Production of palmitoleic acid by oleaginous yeast *Scheffersomyces segobiensis* DSM 27193 using systematic dissolved oxygen regulation strategy

Xinhai Zhou

Nanjing Tech University

Dawei Zhou

Nanjing Tech University

Xinhui Bao

Nanjing Tech University

Jie Zhou

Nanjing Tech University

Fengxue Xin

Nanjing Tech University

Wenming Zhang

Nanjing Tech University

Xiujuan Qian

Nanjing Tech University

Weiliang Dong (✉ dwl@njtech.edu.cn)

Nanjing Tech University

Min Jiang

Nanjing Tech University

Katrin Ochsenreither

Karlsruhe Institute of Technology: Karlsruher Institut für Technologie

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Abstract

Background: Recently, a newly isolated oleaginous yeast strain *Scheffersomyces segobiensis* DSM 27193 was identified with the capability of accumulating high contents of palmitoleic acid (POA) in its intracellular lipid (13.8%). However, high amounts of ethanol as the main by-product were also produced in this system. To eliminate ethanol formation and to further improve POA production, process optimization focused on dissolved oxygen (DO) regulation was conducted in this study.

Results: As a result, agitation control was found to be highly beneficial for cell growth and lipid production. When keeping $pO_2 > 40\%$ through dynamic agitation control, significant improvements toward lipid (70.12% increase) and POA (1.44 fold) production have been obtained, and the accumulation of ethanol was decreased to 2.3 g/L. While increasing the aeration rate to 2 vvm, no significant decrease has been found in ethanol accumulation. Further application of a two-stage agitation regulation (600 rpm in the first 48 h fermentation then adjusted the agitation speed to 1000 rpm) gave 10.23 g/L of lipid (50% increase) with 2.02 g/L of POA (1.15 fold) production. At last, under the best condition of 1000 rpm of agitation and 1 vvm of aeration, no ethanol was detected during the whole fermentation process, while a dry biomass concentration of 44.8 g/L with 13.43 g/L of lipid and 2.93 g/L of POA was achieved. Transcription analysis revealed that ethanol synthetic pathway was down regulated under the condition of high agitation, while the expression of the key enzymes responsible for lipid and POA accumulation were enhanced.

Conclusion: This study introduces the oleaginous yeast chassis *S. segobiensis* DSM 27193 and an effective pO_2 regulation strategy for efficient biological production of POA.

Background

Palmitoleic acid (C16:1 Δ 9) (POA), as an important monosaturated fatty acid (MUFA), has attracted great interest due to its high value in medical and chemical applications [1]. It is reported that POA can reduce nonalcoholic fatty liver disease caused by liver inflammation, decrease the progression of atherosclerosis in obese mice, lower the production of inflammatory cytokines and prevent lifestyle diseases such as diabetes and cardiovascular diseases [1–4]. POA-rich diets have also been shown to be beneficially affects serum lipids/lipoproteins, resulting in the reduction of total and LDL cholesterol [5]. In nature, sea buckthorn and macadamia nut oil contain 20%-40% POA, making them a possible natural source for POA [6]. However, the rare natural occurrence of these plants and their low total lipid content (e.g., 3.5% for the sea buckthorn) limit the commercial development of POA production [1, 7].

Currently, a number of oleaginous microorganisms, including microalgae, yeasts and filamentous fungi, have been screened for their capability of accumulating relatively high amounts of POA [6, 8]. Among those microorganisms, microalgae and cyanobacterium have attracted much attention for their abundant POA content [9]. For example, *Tribonema minus* and *Oscillatoria* sp. can accumulate more than 50% of POA in their intracellular lipids [10, 11]. Some other microalgae like *Dunaliella primolecta* and *Nannochloris* sp. can also accumulate POA in the range of ~ 8% – 25 % of lipid [8]. However, growth rates of microalgae are relatively low, and the lipid production process requires sufficient light and large space, limiting the fermentation process by weather, climate, site, etc.[12]. In addition, lipid extraction of microalgae is very energy-consuming, and gene editing of microalgae is relatively difficult emphasizing the disadvantages of developing microalgae for POA

