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Fatty Acid Profiling During Microbial Lipid Production Under Varying pO_2 and Impeller Tip Speeds

Syed Ubaid Ahmed · Sudheer Kumar Singh · Ashok Pandey · Sanjit Kanjilal · Rachapudi B. N. Prasad

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Abstract The fatty acid profile study was undertaken to study the effect of impeller tip speed-associated shear stress and dissolved oxygen saturation (DO) on the fatty acid composition variation and on total lipid content of the cells. The study was undertaken in a 5-l stirred tank bioreactor using *Mucor* sp. RRL001. To study the interaction of parameters and their effects, a central composite design was used. The fatty acid profiling during the course of study suggested that oleic acid and palmitic acid were two major components with their composition varying between 34–47% and 29–39.1%, respectively, of the total lipid content. The GLA content varied between 3% and 9% of the total lipid. The lipid profile study also revealed the presence of a minor amount of fatty acids of chain length C:12, C:20, C:22, and C:24. The modeling of lipid accumulation suggested that it follows a quadratic model with both impeller tip speed ($p=0.0166$) and dissolved oxygen concentration ($p=0.0098$) following the quadratic order of effect. The fermenter run based on the optimum production zone in response surface plot resulted in the maximum 4.8 g l^{-1} lipid compared with the model-predicted value of 4.49 g l^{-1}. The present study suggests that dissolved oxygen saturation is a more significant contributor to total lipid accumulation. However, the study also suggests that the fatty acid profile of fungal lipid is not directly associated with the shear stress or oxygen availability in *Mucor* sp. RRL001.

Keywords Microbial lipid · Fatty acid profile · Central composite design · Impeller tip speed · Dissolved oxygen concentration

S. U. Ahmed · S. K. Singh · A. Pandey (✉)
Biotechnology Division, National Institute for Interdisciplinary Science and Technology (formerly Regional Research Laboratory), CSIR, Industrial Estate PO, Trivandrum, India
e-mail: ashokpandey56@yahoo.co.in

S. Kanjilal · R. B. N. Prasad
Oils and Fats Division, Indian Institute of Chemical Technology, Hyderabad, India

Introduction

Polyunsaturated fatty acids (PUFAs) are grouped into two major series of omega-3 and omega-6 fatty acids. The gamma-linolenic acid (GLA; 6,9,12,*cis*,*cis*,*cis*-octadecatrienoic acid) comes under the omega-6 series. The polyunsaturated omega-6 fatty acids are among the essential fatty acids (EFAs) needed by the body to regulate activities such as heart function, insulin utilization, and mood balance. The body cannot produce EFAs, so they must be provided with the diet or taken as supplements. The essential fatty acid linoleic acid may play a role in ways that the body utilizes insulin, maintains weight, and resists cancer and heart disease. Linoleic acid is converted into GLA when it is processed in the body. With the emergence of clinical evidences of the favorable effect of GLA administration on various chronic diseases such as rheumatoid arthritis [1], with old age-related complications and anticancer properties [2, 3], the interest of pharmaceutical companies in this product has increased. GLA is formed from linoleic acid (LA) and is the first precursor of arachidonic acid (AA); the delta-6-desaturase enzyme is responsible for the conversion of LA to GLA. Microbial lipid production using oleaginous organisms provides an alternative for plant-based lipid production and reduces our reliance on plant oils, which might be subject to seasonal variations.

Although a number of filamentous fungi of the class Zygomycetes accumulate large amounts of oil, they, however, tend to have a low content of GLA and conversely those with high GLA content have only low level of oil [4, 5]. Hence, optimization is required to improve the oil accumulation or total GLA content. Also, the submerged fermentation of fungal systems is severely affected by proper mixing as the growth of mycelia progresses and higher amount of power is required for the proper distribution of nutrients and oxygen and also for the efficient removal of CO_2 from the system. However, the choice of high agitation speed is limited by a higher cost of power input and also damage to mycelia resulting in loss of production. Given the need of minimum dissolved oxygen saturation for low power input requirement, a process needs to be studied for fungal total lipid content under different process conditions. Hence, to study the effect of process parameters such as impeller tip speed and dissolved oxygen saturation, a central composite design (CCD) was employed to find the optimal lipid production conditions. The CCDs are constructed by adding additional axial or star points and center point runs to two-level full or fractional factorial designs. The CCD is a better alternative to the full factorial design as it demands a smaller number of experiments whereas providing comparable results [6, 7].

