Optimization of cultural conditions for lipid accumulation by *Aspergillus wentii* Ras101 and its transesterification to biodiesel: application of response surface methodology

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Abstract

The present study is aimed to maximize biodiesel production by using the fungal strain *Aspergillus wentii* Ras101 as a feedstock. Response surface methodology was used to relate the interaction between some nutritional and environmental factors affecting the lipid productivity by *A. wentii* Ras101. By applying LINGO optimization program, the maximum lipid production of 40% dry biomass of this fungal isolate has been attained in a fermentation medium composed of 50 g/l glucose, 1 g/l nitrates, 1.5 g/l phosphorous, and 0.5 g/l NaCl. This medium was adjusted at pH of 6, and incubated at 28 °C for 7 days. The values of correlation errors between the experimental and estimated values are less than 1%; this proves that the proposed correlation could be used effectively for estimating the fungal lipid production. Consequently, the effects of time and temperature on the amount of biodiesel produced in the extraction and transesterification one-step process have been investigated. The maximum biodiesel production of 28% dry biomass (80% lipid) has been achieved in the transesterification process at 70 °C for 30 min. Additionally, it is found that the combination of glucose, nitrogen and phosphorous contents has a positive influence on lipid production in the fungal biomass. The density, kinematic viscosity, water content and calorific value of the produced biodiesel were 800 kg/m³, 2.8 mm²/s, 66 ppm and 10122 kcal/kg, respectively that matched well with biodiesel and fossil standard specifications.

Keywords Fungal lipids · Alternative fuel · Biodiesel production · Renewable energy · Optimization

Introduction

Current chemical and energy industries are heavily reliant upon fossil fuels, which are unsustainable and contribute to economic and political vulnerability. The exploitation of fossil fuels for the human requirements has increased last years due to the population increase and huge consumption of energy; their usage contributes to greenhouse gas (GHG) emission (Matsakas et al. 2017). This consequently

triggers the world energy crisis (Pérez-Lombard et al. 2008). According to Hirsch et al. (2005), world demand for fuels is expected to accelerate to 40% by 2025. Moreover, fossil fuels influence the environment by their negative effects, such as climate change and atmospheric pollution (Rahimnejad et al. 2012). Therefore, it is necessary to discover other renewable energy resources which would reduce environmental pollution, as well as decrease the dependence on fossil fuels, such as solar energy, wind energy, water energy and biomass.

Biorefinery uses different technologies to separate biomass into its lipid (triglycerides) content from other constituents, which can be transformed into biodiesel through other different processes (González-Delgado and Kafarov 2011). Biodiesel is the main product from the transesterification process of triglycerides (TG) and methanol, in the presence of a catalyst, giving fatty acid methyl esters (FAME), in addition to glycerol as a by-product (Parawira 2009; Fjerbaek et al. 2009).

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Biodiesel is considered as a renewable fuel which can replace petrodiesel, since it is easy to manufacture, compatible with existing engines, biodegradable, non-toxic, better in flash point and sulfur free (Ma and Milford 1999; Antolin et al. 2002; Vicente et al. 2004; Demirbas 2009). Sharp (1996) estimated that the use of biodiesel as fuel would reduce sulfur emission, carbon monoxide emission, air toxicity and cancers by 30, 10, 90 and 95%, respectively, compared to petrodiesel. Furthermore, the resulted byproducts, such as glycerin produced during the transesterification process, are of an economic value. They can also help to increase the energy efficiency of the whole process (Rutz and Rainer 2007). Shoaib and Bahrán (2013) have introduced an optimization program aiming at maximizing the biodiesel mixing ratio with petrodiesel, considering all the constraints imposed on each property of the final biodiesel/petrol diesel blends.