production. In our previous study, the oleaginous yeast *Scheffersomyces segobiensis* DSM 27193 has been recognized for accumulating high amounts of POA (approximately 16% in its intracellular lipid) [13]. Compared with microalgae, oleaginous yeasts possess the advantages of easy cultivation, fast growth rates resulting in high biomass concentrations, high lipid content, wide substrate spectrum, and strong adaptability in industry [14], which endow oleaginous yeasts great potential in functional MUFA production.

The level of dissolved oxygen (DO) is one of the most important factors affecting the cell growth and lipid accumulation during the fermentation process. Besides, The degree of unsaturation can also be regulated by the variation of DO, due to the oxygen requirements of key enzymes responsible for regulating lipid unsaturation [15]. The requirements of DO concentration seems to be strain-related and appear to be variable among oleaginous yeasts. For example, low DO concentration led to a high final lipid content in *Yarrowia lipolytica*, while its cell growth was enhanced at high DO concentration [16]. The same phenomenon has also been observed in *Rhodotorula glutinis* [17]. In contrast, low lipid yields have been obtained by *Apiotrichum curvatum* under lower DO level [18]. In addition, the influence of DO concentration on lipid unsaturation also needs to be investigated for every strain. For example, low DO conditions decreased the lipid unsaturation in *Lipomyces starkeyi* lipid [15], while it increased the lipid unsaturation in *Saccharomyces cerevisiae*, since the expression of desaturase was induced under low DO condition [19].

Aeration and agitation are the two common ways for regulating the DO concentration in medium. Higher aeration rates are usually beneficial for cell growth by improving mass transfer of substrate, product and oxygen and enhancing the oxygen availability since oxygen is an electron acceptor [20]. Agitation can also facilitate the transfer of mass and heat in the reactor, but high agitation rate will inevitably cause stronger shear forces, leading to morphological changes and might even damage the cell structure [21, 22]. In this study, the effect of different DO concentrations expressed as partial pressure of oxygen (pO_2) regulated by agitation and aeration on cell growth, lipid production, POA accumulation and ethanol generation were comprehensively investigated. Furthermore, the transcription analysis indicated that the key enzymes of POA production were significantly activated under the best condition of 1000 rpm of agitation and 1 vvm of aeration, which will provide more information and experience for POA, even more MUFAs biological production.

Results And Discussion

Characteristics of *S. segobiensis* DSM 27193 fermentation using fed-batch culture

Fig. 1 displays the cell growth and lipid accumulation of *S. segobiensis* DSM 27193 using fed-batch culture with glucose as sole carbon source. The fermentation process of general oleaginous yeast was usually be divided into two phases: cell growth phase and lipid accumulation phase [23]. Surprisingly, *S. segobiensis* DSM 27193 kept the cell growth during whole the fermentation time. At the end of the first phase, 9 g/L of biomass with only 5.78% of lipid has been obtained. Nitrogen depletion induced the lipid accumulation phase at ~50 h. Finally, 28.6 g/L of dry biomass and 19.2% per dry biomass of lipid has been obtained, corresponding to 5.49 g/L of lipids. Fatty acid profile analysis revealed that *S. segobiensis* DSM 27193 contained 13.8% of POA in its intracellular lipid. At present, high content of POA is usually reported in *Kluyveromyces polysporus* and *S. cerevisiae*, however, the total lipid content in these non-oleaginous yeasts is much lower than *S. segobiensis* DSM 27193[24]. In contrast, oleaginous yeasts like *R. glutinis* and *Trichosporum cutaneum* can accumulate

higher lipid amounts, but their POA content is much lower, as <5% of POA have been detected in their intracellular lipid [24]. The high POA content in the lipid profile of *S. segobiensis* DSM 27193 endows it promising potential in developing POA commercial production.