Although the effect of different amounts of inoculum [8], pH, temperature, carbon, nitrogen, addition of metal ions and oil supplementation [9], and morphology [10] have already been investigated for oleaginous systems, not much information is available on lipid profile variation and its relationship with total lipid in oleaginous mold during different agitation–aeration regimes. In general, fatty acid composition variations have been observed between two subgenus of *Mortierella*. The average fatty acid composition reported for subgenus *Mortierella* ranged from 6.6% to 20.3% for palmitic acid, 4.0% to 14.0% for stearic acid, 6.9% to 36.5% for oleic acid, 4.9% to 14.4% for linolenic acid, and 3.8% to 10.9% for GLA, whereas the fatty acid composition for subgenus *Micromucor* varied from 16.0% to 25.2% for palmitic acid, 1.7% to 5.4% for stearic acid, 21.4% to 41.75% for oleic acid, 16.1% to 25.0% for linolenic acid, and 6.9% to 31.4% for gamma-linolenic acid [9]. However, no further study was performed to study the effect of process variable on overall fatty acid profile of *Mucor* sp. suggesting a need to investigate the change in fatty acid profile under varying shear and oxygen stress conditions. In the present study, an effort was made to investigate the effect of impeller tip speed and dissolved

oxygen concentration on total microbial lipid and its fatty acid composition during different agitation–aeration regimes to asses their roles in the *Mucor* sp. RRL001.

Materials and Methods

Microorganism and Inoculum Preparation

The *Mucor* sp. RRL001 was isolated from Western *Ghats* of Kerala, India and was maintained on the potato dextrose agar (PDA) at 30 °C for 4 days for growth and sporulation. The spore suspension was prepared by scrapping the spores from individual slants in 2 ml of sterile distilled water. The suspension was transferred to 250 ml sterile Erlenmeyer flask containing 50 ml of medium for inoculum preparation. The medium used for inoculum preparation consisted of (in g l^{-1}): glucose 100, yeast extract 10, and peptone 1 with pH adjusted to 5.4±0.1 and was incubated at 30 °C for 24 h at 200 rpm. The 24-h-old inoculum was used to inoculate the fermenter with 10% inoculum (*v*/*v*) [11].

Bioreactor and Experimental Setup

The production media consisted of 100 g l^{-1} glucose and 10 g l^{-1} yeast extract; pH was initially adjusted to 6.5 by using 0.5 M HCl. The experiments were carried out in a bench scale 5-l stirred tank bioreactor-STB (Biostat B-5; B. Braun Biotech-Sartorius) with 3-l of final working volume, fitted with two-stage six-blade Ruston turbine impeller (64 mm diameter) and a stainless steel ring sparger at the base of the impeller. The height to diameter ratio of the bioreactor was 2:1. No pH control was exerted during the fermentation run. The fermentation runs were carried out at different impeller tip speeds of 0.335, 0.670, 1.340, 2.011, and 2.681 m s^{-1}. The impeller tip speed was initially kept constant during the entire run. A specific air or oxygen flow rate was maintained at 0.5 vvm, and a temperature of 30 °C was maintained by circulating the chilled water, whereas foaming in the fermentation broth was controlled by adding silicon oil.

The fermenter was operated in cascade for the dissolved oxygen saturation, which was maintained by supplying air/oxygen. The minimum dissolved oxygen saturation was maintained during the entire fermentation run, and five different minimum dissolved oxygen levels of 5%, 20%, 40%, 60%, and 75% were studied by continuous monitoring using a sterilizable polarographic electrode (Mettler-Toledo InPro6000 Series). The DO electrode was calibrated by two-point calibration method between 0% and 100% oxygen saturation. The fermentation run was terminated after 168 h, and end volume was measured. During the course of fermentation, samples withdrawn periodically at 24 h interval were studied for mycelia breakage and biomass estimation.