Different natural vegetable oils and waste cooking oils were used as feedstocks for biodiesel production (Kulkarni and Ajay 2006; Felizardo et al. 2006; Gui et al. 2008; Azócar et al. 2010). Particularly, the oils extracted from soybean, olive, rapeseed, and palm are the major feedstocks for biodiesel production (Giakoumis 2018). However, the use of vegetable oils and animal fats, as primary raw materials for biodiesel production, will increase the world demand for vegetable oil production. Consequently, large agricultural land areas are needed for cultivation of oilseed crops targeted away from food production (Chisti 2007; Gustone 2009). Additionally, cooking oils and fats may contain high quantity of free fatty acids, which require additional processing; this consequently will raise the biodiesel production cost (Demirbaş 2003; Haas 2005). Therefore, it is necessary to explore new raw materials which are economically competitive; can be produced in quantities sufficient to meet the energy demands, provide a net energy gain over the energy sources used to produce it, and moreover, do not compete with food production (Meng et al. 2009).

Recently, much attention has been paid to the FAME production on a global scale from single cell oils produced by oleaginous microorganisms (Chisti 2008; Lu et al. 2008; Meng et al. 2009) such as yeast, fungi, bacteria, and microalgae (Li et al. 2008; Subramaniam et al. 2010). They are able to accumulate more than 20% lipid in their biomass during growth on high content of carbon and limitation of nitrogen (Ratledge and James 2002; Athenaki et al. 2018). Single cell oil producers are characterized by low cost, short life cycle, available from different carbon sources and wastes and easier to scale up (Li and Wang 1997). Therefore, microbial lipid will become one of potential feedstocks for biodiesel production in the future, though, there are several studies concerning with microorganism producing high amounts of lipid of unsaturated fatty acids that need to be carried out further (Liao et al. 2016).

The microbial lipid production cost depends mainly upon the microbial species selected for cultivation (Borowitzka 1997) and the environmental and nutritional culture conditions, such as C/N ratio, nitrogen source, temperature, pH, oxygen, and inorganic salts concentration (Li et al. 2008). Higher C/N ratio and organic nitrogen sources have been shown to favor an oil accumulation in microbial biomass (Hassan et al. 1996; Huang et al. 2010). Several studies must be developed to optimize the cultural conditions for lipid accumulation in the microorganism biomass and determine the process that permits an effective oil extraction in terms of efficiency, product purity, energy requirements, costs and environmental impacts (González-Delgado and Viatcheslav 2013).

The objective of the present work is to study experimentally and mathematically the biodiesel production process; concentrating on the optimization of lipid accumulation and FAME transesterification process from a novel fungal strain of *Aspergillus wentii*. The optimization of biodiesel production process was carried out using Lingo optimization software version 14.0. Response surface methodology (RSM) has been applied to derive two correlations relating lipid and biodiesel production to the affecting parameters in either the growth process and the extraction and transesterification one-step direct process. These correlations helped in building an optimization programs aiming at determining the optimum operating conditions those result in maximum lipid and biodiesel production in the growth stage as well as the extraction and transesterification one-step direct process. Furthermore, RSM has been used to study the interaction between the affecting variables on lipid and biodiesel production.

Materials and methods

Microorganism preparation

The fungal strain; *A. wentii* Ras101 was used in the present study as a feed stock for biodiesel production due to its potential ability to accumulate and produce high amount of lipids in its biomass. It was isolated from the soil by members of the microbiology research laboratory (Faculty of Science-Suez University); it has been picked up among several oleaginous fungal strains.

Aspergillus wentii Ras101 was maintained on Sabouraud dextrose agar (SDA) slants and stored at 4 °C with regular transfer at monthly intervals. For long time preservation, spore suspension of new culture with 25% glycerol was used and stored at − 20 °C. The fungal spore suspension of *A. wentii* Ras101 was prepared from 5 days old cultures on SDA slants using sterile distilled water and used in about 4×10^6 spores/ml fermentation medium.