In addition to intracellular lipids, 6.26 g/L of ethanol has been detected as the main by-product in the fermentation process. As shown in Fig 1, ethanol began to accumulate at ~48 h, when pO_2 decreased to 0%. The limited oxygen availability might have stimulated the production of ethanol, characterizing this yeast as Crabtree negative [25], contrary to Crabtree positive yeast like *S. cerevisiae*, which only produces ethanol under the condition of excess carbon source [26]. Ethanol production might lower the carbon flux toward lipid production. Therefore, process optimization strategies should focus on DO regulation to improve lipid and POA production and to decrease ethanol accumulation.

Effect of dissolved oxygen concentration on cell growth and lipid accumulation

DO concentration plays a key role in the production of lipid and is also reflected by cell growth during the fermentation process. In the early phase of cultivation, pO_2 of the medium decreased rapidly even to zero and maintained at a low level throughout the exponential phase (data not known), which inhibited cell growth and lipid production, while promoting ethanol formation[13]. Therefore, sufficient oxygen supply in the early phase (approximately at 48 h) should be helpful for enhancing cell growth and lipid accumulation.

In this study, three different pO_2 levels of $pO_2 > 20\%$, $pO_2 > 40\%$ and without pO_2 control (constant agitation at 600 rpm) were carried out through dynamic agitation regulation. The initial agitation speeds were set at 600 rpm. Fast cell proliferation led to rapid pO_2 decrease. Within 30 h and 72 h cultivation, pO_2 level decreased to 40% and 20%, respectively. Subsequently, the dynamic agitation regulation was launched. After around 2 days of cultivation, 850 rpm and 750 rpm were necessary to maintain $pO_2 > 40\%$ and $pO_2 > 20\%$, respectively. In terms of cell growth, 33.9% improvement in cell growth has been achieved by maintaining $pO_2 > 40\%$ compared to no pO_2 control, indicating the limitation of DO in the medium during the experiment without pO_2 control; however, no obvious difference has been found between $pO_2 > 20\%$ (28.6 g/L) and without pO_2 control (29.55g/L) (Fig. 2A).

Fig. 2C showed the lipid content variation under different pO_2 conditions. 19.2%, 21.7% and 24.4% of lipid content per dry biomass has been achieved without pO_2 control, $pO_2 > 20\%$ and $pO_2 > 40\%$, respectively, corresponding to lipid concentrations of 5.49 g/L, 6.41g/L and 9.34 g/L, respectively (Fig. 2D) (Table.1). Lipid composition analysis determined that the main fatty acids in *S. segobiensis* DSM 27193 are palmitic acid (C16:0), POA (C16:1), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and eicosanoic acid (C20:0) regardless of the oxygen supply strategy (Table.2). Thereof, POA and C18:1 were the major components constituting 13.8% and 60.1% of the total lipid, respectively. POA ratio could be increased from 13.8% to 16.4% and 19.9% when maintaining $pO_2 > 20\%$ and $pO_2 > 40\%$ compared with the without pO_2 control, respectively. In contrast, C18:1 decreased from 60.1% to 54.7% when keeping $pO_2 > 40\%$. We also noted that C16:0, the precursor of POA slightly increased from 12.95% to 16.4% once keeping $pO_2 > 40\%$. The increase of DO concentration regulated by agitation might prevent the transformation of C16:0 to C18:0, so that more POA could be accumulated. The phenomenon that variation in pO_2 levels affects the fatty acid profile has also been observed in *Lipomyces starkeyi*, *Y. lipolytica* and *Rhodotorula gracilis* [15, 27, 28]. While in these studies, fatty

acid desaturases were considered to be oxygen-related enzymes and oxygen-rich conditions reflected relative activity of desaturases and finally led to an increase in the degree of unsaturation of fatty acids.