Central Composite Experimental Design

The central composite experimental design was used to study the effects of impeller tip speed and dissolved oxygen saturation on total lipid content and fatty acid profile. The independent variables were specified at five different levels coded as −1, +1, 0, $-\alpha$, and α. The design consisted of a total of 10 runs including two center point runs and was divided into two blocks. The experimental design was developed using Statistica version 7 (StatSoft). The impeller tip speed varied from 0.335 to 2.681 m s^{-1}, whereas dissolved oxygen saturation varied from 5% to 75% (Table 1). The analysis of variance (ANOVA) was performed at a p=0.05.

Table 1 Experimental design with predicted, actual, and residual values of total lipid.

Run number	Block	Coded values A	Coded values B	Actual values A	Actual values B	Biomass ($g\ l^{-1}$)	Total lipid actual ($g\ l^{-1}$)	Total lipid predicted ($g\ l^{-1}$)	Residual	% Lipid/ biomass
1	1	1	1	2.011	60	29.4	3.29	3.42	−0.13	11.19
2	1	−1	−1	0.670	20	12.25	3.00	2.68	0.32	24.49
3	1	0	0	1.340	40	39.05	3.94	4.32	−0.38	10.09
4	1	1	−1	2.011	20	21	3.70	3.59	0.11	17.62
5	1	−1	1	0.670	60	13	1.80	1.72	0.08	13.85
6	2	$-\alpha$	0	0.335	40	7.5	1.00	1.23	−0.23	13.33
7	2	α	0	2.681	40	50	2.40	2.36	0.04	4.8
8	2	0	$-\alpha$	1.340	20	28	1.50	1.70	−0.20	5.36
9	2	0	α	1.340	75	16.6	0.80	0.72	0.08	4.82
10	2	0	0	1.340	40	23.3	4.00	3.68	0.32	17.17

A: impeller tip speed ($m\ s^{-1}$), *B*: dissolved oxygen (%)

Lipid Extraction

The mycelia were harvested from the medium by filtration through Whatman no. 1 filter paper, thoroughly washed using distilled water, and then freeze dried (Operon freezer dryer, Korea). The total lipid was extracted from the dried mycelia using chloroform and methanol [12] and residual moisture was removed by adding anhydrous sodium sulfate. The total lipid was finally filtered with Whatman filter paper and was concentrated by vacuum drying (Buchi Rotavapor, Germany).

Methyl Ester Preparation and Fatty Acid Profile

The fatty acid methyl ester (FAME) was prepared by standard protocol [13]. This included refluxing the lipid with boron trifluoride and dried methanol followed by extraction with hexane and washing with distilled water. The hexane layer was separated after brief mixing, and residual moisture was removed from the FAME by adding anhydrous sodium sulfate, then filtered using Whatman no. 1 filter paper, and concentrated by evaporating the hexane on a boiling water bath.

Gas Chromatographic Conditions

The methyl ester samples were analyzed by Agilent 6890 Series Gas Chromatograph equipped with a FID and the capillary column DB-23 (30 m length, 0.25 mm i.d., 0.5 mm film thickness; J & W Scientific, USA). The analytical conditions included injector and detector temperatures at 230 and 250 °C, respectively, whereas the oven was programmed for 2 min at 160 °C, and then increased to 180 °C at 6 °C min^{-1}, maintained for 2 min at 180 °C, increased further to 230 °C at 4 °C min^{-1}, and finally maintained for 10 min at 230 °C. Nitrogen was used as a carrier gas at a flow rate of 1.5 ml min^{-1}. The injection volume of the sample was 1 μl with a split ratio of 50:1.

The fatty acid identification was done by GC-MS using Agilent 6890N Gas Chromatograph and Agilent 5973 Mass Spectrometer at 70 eV (*m*/*z* 50–550; source at 230 °C and quadruple at 150 °C) in the EI mode with an HP-5 ms capillary column (30 m length, 0.25 mm i.d., 0.25 mm film thickness; J & W Scientific, USA). The carrier gas used

was helium and maintained at a flow rate of 1.0 ml min^{-1}. The inlet temperature was maintained at 300 °C and the oven was programmed for 2 min at 150 °C, then increased to 300 °C at a rate of 4 °C min^{-1}, and maintained for 20 min at 300 °C. The injection volume was 1 μl with a split ratio of 50:1. The structural determinations were based on the interpretation of the mass spectrometric fragmentation and by comparison of the retention times as well as the fragmentation pattern of the methyl gamma-linolenate (Sigma). The mass fragmentation patterns were compared with spectral data from the Wiley and NIST libraries.

