisms. Martek Bioscience Corporation, Columbia, pp 121–153

Melickova K, Roux E, Athenstaedt K, Andrea S, Daum G, Chardot T, Nicaud JM (2004) Lipid accumulation Lipid body formation and acyl coenzyme A oxidases of the yeast Yarrowia lipolytica. Appl Environ Microbiol 70(7):3918–3924

Zlatanov M, Pavlova K, Grigrova D (2001) Lipid composition of some yeast strains from Livingston island, Antarctica. Folia Microbiol 46(5):402–406