Table 1. Product titers and yields obtained at different pO₂ levels

pO ₂	Biomass (g/L)	Lipid content (%per CDW)	lipid production (g/L)	POA ratio (%)	POA production (g/L)	$Y_{L/g}$ (g lipid/g glucose)	$Y_{L/x}$ (g lipid/g biomass)
600 rpm	28.6±1.53	19.2±0.45	5.49±0.42	13.8±1.05	0.76±0.12	0.04	0.19
pO ₂ >20%	29.5±2.12	21.7±0.73	6.41±0.68	16.7±1.61	1.07±0.22	0.04	0.22
pO ₂ >40%	38.3±3.16	24.4±0.23	9.34±0.86	19.94±1.10	1.86±0.27	0.07	0.24

$Y_{L/g}$ (g lipid/g glucose) was estimated by the formula $X_L/g = \text{Lipid production}/\text{glucose consumption}$

$Y_{L/x}$ (g lipid/g biomass) was estimated by the formula $X_L/x = \text{Lipid production}/\text{biomass}$

Table.2 Fatty acid composition profile in % of total fatty acids of *Scheffersomyces segobiensis* DSM 27193 under different pO₂ levels

Fatty acid species	600 rpm	pO ₂ >20%	pO ₂ >40%
C16:0	12.9±1.14	14.1±0.90	16.4±0.50
POA	13.8±1.05	16.4±1.64	19.9±1.10
C18:0	0.7±0.07	0.6±0.22	0.4±0
C18:1	60.7±0.78	57.7±0.61	54.7±0.88
C18:2	10.8±1.54	9.7±1.14	7.6±0.30
C20:0	1.1±0.04	1.5±0.24	1.0±0

In terms of ethanol production, high DO concentration lowered ethanol production, as the highest ethanol concentrations of 6.26 g/L, 5.76 g/L and 2.3 g/L have been detected under the conditions of constant agitation at 600 rpm (no pO₂ control), pO₂>20% and pO₂>40%, respectively (Fig. 2B). Interestingly, the highest ethanol accumulation was found in the middle period of fermentation process, and the produced ethanol was reassimilated in the later fermentation stage in all experiments. Speculatively, on one hand, the decrease of oxygen demand in the later stage of fermentation led to the DO increase in the fermentation medium, therefore, the prerequisites of DO limitation for ethanol production has been rescinded, hence limiting the further production of ethanol; while on the other hand, the increased oxygen supplied to medium caused an increasing oxidation of the ethanol produced even in the presence of glucose in the fermentation medium. The same ethanol reassimilation phenomenon has also been described in *Candida tropicalis* and *Pachysolen tannophilus* [29, 30]. Despite the fact that high DO control decreased the ethanol production, 2.3 g/L of ethanol was still

produced at 69 h under the condition of $pO_2 > 40\%$. Therefore, more considerate DO regulation strategy was required.

Effect of aeration rate on cell growth and POA production

Aeration control is another important DO regulation strategy. Compared with agitation, aeration regulation imposes lower shear stress to the cells and energy consumption. In this study, three different aeration rates of 0.1 vvm, 1 vvm and 2 vvm were tested with a constant agitation speed of 600 rpm. Fig.3A shows the cell growth under different aeration conditions. When aerating with 0.1 vvm, *S. segobiensis* DSM 27193 grew slowly, and only 8.8 g/L of dry biomass were obtained at the end of fermentation process. In contrast, high aeration invigorated cell growth throughout the fermentation process, and maximal dry biomass concentration of 46.8 g/L was obtained under the condition of 2 vvm, whereas 33 g/L of dry biomass has been reached with 1 vvm aeration.