Fungal strain growth and its fermentation medium

The fermentation medium used in the current work is the modified Czapek Dox Broth medium of rich carbon and limited nitrogen to induce lipid accumulation. This medium contains $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/l dist. H_2O ; and $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$, 0.01 g/l dist. H_2O . Aliquots of 50 ml medium was dispensed in 250 ml Erlenmeyer flasks, autoclaved at 121°C for 15 min and inoculated after cooling with a spore suspension of *A. wentii* Ras101.

Other operating conditions such as glucose concentration, pH, nitrates concentration, sodium chloride concentration, phosphorous concentration, incubation time and incubation temperature, were studied in this work to show their effects on fungal growth and lipid accumulation. These seven factors which influence the biomass production have been denoted as A, B, C, D, E, F and G, respectively. Batch experiments for fungal lipid synthesis were conducted by employing 16 selected experimental variations in combination with these seven factors at 5 levels as presented in Table 1.

Biomass dry weight and lipid content determination

The dry weight of mycelium was determined after the desired incubation period by filtration of the fungal mycelium of each flask using a filter paper (Whatman no. 1; 15 cm diameter). The filtrate was washed three times with distilled water and dried for 24 h at 85°C until constant weight is reached. The lipid content of the obtained dry biomass of *A. wentii* Ras101 was quantitatively determined by sulfo-phospho-vanillin assay according to the method of Mishra et al. (2014).

Lipid extraction and transesterification

The fungal lipid of the obtained dry biomass was extracted for transesterification to fatty acid methyl esters (FAME) by a one-step direct process, according to the method of Reis et al. (2014). The variables affecting the produced biodiesel are temperature, time and biomass/solvent ratio (g/ml). Three levels are taken for both temperature and time,

while biomass/solvent ratio is fixed at 0.2 g of dry biomass per 30 ml of solvent. Table 2 shows levels taken of both temperature and time. Thus, batch experiments for fungal lipid synthesis were conducted by employing nine experimental variations with two variables at three levels.

In situ transesterification of the fungal lipid; 200 mg dry biomass were dissolved in 30 ml of methanol–hydrochloric acid–chloroform mixture (10:1:1, v/v/v) and kept in a thermostatic bath at a specified temperature and time. The ester produced from transesterification is extracted three times by adding 20 ml of hexane–chloroform mixture (4:1, v/v) and 10 ml of water. Next, the mixture was separated by shaking and settling for 10 min in a separation funnel to complete phase separation. The light phase (hexane and methyl ester) is separated, then dried on anhydrous sodium sulphate, while the solvent was evaporated under reduced pressure in a rotary vacuum evaporator. The methyl esters fraction (biodiesel) is dried, weighed and gravimetrically determined as a percentage of dry biomass and initial lipid.

Methodology for obtaining optimum operating conditions

To maximize biodiesel production from the tested fungal strain, it is necessary to get the optimum affecting parameters both in the fungal growth stage as well as in the extraction and transesterification one-step stage. The best method to achieve that target is to derive two correlations; one of these correlations is to relate the lipid production to the seven previously mentioned affecting variables in the growth stage; the second correlation is to relate the biodiesel production to the affecting variables in the extraction and transesterification one-step direct process; time and temperature. These derived correlations are based on the obtained experimental results. Response surface methodology (RSM)

Table 1 Factors affecting lipid production and their levels

Factors	Level 1	Level 2	Level 3	Level 4	Level 5
Glucose (A), g	20	40	60	70	90
pH (B)	3	4.5	5.5	6	7
Nitrates(C), g	1	2	4	5	6
NaCl (D), g	0	0.5	1	1.5	2
Phosphorous (E), g	0	0.2	0.5	1.5	2
Incubating time (F), h	48	72	96	120	144
Incubation temperature (G), $^\circ\text{C}$	15	20	28	30	35

Table 2 Levels of variables affecting lipid extraction

Variables	Level 1	Level 2	Level 3
Temperature, $^\circ\text{C}$	50	70	90
Time, h	0.5	1	1.5

through Design Experts software was used to derive the introduced correlations.