Results and Discussion

The analysis of lipid yield based on experimental design revealed lipid yield variations with run parameters. The data were analyzed using Statistica release version 7 (StatSoft, USA) by considering the total lipid yield as the response, whereas impeller tip speed and dissolved oxygen concentrations were considered as independent variable. A polynomial equation was developed, which included both linear and quadratic order of effect and interaction of linear effects to predict the total lipid yield (Table 1). The polynomial equation was provided as:

$$Z = -1.804 + 4.295 \times A - 1.459 \times A^2 + 0.128 \times B - 0.002 \times B^2 + 0.015 \times A \times B - 0.317$$

where Z is the predicted response, −1.804 is the intercept, A is the impeller tip speed, and B is the dissolved oxygen concentration. The predicted and actual values were used for the estimation of residual values which are the difference between actual and model-predicted yield. The study revealed a maximum lipid content of 4.0 g l^{-1} at 40% dissolved oxygen saturation and 1.340 m s^{-1} impeller tip speed, whereas a minimum oil yield of 0.80 g l^{-1} was observed at 1.340 m s^{-1} rpm and 75% dissolved oxygen (Table 1). It was also observed that the center point run resulted in a lipid yield of 3.94 and 4.0 g l^{-1}, respectively. The validation studies with an impeller tip speed of 2.013 m s^{-1} and 45% dissolved oxygen saturation resulted in an oil yield of 4.8 g l^{-1}, which was very close to the model-predicted yield of 4.49 g l^{-1} for 1.6621 m s^{-1} for impeller tip speed and 37.7% dissolved oxygen saturation, suggesting the robust response zone provided by the model. The study suggested that both the impeller tip speed and dissolved oxygen concentrations were best explained by quadratic order and were significant for total lipid content (Table 1). The quadratic behavior suggested that both the responses had curvature in response and were not linear in nature. This also confirmed that both impeller tip speed and dissolved oxygen saturation are important for maximum lipid yield. The present study also revealed that both very high and low oxygen saturation were detrimental to the total lipid yield. Similarly, very low and very high impeller tip speeds were not favorable for good lipid yield. The low impeller tip speed may lead to poor agitation of broth whereas a very high impeller tip speed may lead to cell damage resulting in poor lipid yield. The earlier studies for lipid accumulation and fatty acid profile during the fatty acid turnover in *Cunninghamella echinulata* suggested that triacylglycerols (TAGs) containing GLA were hydrolyzed with a slower rate than the other TAG molecular species [14]. Similarly, the study to observe the effect of aerobic and anaerobic conditions on the fatty acid profile of *Mucor rouxii* revealed that the difference in fatty acid profiles obtained under aerobic or anaerobic growth were not directly related to any particular morphology and oxygen limitation led to a decrease in long-chain fatty acids

and an increase in medium-chain saturated fatty acids [15]. In agreement with the current investigation, lipid accumulation by *Yarrowia lipolytica* was clearly enhanced and very high quantities of lipid were accumulated in batch bioreactor experiments in which moderate aeration and agitation regimes were employed; in contrast, at high aeration and agitation rate media, insignificant lipid quantities were indeed accumulated inside the cell structures and metabolism was shifted toward the synthesis of lipid-free material [16]. The strain showed the tendency to degrade its storage lipids with fatty acids C12:0, C14:0, and C16:0 being rapidly incorporated and mainly used for growth needs, whereas C18:0 was incorporated with reduced rates and was mainly accumulated as storage material. Reserve lipids, principally composed of TAGs (55% *w*/*w* of total lipids) and free fatty acids (35% *w*/*w*), were rich in stearic acid (80% *w*/*w*) [16]. In another study using a mixed carbon source of saturated free fatty acids (an industrial derivative of animal fat called stearin), technical glycerol (the main by-product of biodiesel production facilities) and glucose with *Yarrowia lipolytica* resulted in an interesting finding. It was observed that the utilization of technical glycerol and stearin as co-substrates resulted in higher lipid synthesis and increased citric acid production than the combination of glucose and stearin. The lipids produced contained significant amounts of stearic acid (50–70% *w*/*w*) and lower ones of palmitic (15–20% *w*/*w*), oleic (7–20% *w*/*w*), and linoleic acid (2–7% *w*/*w*) [17].