As expected, higher aeration rates did also increase the lipid accumulation, as 18.91%, 20.05% and 28.26% lipid per cell dry mass was reached when employing aeration rates of 0.1 vvm, 1 vvm and 2 vvm, respectively (Fig. 3C). In addition, lipid accumulation was accelerated when using the highest aeration rate. Lipid composition analysis revealed, that the accumulation of POA and C18:2 was slightly higher at the lowest aeration rate of 0.1 vvm, while the amount of C16:0 and C18:1 decreased from 12.95% to 7.91% and from 60.10% to 52.47%, respectively (Fig. 5A). Low DO was speculated to induce the expression of Δ -9 fatty acid desaturase, which is responsible to form a double bond in saturated fatty acyl-CoA substrates, thereby increase the unsaturation level of lipid, which has been confirmed in yeast *S. cerevisiae* [19]. However, POA and linoleic acid content was more or less constant regardless of the aeration rate.

In terms of ethanol production, high ethanol levels up to 20 g/L were produced with the lowest aeration rate (0.1 vvm) (Fig.3B), which seems not to be reassimilated in the later fermentation stages. Instead, it stagnated at a high level. This might be due to the low oxygen availability at the lowest aeration rate used. A similar phenomenon is known for *P. stipites*, where the volumetric oxygen transfer coefficient (KLa) of less than 11.7 h^{-1} inhibited ethanol assimilation [31]. However, even at higher aeration rates, about 6 g/L (1 vvm) and 7.4 g/L (2 vvm) of ethanol was produced around 120 h fermentation time, indicating DO limitation. Therefore, higher applying aeration rates are seemingly not sufficient for decreasing ethanol production. Further combination of both aeration and agitation regulation might not only favor cell growth and lipid production, but might also decrease ethanol production, and was therefore tested in the next experiments.

Two-stage agitation regulation to enhance POA production

Considering that higher agitation speed did significantly improve cell growth and lipid production without exerting damaging stress to the yeast cells, and the high DO demand in the early fermentation period, two high agitation regulation strategies were applied: ① constant high agitation speed of 1000 rpm; ② two-stage agitation regulation with 600 rpm in the early fermentation phase which was switched to 1000 rpm after 48 h to avoid reduction of cell growth. For both strategies 1 vvm was selected as the aeration rate, when taking all factors (cell growth, lipid and POA production, ethanol production) into consideration.

As shown in Fig. 4A, high agitation speed had a positive effect on cell growth as expected, as the biomass concentration of 44.8 g/L and 42.1 g/L were obtained under the condition of 1000 rpm and the two-stage agitation regulation, respectively. Additionally, nearly no ethanol was detected throughout the fermentation when using constantly high agitation speed of 1000 rpm (Fig. 4B). In contrast, with the two-stage agitation regulation ~2 g/L of ethanol were produced at 45 h, which was quickly reassimilated after the agitation speed increase. Constant agitation at 1000 rpm could keep pO_2 level always >40%, while in the two-stage agitation regulation, pO_2 was decreased to 10% during the early fermentation period.

In terms of lipid production, 1000 rpm and two-stage agitation regulation increased lipid content to 30.07% and 25.3%, respectively (Fig. 4C). In this case, high pO_2 condition had both positive effects on cell growth and lipid accumulation for *S. segobiensis* DSM 27193. In addition, POA content was greatly improved from 13.8% to 21.78% with constant high agitation of 1000 rpm compared to the control condition (Fig. 5B), and the final POA production of 2.93g/L was achieved, corresponding to a 2-fold increase.