To validate these correlations, four extra runs for each stage have been carried out experimentally and their results are compared to the calculation results. After validation of the proposed correlations, they have been introduced into a mathematical formulation program; the affecting variables have been optimized through LINGO optimization software, version 14. LINGO is a comprehensive tool designed to build and solve linear, nonlinear (convex and nonconvex/global), quadratic constrained, second order cone, semi-definite, stochastic, and integer optimization models fastely, easily and efficiently.

In the present work, the optimization program solves a non-linear program (NLP) to maximize both lipid production, and biodiesel production formed in the growth and extraction–transesterification stages respectively. The lower and upper limits of each variable under consideration should be specified and introduced as the program constraints.

Mathematical formulations for maximum lipid and biodiesel production

It is desired to determine optimal factors corresponding to the maximum lipid production without running a large number of experiments. This could be attained through using the derived correlation relating lipid production to the affecting operating variables. The mathematical formulation to maximize the lipid production in the optimization program could be summarized as follows:

$$\text{Objective} = \text{Max (Lipid production)}, \quad (1)$$

where the correlation relating the lipid production to the different affecting variables is clarified in Eq. (5).

Constraints for upper and lower bounds of each affecting variable are formulated as follows:

$$P^L \leq P \leq P^U, \quad (2)$$

where P is a set corresponds to the process variables. P^L and P^U correspond to lower and upper bounds with each affecting variable respectively. Table 3 addresses the lower and upper limits of each variable under consideration.

The resulted correlation which relates the biodiesel production to the extraction affecting variables has been used to find the optimum temperature and time corresponding to the maximum production of biodiesel. Constraints for upper and lower bounds of each affecting variable are formulated as follows:

$$X^L \leq X \leq X^U, \quad (3)$$

$$Y^L \leq Y \leq Y^U, \quad (4)$$

Table 3 lower and upper limits of each variable under consideration

Variables	Lower value	Upper value
A, g/l	30	60
B, PH	4.5	8
C, g/l	0.5	1.5
D, g/l	0	1
E, g/l	0.5	2
F, h	72	192
G, °C	20	35

where X is temperature (°C); X^L and X^U are the lower and upper limits of temperature, respectively, Y is time (h), Y^L and Y^U are the lower and upper limits of time respectively. In the present case study, the temperature limitation was between 50 and 90 °C; while the time limitation was between 0.5 and 1.5 h.

Biodiesel characterization methods

The quality of the fungal biodiesel was determined by analyzing the density (ASTM, D 4052), the kinematic viscosity (ASTM, D 445), the water content (ASTM, D 4928) and the calorific value (ASTM, D 240).

Results and discussion

This section begins with studying the optimization of lipid and biodiesel production; then, the influence of the studied nutritional and environmental factors on the fungal growth and lipid production are discussed. These factors contain the medium composition, initial pH, and incubation temperature and period. Additionally, the effect of temperature and time of extraction and transesterification one-step process on the produced amount of biodiesel is investigated. Finally, the produced biodiesel properties are determined and compared to the standard properties of both biodiesel and fossil diesel.

Lipid and biodiesel production correlations and optimization

Response surface methodology (RSM) is used to relate the lipid productivity to the nutrition and environmental conditions of growth medium for the fungal strain (*A. wentii* Ras101) by applying Design Experts version 10.0 software program. This growth medium contains glucose, nitrates, phosphorous, and NaCl at different levels of pH, incubation time and temperature. The following quadratic equation was derived:

$$\begin{aligned}
 \text{Lipid production} = & 1.366C^2 - 0.142A^2 + 876.671E^2 \\
 & + 132.486D^2 + 39.864B^2 \\
 & + 1.91G^2 - 0.231F^2 - 0.224AC \\
 & + 12.34A - 64.493C - 494.303E \\
 & - 157.258D - 339.35B - 85.876G \\
 & + 39.626F + 27.262
 \end{aligned} \quad (5)$$

The comparison between the experimental and estimated lipid production results are presented in Table 4.