Likewise, in the study with *Candida lipolytica* cultivated in batch bioreactor experiments, lipid accumulation was clearly favored at low dissolved oxygen (D.O.) saturation media, whereas at high D.O., storage lipid concentration decreased and a shift toward extracellular citric acid production occurred [18]. In the above papers, fatty materials (and not glucose, as it was the case of the current manuscript) were used as carbon source. Finally, in disagreement with the results obtained by the authors in the present study, the lipid accumulation process was favored in high oxygen saturation media when glucose was used as substrate by *Rhodotorula gracilis* in chemostat experiments [19].

Although the total lipid yield showed variation with oxygen saturation and impeller tip speed, the fatty acid profile did not show any definite trend in accumulation of short- or long-chain fatty acids, suggesting that the fatty acid profile may not be directly related with oxygen saturation or impeller tip speed-associated shear stress under defined experimental

Table 2 Fatty acid profiles of microbial lipid during varying impeller tip speeds and dissolved oxygen saturation.

Run number	A C12:0	B C14:0	C C15:0	D C16:0	E C16:1	F C18:0	G C18:1	H C18:2	I C18:3	J C20:0	K C20:1	L C22:0	M C24:0	N C24:1
1	–	0.7		30.7	1.1	6	43.5	6.8	8.9	0.9	–	0.4	1	–
2	–	1.1		33.3	4.3	3.2	44.5	5.1	5.8	0.9	–	0.5	1.3	–
3	–	1.2		38.1	3.1	4.6	41.4	4.4	3.7	1.4	–	0.7	1.4	–
4	Traces	1	0.2	34.9	2	3.2	39.2	7.7	9	0.8	0.2	0.4	1.2	0.2
5	Traces	1.2	0.3	29.7	4.7	3.2	46.3	5.3	5.8	0.9	0.3	0.5	1.4	0.4
6	0.1	2.1	0.2	29.1	13.2	2.6	40	5.3	5.3	0.4	0.3	0.2	0.7	0.5
7	–	1.3	0.2	39.1	2.4	2.1	38.1	6.8	7.7	0.5	0.1	0.3	1.2	0.2
8	–	2.1	0.2	38.6	8.9	1.3	38.3	4.4	4.4	0.4	0.2	0.2	0.7	0.3
9	0.7	2.1	0.2	36.6	5.4	2.2	34.9	7.5	8.6	0.3	–	0.2	1	0.3
10	–	1.5	0.1	35.5	7.1	1.7	41.3	5.1	5.8	0.4	0.2	0.2	0.7	0.4

A: lauric acid, *B*: myristic acid, *C*: pentadecanic acid, *D*: palmitic acid, *E*: palmitolic acid, *F*: stearic acid, *G*: oleic acid, *H*: linoleic acid, *I*: gamma-linolenic acid, *J*: arachidic acid, *K*: ecosenoic acid, *L*: docosanoic acid, *M*: tetracosanoic acid, *N*: tetracosaenoic acid