The synthesis pathway of POA in *S. segobiensis* DSM 27193 was displayed in Fig.6A. Transcription analysis of the key enzymes in ethanol production pathway (ALDH1, ADH1, PDC1) and POA synthetic pathway (ACC, DGAT1, DGAT2, OLE1, OLE2) in the early fermentation phase were displayed in Fig.6B. ALDH1, ADH1, PDC1 were strongly induced at 600 rpm compared with high agitation condition. In detail, the transcription levels of ADH1 at 600 rpm was 3.6 times higher than that at 1000 rpm; Also, the transcription of PDC1 under the condition of 600 rpm displayed 3 times higher than that achieved in 1000 rpm, indicating ethanol synthetic pathway was significantly activated at low DO condition in the early fermentation phase. Unsurprisingly, the transcription of the key enzymes such as DGAT1 and OLE2 responsible for lipid synthesis and desaturation in *S. segobiensis* DSM 27193 were increased at higher DO condition in the early fermentation phase. In specific, the transcription of DGAT1 was up-regulated by 5.4 times and OLE2 was up-regulated by 3.76 times under the condition of 1000 rpm. This may explain why increasing the agitation speed can effectively increase the total lipid production and the proportion of POA.

Conclusion

In this work, *S. segobiensis* DSM 27193 was evaluated as a candidate for lipid and POA production. The study revealed that DO concentration has a great impact on the cell growth, POA accumulation, as well as the ethanol production in the strain DSM 27193. Applying the optimized conditions, a moderate POA production (2.93 g/L) was achieved. To our knowledge, this is the first report of POA as the target product in a wild-type yeast strain. Current advances in yeast engineering for POA production will establish *S. segobiensis* DSM 27193 as a reliable source for further improvement in POA production.

Methods

Strains and media

Scheffersomyces segobiensis (syn. *Pichia segobiensis*) DSM 27193 used in this study was deposited at DSMZ culture collection in Braunschweig, Germany. The glycerol stocks were stored at -80°C in the in-house culture collection. For plate cultivation, Yeast Malt (YM) Agar plates were used (g/L): yeast extract 3.0, malt extract 3.0, peptone 5.0, glucose 10.0, agar 20.0. For lipid and POA production, a mineral salt medium was used as

described by Ines Schulze et al. [13]. 50 g/L of glucose was used initially, from the 2nd day, glucose was replenished to a maximum concentration of 90 g/L after determining the actual concentration.

Batch fermentation in bioreactor with controlled dissolved oxygen levels

A single colony of yeast cells was picked from YM agar plates and then inoculated into 20 mL mineral salt medium in a 200 ml baffled shake flask as pre-culture I. Pre-culture II was prepared with 200 ml using the same medium in 2 L shake flasks and inoculated with pre-culture I to obtain an initial OD₆₀₀ of 0.5-1.0. The seed cultures were cultivated at 25°C and 150 rpm for 24 h.

Fermentation was performed in a 2.5 L fermenter (Infors HT, Bottmingen, Switzerland; Minifors fermenter) with working volume of 1.2 L, an initial OD₆₀₀ of 0.5–1.0. The Cultivation temperature was 25°C. A constant pH of 5.0 was maintained automatically by 4 M H₃PO₄ and 4 M NaOH. In each fermenter, Contraspum A 4050 HAC was applied as antifoam agent.

The initial fermentation condition (control) was set at 600 rpm and 1 vvm without control of pO₂. The pO₂ value was measured constantly by a probe. The condition of pO₂>20% and pO₂>40% was automatically maintained by continuously increasing the agitation if pO₂ dropped below the set-point. In the two-stage strategy, fermentation was started with 600 rpm and the agitation was enhanced to 1000 rpm after the first 48 h.

Determination of biomass

The cell concentration in the culture medium was determined by the optical density at 600 nm (OD₆₀₀) with the initial culture medium as blank. All samples were diluted to an optical density of 0.2–0.8 at 600 nm for measurement.

Biomass was analyzed gravimetrically. 1 mL aliquot of the culture broth was transferred into a pre-dried and pre-weighed 1.5 mL reaction tube and centrifuged at 13,000 rpm for 5 min. The supernatant was collected and used for the determination of glucose. The cell pellet was washed with 1 mL saline (0.9% NaCl), dried at 60 °C for 24 h and weighed.

Lipid analysis

The extraction of cellular lipids and determination of fatty acid composition were modified according to the method of Qian et al[23].