For validation of the introduced Eq. (5), it is better to look at adjusted and predicted R^2 in conjunction with R^2 and to look at the standard error of the regression rather than the standard deviation of the errors (Weisberg 1998; Neter et al. 1996; Mapiour et al. 2010; Sen and Muni 2012). R^2 , predicted R^2 and adjusted R^2 of Eq. (5) that estimated the lipid production are found to be 1, 1 and 0.857 respectively. This consequently indicates the good agreement between the experimental lipid productions and that obtained by the proposed correlation. The error % between

experimental and estimated values of lipid production was determined by applying the following equation:

$$\text{Error \%} = \frac{\text{ABS}(Lp_e - Lp_c)}{Lp_e} \times 100\%, \quad (6)$$

where Lp_e and Lp_c are the experimental and estimated lipid production, respectively. According to Table 4, it is clear that all values of error are less than 1%; this also proves that the proposed correlation can be used effectively in estimating the lipid production derived from the fungal strain *A. wentii* Ras101 within the studied operating conditions.

For further verification of the proposed correlation for lipid production, extra four experiments with different sets of the studied variables were carried out. Experimental results of these four experiments are shown in Table 5. Results presented in Table 5 reveal that all values of error are less than 5%. This consequently proves that the proposed correlation can be used effectively in estimating the lipid production content within the limits of the investigated affecting variables. By applying LINGO optimization software, the optimal values for process parameters at the optimum value (40%) of lipid production are listed in Table 6.

Table 4 Comparison between experimental and estimated lipid production

Exp. no.	Factors							Lipid (% of dry biomass)		Error (%)
	A	B	C	D	E	F	G	Experimental	Estimated	
1	20	4.5	1	0.5	0.2	72	20	21.4	21.39	0.041
2	20	3	2	0.5	0.2	72	20	17.09	17.08	0.058
3	20	5.5	4	1	0.5	96	28	15.85	15.83	0.118
4	20	3	4	1	0.5	96	28	17.13	17.11	0.113
5	40	3	1	0.5	0	48	15	28.09	28.11	0.001
6	40	4.5	2	0.5	0.2	72	20	24.34	24.31	0.028
7	40	5.5	4	1	0.2	96	28	38.89	38.87	0.041
8	40	5.5	4	1	0.5	96	20	28.43	28.42	0.058
9	60	3	1	0	0.5	48	15	4.50	4.51	0.103
10	60	5.5	1	0	0	48	15	31.21	31.21	0.017
11	60	5.5	2	0.5	0.2	72	20	38.01	38.01	0.004
12	60	5.5	4	1	0.5	96	28	20.16	20.15	0.055
13	70	3	1	1.5	0	48	15	31.61	31.62	0.023
14	70	3	1	0	0	120	15	25.70	25.67	0.084
15	70	4.5	2	0.5	0.2	72	30	9.43	9.44	0.020
16	70	5.5	4	1.5	0.5	96	28	37.35	37.34	0.022

Table 5 Comparison between experimental and estimated lipid production for four experiments carried out at different values of each affecting variable

Exp. no.	Variables							Lipid (% of dry biomass)		Error (%)
	A	B	C	D	E	F	G	Experimental	Estimated	
1	40	4.5	4	1	0.5	96	28	15.51	15.40	0.782
2	60	4.5	2	0.5	0.5	72	20	13.99	14.54	3.772
3	70	6	2	0.5	0.2	72	20	32.71	32.24	1.435
4	70	4.5	2	1.5	0.2	72	20	20.85	21.13	1.343

Table 6 The optimal variables at maximum lipid production (40%)

Factor	Value
Glucose (A)	50.0
pH (B)	6.1
Nitrates (C)	1.0
NaCl (D)	0.5
Phosphorous (E)	1.5
Incubating time (F)	168.0
Incubating temperature (G)	28.0

Table 7 ANOVA for response surface methodology

Factors	<i>P</i> value
Glucose (A)	0.702
pH (B)	0.004
Nitrates (C)	0.009
NaCl (D)	0.008
Phosphorous (E)	0.109
Incubation time (F)	0.248
Incubation temperature (G)	0.155

The significance of the variables affecting lipid production by *A. wentii* Ras101 is identified using the analysis of variance (ANOVA) method. It should be declared that the lower the *P* value of a factor, the higher the effect of the variable on the lipid production content. As shown in Table 7, the *P* test shows that the pH factor has a significant effect on the lipid production content, while glucose has a minor effect on the lipid production.