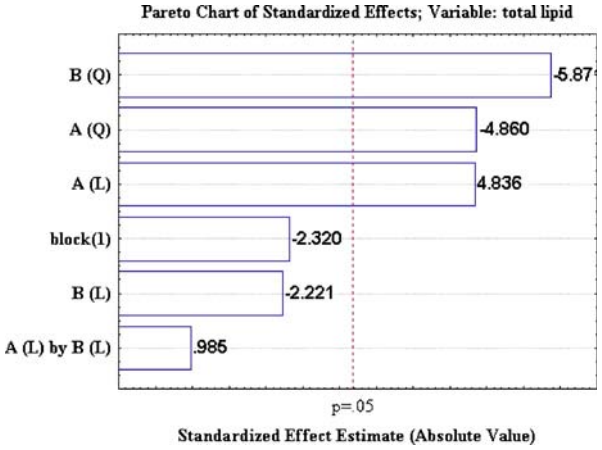

Fig. 1 Pareto chart of effects: *A* impeller tip speed (m s^{-1}), *B* dissolved oxygen saturation, *L* linear order of effect, *Q* quadratic order of effect, *A (L) by B (L)* interaction of linear effects

conditions. However, with both lipid accumulation and GLA biosynthesis being related to the secondary metabolic growth and new biomass production [5], the study of lipid accumulation and fatty acid profile under different impeller tip speeds and oxygen saturations provides process conditions suitable for improved PUFAs profile upon scale up as higher impeller tip speed-associated shear is known to be detrimental to microbial systems [20], which might have implications for microbial lipid production.

The fatty acid analysis of microbial lipid suggested the presence of palmitic (29.1–39.1%) and oleic acid (34.9–46.3%) as a major component (Table 2) along with gamma-linolenic acid (3.7–8.9%, and linoleic acid (4.4–7.7%) as other contributors. It was also observed that the profile of major fatty acid components varied with variations in dissolved oxygen concentration and impeller tip speed. The presence of other fatty acids such as stearic acid, lauric acid, myristic acid, pentadecanic acid, palmitolic acid, arachidic acid, ecosenoic acid, docosanoic acid, tetracosanoic acid, and tetracosaenoic acid were also detected in small amounts. The earlier studies with lipid production and fatty acid profiling of *Mucorales* grown either in submerged or solid-state fermentation conditions have seldom revealed the presence of fatty acids with chain length higher than C:20 [14, 15, 21–23] as was the case in the present investigation in which fatty acids of higher chain length were also observed in trace amounts. The fatty acid profile studies from *Mucorales* especially *Mortierella vinacea* reported higher percentage values for palmitic, stearic, and linoleic

Table 3 ANOVA showing factors and their significance to response.

Factor	SS	*df*	MS	*F*	*p*
Blocks	0.86511	1	0.865105	5.38034	0.103126
Impeller tip speed (L)	3.76047	1	3.760475	23.38750	0.016861
Impeller tip speed (Q)	3.79748	1	3.797476	23.61762	0.016637
Dissolved oxygen (L)	0.79329	1	0.793295	4.93373	0.112917
Dissolved oxygen (Q)	5.54127	1	5.541270	34.46279	0.009859
1L×2L	0.15588	1	0.155881	0.96947	0.397413
Error	0.48237	3	0.160790		
Total SS	13.25921	9			

L: linear order of effect, *Q*: quadratic order of effect, *SS*: sum of squares, *df*: degrees of freedom, *MS*: mean square, *F*: *F* values, *p*: *p* values, *1L× 2L*: interaction of linear effects

Table 4 Factors and their effect on lipid production.

Factor	Effect	SE	*t*	*p*
Mean/interaction	4.002	0.27	14.66	0.0006
Block	−0.63	0.27	−2.32	0.1031
Impeller tip speed (L)	1.30	0.27	4.84	0.0169
Impeller tip speed (Q)	−1.31	0.27	−4.86	0.0166
Dissolved oxygen (L)	−0.56	0.25	−2.22	0.1129
Dissolved oxygen (Q)	−1.61	0.27	−5.87	0.0098
1L×2L	0.39	0.40	0.98	0.3974

L: linear order of effect, *Q*: quadratic order of effect, *1L*× *2L*: interaction of linear effects, *SE*: standard error, *t*: values for t distribution, *p*: *p* values

acids and lower values for the relative percentages of oleic and linolenic acids with the presence of C14:0 and C15:0 fatty acids [24], whereas the fatty acid profile reported from the study of Amano et al. suggested the presence of only C:16 and C:18 fatty acids [21]. Another study with *Mortierella alpina* reported longer-chain fatty acids with chain length more than C:20 comprising 3.7% of the total fatty acid content [25].