Quantitative PCR

Total messenger RNA (mRNA) was extracted using Vazyme's FastPure Cell/Tissue Total RNA Isolation Kit. Total mRNA (2 µg) was then treated with TURBO DNase (Ambion) to remove genomic DNA. Total mRNA (2 µg) was then treated with

TURBO DNase (Ambion) to remove genomic DNA. cDNA was synthesized from mRNA using Tiangen's QuantiTect Reverse Transcription kit. Primers were designed using Primer Premier 6 and are listed in Table S1. The ITS gene was used as the reference gene for internal control. Standard curves were generated using serial

dilutions of *Scheffersomyces segobiensis* DSM 27193 genomic DNA as the template. All data points were collected from three biological replicates.

Abbreviations

POA: Palmitoleic acid; **DO**: dissolved oxygen; **MUFA**: monosaturated fatty acid; **pO₂**: partial pressure of oxygen; **Pyr**: pyruvate; **G6PD**: Glucose-6-phosphate dehydrogenase; **ALDH**: aldehyde dehydrogenase; **ADH**: alcohol dehydrogenase; **PDC**: pyruvate decarboxylase; **CAT**: catalase; **ACC**: acetyl-CoA carboxylase; **DGAT1**: diacylglycerol acyltransferase; **DGAT2**: 2-acylglycerol acyltransferase; **OLE**: stearyl-CoA desaturase

Declarations

Ethical Approval and Consent to participate

Not applicable.

Consent for publication

All authors consent the manuscript for publication in Biotechnology for Biofuels.

Availability of supporting data

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZXH conceived, designed the experiments, performed the laboratory work, analyzed, and interpreted the data and drafted the paper. ZDW and BXH performed the sample determination. ZJ, XFX, ZWM critically revised the manuscript. MJ, QXJ, DWL and KO contributed to experimental design and data interpretation and also critically revised the manuscript. All authors read and approved the final manuscript.

Availability of data and material

The information about accession numbers is given in the manuscript and its additional files.

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Authors' information

All of the information of the authors has been listed on the title page.

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Figures

Figure 1

Scheffersomyces segobiensis DSM 27193 cultivated on glucose in 2.5 L-bioreactor in mineral salt medium at pH 5 and 25°C; Aeration rate of 1vvm and agitation speed of 600 rpm were applied and glucose was fed to 90 g/L daily.

Figure 2

Influence of different pO_2 levels of *Scheffersomyces segobiensis* DSM 27193 on the production of lipid. A cell growth. B ethanol accumulation. C Lipid content per dry biomass. D Lipid concentration

Figure 3

Cultivation of *Scheffersomyces segobiensis* DSM 27193 with varying aeration rates (0.1 vvm, 1 vvm, 2 vvm). A cell growth. B ethanol accumulation. C lipid content per dry biomass. D lipid concentration

Figure 4

Cultivation of *Scheffersomyces segobiensis* DSM 27193 with varying agitation speeds (600 rpm, 600 rpm for 48 h and subsequent increased to 1000 rpm, 1000 rpm). A cell growth. B ethanol accumulation. C lipid content per dry cell weight. D lipid concentration

Figure 5

Fatty acid compositions (%) of *Scheffersomyces segobiensis* DSM 27193 at varying aeration rates (0.1 vvm, 1 vvm, 2 vvm) (A) and agitation speeds (600 rpm, 600 rpm at first 48 h and subsequent increased to 1000 rpm, 1000 rpm) (B).

A

Figure 6

Metabolic pathway for POA and ethanol production within *Scheffersomyces segobiensis* DSM 27193 (A) and relative mRNA expression level under high agitation speed condition (1vvm, 1000 rpm) at 70h (B). mRNA expression levels are normalized to ITS mRNA expression, which was used as an internal standard. The data show the mean and standard deviation resulting from three biological replicates.

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