To obtain the maximum amount of biodiesel, it is necessary also to optimize different variables affecting the one-step extraction and transesterification process. This step was carried out at different levels of time and temperature. The correlation that relates the studied variables of time and temperature to the biodiesel production was achieved by applying Design Expert software. The obtained quadratic equation is indicated below.

$$\text{Biodiesel production} = -0.1792X^2 - 4.66667Y^2 - 0.2375XY + 2.5708X + 21.29167Y - 61.7222, \quad (7)$$

where *X* is temperature (°C) and *Y* is time (h).

It should be noted that the biodiesel production is measured in % of dry biomass weight; values of *R*², adjusted *R*² and multiple *R* of the above correlation are found to be 0.9, 0.74 and 0.95 respectively; this confirms that there is a good agreement between the experimental and estimated values of produced biodiesel. For further verification, the error % between experimental and estimated values of biodiesel is determined at different values of the studied variables of time and temperature.

Table 8 Comparison between experimental and estimated amount of produced biodiesel

Exp. no.	Variables		Biodiesel (% of dry biomass)		Error (%)
	Temperature, °C	Time, h	Experimental	Estimated	
1	50	0.5	24	25.56	6.51
2	50	1	28.5	26.77	6.08
3	50	1.5	25.5	25.64	0.56
4	70	0.5	34.5	31.59	8.43
5	70	1	27.5	30.43	10.64
6	70	1.5	27	26.93	0.28
7	90	0.5	22	23.29	5.86
8	90	1	21	19.75	5.96
9	90	1.5	14	13.88	0.91

Table 9 Comparison between experimental and estimated extraction and transesterification process for the selected four experiments at different values of the affecting variables

Exp. no.	Variables		Biodiesel (% of dry biomass)		Error (%)
	Temperature, °C	Time, h	Experimental	Estimated	
1	60	0.5	30.5	31.53	3.28
2	60	1	31.25	30.39	2.83
3	80	0.5	31.05	30.41	2.14
4	80	1	26	26.88	3.27

As shown in Table 8, it is clear that most of the percent errors calculated values are less than 7%; this proves that the proposed quadratic correlation can be used effectively in estimating the amount of biodiesel produced in the extraction stage within the limits of the studied operating conditions.

For further confirmation of the biodiesel production correlation, additional four experiments at different values of studied variables (time and temperature) were carried out; the experimental results are compared with the correlation results. From Table 9, it is clear that all values of the calculated error % are less than 5%. This confirms that the proposed correlation which relates biodiesel production in the one-step direct extraction and esterification to the affecting variables of time and temperature can be used effectively in estimating the amount of the produced biodiesel. By applying LINGO optimization software, it is found that the maximum amount of biodiesel produced in the extraction and transesterification stage is 31.65% of dry biomass which could be achieved at optimum conditions of 70 °C and 0.5 h.

Effects of the nutritional and environmental factors on Lipid production

The medium composition, initial pH, incubation temperature and incubation period are the main factors affecting the fungal growth and lipid accumulation (Wu et al. 2005). In the following sections, all of these factors were investigated.