The statistical analysis of lipid yield using ANOVA (Table 3) and Pareto chart of effects (Fig. 1) suggested that both impeller tip speed (p=0.0166) and dissolved oxygen (p= 0.0099) quadratic effects were significant effects. To test the statistical significance of various factors on response, it is considered that a factor response predictability greater than 95% or a p value less than or equal to 0.05 is significant. The effect estimate (Table 4) study revealed that dissolved oxygen saturation quadratic effect (−1.614) was contributing more to the total lipid production than impeller tip speed quadratic effect (−1.311). The presence of the negative sign suggested that both were negative effects, but as the order was quadratic in nature, hence, incorporated curvature in response and optimum response zone was observed to be an impeller tip speed of 1.6621 m s^{-1} and 37.7% dissolved oxygen saturation. The block effects (p=0.1031), dissolved oxygen linear order (p=0.1129), and linear interaction effect of dissolved oxygen and impeller tip speed were not significant (p= 0.3974) whereas impeller speed linear order (p=0.0169), although being significant, the response can be better explained by a quadratic effect. The regression analysis of the

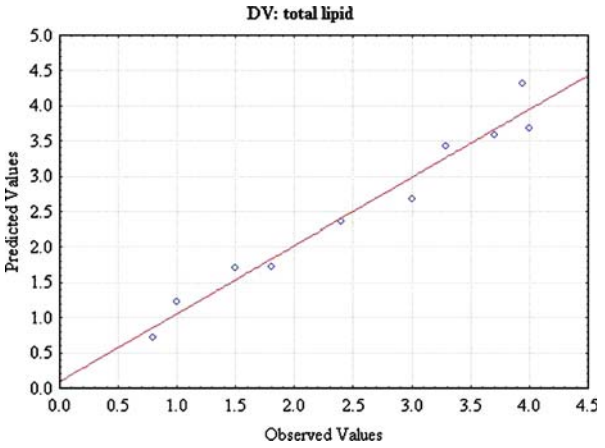

Fig. 2 Graph of observed and predicted values: observed values are experimentally determined lipid yields whereas predicted values are model-predicted lipid yields

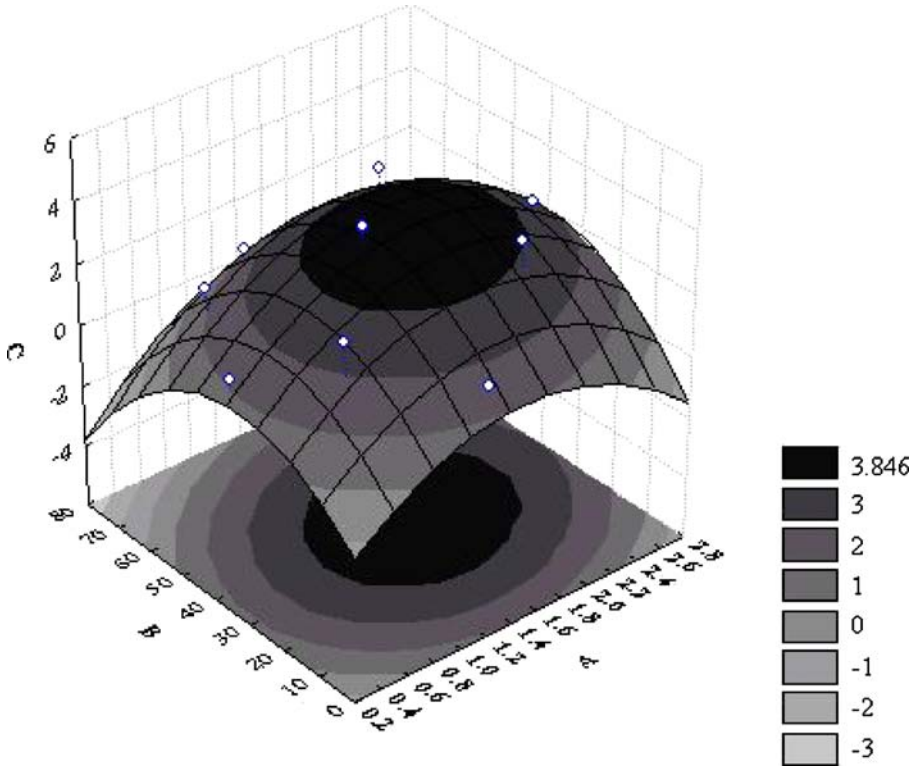

Fig. 3 Response surface providing effects of impeller tip speed (m s^{-1}) and DO (percent of dissolved oxygen saturation) on total lipid yield. *Hollow squares* are experimental values. *A* impeller tip speed values (m s^{-1}), *B* percent dissolved oxygen saturation, *C* predicted lipid yield (g l^{-1})