Effect of glucose concentration

The carbon source in the fermentation medium is the main nutrient for the fungal growth; it strongly affects the accumulation and composition of lipid in the different fungi due to dissimilarities in their metabolism. Glucose is the most common carbon source in the growth medium and was used most commonly for the growth and lipid production of oleaginous fungi under nitrogen limitation in different other studies (Carvalho et al. 2018). Figure 1a shows that at low glucose concentrations in the culture medium of the studied

fungal strain, the produced percentage of lipid is low. On the other hand, increasing the glucose content, will increase the lipid content until it reaches its maximum value at glucose content around 50 g/l. This corresponds to a lipid production content of 38.02% of dry biomass. The preferred carbon source concentration for highest lipid production by the oleaginous fungi is varied from 20 to 80 g/l (Al-Hawash et al. 2018). The supply of carbon in the growth medium is important for synthesis of triacylglycerol (TAG) in fungi. Fungi use the available carbon source in the medium for growth and production of lipid-free biomass. If the medium contains sufficient amount of carbon, it will satisfy the growth need; balanced growth occurs without any accumulation of storage lipids. When carbon is excessed, a part of the carbon flow is directed towards the generation of cells and the remaining part of carbon source is channeled into the production of storage lipids (Subhash and Mohan 2014).

Fig. 1 Effect of **a** glucose content, **b** initial pH value, **c** incubation temperature and **d** incubation time on lipid production

Effect of pH

The effect of pH on the lipid production was studied in the present research work; the results are presented in Fig. 1b. This figure reveals that the lipid production is increased with increasing the pH. The optimized value obtained from LINGO optimization program is about 6.1 at which the optimum value of the produced lipid is 40% of the obtained dry biomass. Minhas et al. (2016) reported that the pH of the fermentation medium is an important environmental factor affecting cell growth and products formation. Lilly and Horace (1951) reported that the hydrogen ion concentration of the culture medium is strongly affecting the growth, sporulation and metabolic activities of fungus. Besides, the external pH of the medium may affect the plasma membrane permeability; consequently, the change of the external pH affects the membrane osmosis towards the absorption of the different ions and nutrients from the surrounding medium (Amanullah et al. 2001).

Effect of incubation temperature on lipid production

Incubation temperature is one of the most important factors which affect the lipid accumulation. Therefore, the fungal strain (*A. wentii* Ras101) was incubated at varying temperatures ranging from 15 to 35 °C (Liu et al. 2010). As shown in Fig. 1c, lipid content increases with increasing the incubation temperature until it is reached the value of 28 °C. At incubation temperatures higher than 28 °C, the lipid production is decreased. The optimum temperature achieved by Lingo program was 28 °C, which corresponds well with the experimental value shown in Fig. 3.

Effect of incubation time on lipid production

The incubation time is also an important factor for the fungal strain growth and biomass formation. It has an observable effect on lipid production of biomass. The determination of the optimum incubation time can guarantee the efficient utilization of available carbon source in the growth medium, which leads to a higher accumulation of lipid content. Figure 1d shows the relationship between lipid production percentage and incubation time. According to this figure the lipid production content is increased when the incubating time is increased up to about 170 h. According to LINGO software, the optimum time required for the fungal strain growth is about 168 h (7 days). These results are in agreement with data obtained by Ali et al. (2017), who reported that the maximum lipid production by *Aspergillus* spp. was obtained after 5 days of incubation.

Fig. 2 Effect of glucose and nitrates contents on lipid production

Fig. 3 Effect of phosphorous and nitrates contents on lipid production content

Effect of nutrients interaction on lipid production

Factors such as glucose, pH, incubation temperature and time have individual influences on the lipid production content, whereas other factors such as nitrates, NaCl and phosphorous contents do not have any impact, neither on the fungal growth nor lipid accumulation when they are studied individually; however, their effects are appeared when these factors are combined together. The integrated effects of glucose, nitrates, and phosphorous on the lipid production were studied using RSM. Figure 2 (3D diagram) illustrates the interaction between glucose content, nitrates content and lipid production (% of dry biomass). In addition, Fig. 3 shows the influence of phosphorous and nitrates contents on lipid production. According to these two figures, it is

noticed that the lipid production is increased when glucose and nitrates or glucose and phosphorous are increased.