individual effect suggested that a coefficient of determination (R^2) value of 0.964 and adjusted R^2 values of 0.891 were in close agreement. This ensured a satisfactory adjustment of the proposed model. The coefficient of determination values greater than 0.9 suggest that 90% values of the response can be explained by the proposed model. In the present

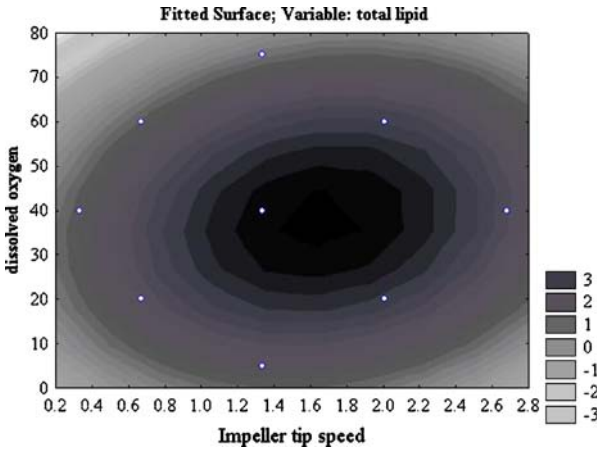

Fig. 4 Contour graph providing the total lipid yield (g l^{-1}) against different impeller tip speeds (m s^{-1}) and DO (percent of dissolved oxygen) saturations. The contours are regions defined for lipid yield; *hollow squares* refer to experimental run conditions

investigation, a R^2 value of 0.964 suggested that more than 96% variation in the response can be explained by the proposed model. The plot of predicted vs observed yield values (Fig. 2) and Table 1 shows both positive and negative dispersion of observed values. The three-dimensional response surface (Fig. 3) and contour graphs (Fig. 4) were plotted based on the model to study the impeller tip speed and dissolve oxygen saturation effects on the total lipid content and fatty acid profile of *Mucor* sp. The contour plot showed a plateau region with lipid production showing gradients at different levels of two parameters and maximum response zone lying near the center point of two parameters.

In the present investigation with *Mucor* sp., we were able to identify fatty acids with chain lengths up to C:24 with total average C:20 to C:24 fatty acid content being in the range of 1.8–3.5% of the total lipid. The GLA content varied from 3.75% to 8.9% of total lipid with specific growth rate during validation run being 0.0125 h^{-1}. The study by Chen and Liu revealed that GLA content was 11.2–13.4% of the total lipid for *Cunninghamella echinulata* CCRC 31840 [8] whereas the study by Kavadia et al. revealed that strains of Zygomycetes belonging to genera *Zygorhynchus*, *Morterella*, *Rhizopus*, *Mucor*, and *Cunnighamella*, when cultivated on glucose, accumulated lipids ranging from 10% to 28% (oil/dry mycelium) [5]. The lipid yields from the present isolate compare favorably (4.8–24% oil/dry biomass) with the reported lipid production. Also, the presence of smaller quantities of the C:12 chain length fatty acid was detected, which has not been reported earlier as a part of mold total lipid. This suggests that the culture is capable of synthesizing smaller, intermediate, and higher chain length fatty acids and may be studied for higher chain length fatty acid production. The ability of the organism to withstand higher impeller tip speed-associated shear stress suggests that the culture is suitable for single-cell oil production [26]. The present investigation reveals that the isolate used in the study, *Mucor* sp. RRL001, is a potential isolate for further strain improvement for enhanced lipid accumulation and for industrial exploitation for PUFAs production.

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