The combination of both glucose content and nutrients content (nitrogen and phosphorous contents) has a positive influence with regard to lipid accumulation. This behavior agrees with Dean et al. (2010) and Kong et al. (2010) research works, which indicate that the limitation of nitrogen and phosphorus in the fermentation medium lead to the increase of lipid accumulation and lipid productivity in the microbial cell. It is worth to note that the representation of the effect of sodium chloride (NaCl) on lipid production content has been excluded because it is found that sodium chloride (NaCl) influence on lipid production content is very weak.

Effect of temperature and time of extraction and transesterification one-step process on the produced amount of biodiesel

The relationship between temperature of the simultaneous extraction and transesterification one-step processes and the produced amount of biodiesel is depicted in Fig. 4a. It is clear that the produced biodiesel amount is increased by increasing temperature until it reaches its optimum amount at a temperature of about 70 °C. After this temperature, the biodiesel tends to decrease with further increasing in temperature; this could be ascribed to the possibility of methanol loss as a

result of vaporization at higher temperatures (Leung et al. 2010). According to Ramadhas et al. (2005), further higher temperature could result in an increase of product cloudiness and production cost. Figure 4b addresses the influence of time of extraction and transesterification processes on the biodiesel amount. It is shown that the optimum amount of biodiesel is achieved at the short time of about 0.5 h. Further increase in time has insignificant effect on the biodiesel production; this could be attributed to the generation of water, which barred further reaction (Ghadge and Hifjur 2005).

Properties of the produced biodiesel

The most important properties of biodiesel needed to be determined are density, water content, calorific value, and viscosity. These properties were determined for the produced biodiesel at Nasr Petroleum Company, Suez, Egypt. The determined properties of the produced biodiesel are compared to the standard properties of both biodiesel and fossil diesel derived from crude oil. As shown in Table 10, kinematic viscosity and water content of the produced biodiesel are in the required limits for both biodiesel and fossil diesel. In addition, the calorific value of biodiesel under consideration is higher than that of both standard biodiesel and fossil diesel. The higher calorific value of the produced biodiesel is an advantage. It is

Fig. 4 Effect of **a** temperature and **b** time of extraction and transesterification processes on the produced amount of biodiesel

Table 10 Properties of the produced biodiesel compared to standard biodiesel and fossil diesel

Properties	Produced biodiesel	Standard biodiesel	Standard fossil diesel
Density at 15 °C, kg/m ³	800	880	850
Kinematic viscosity at 40 °C, mm ² /s	2.8	1.9–6	1.3–4.1
Water content, ppm	66	Max 500	1610
Calorific value, kcal/kg	10,122	9600	10,700

also noted that the density of the produced biodiesel is lower than densities of the two standard diesel types; however, it is nearly close to their standards (Uddin et al. 2013).

Conclusion

The present work was conducted to maximize the lipid production and, consequently, the produced biodiesel derived from the investigated oleaginous fungal isolate *A. wentii* Ras101. This study considers also deriving correlations those relate extracted lipid and produced biodiesel contents to their studied affecting factors. This was achieved using response surface methodology (RSM) through applying the Design Expert software. Analysis of variance method (ANOVA) of the proposed correlations illustrates that there is a good agreement between the experimental and estimated results of lipid and biodiesel production; this, in turn, indicates the validity of the proposed correlations. The optimum lipid and biodiesel production affecting variables could be predicted by using Lingo software version 14. The maximum lipid production of 40% dry biomass is realized when using 50 g/l glucose, 1 g/l nitrates, 1.5 g/l phosphorous, 0.5 g/l NaCl, pH of 6, time of 7 days and temperature of 28 °C. The biodiesel maximum amount of 28% dry biomass (80% lipid) was achieved at 70 °C and 0.5 h. Finally, the required specifications of the produced biodiesel such as density, viscosity, water content and calorific value were determined. The results showed that the produced biodiesel is matched well with the standard specifications of both standard diesel types. Moreover, as an advantage, it is noticed that the calorific value of the produced biodiesel is higher than that of the standard biodiesel.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

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