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Designing of a "cheap to run" fermentation platform for an enhanced production of single cell oil from *Yarrowia lipolytica* DSM3286 as a potential feedstock for biodiesel

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HIGHLIGHTS

- Different culture media were tested for lipid production.
- The feasibility of produced oils for biodiesel production were evaluated.
- A cost-effective minimal culture medium for lipid was obtained.
- The scale-up of the designed minimal medium led to a lipid content of 65%.
- *Yarrowia lipolytica*'s oils were highly suitable for quality biodiesel production.

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ABSTRACT

In this study, the culture medium components screening and filtering were undertaken in order to set up efficient and cost effective minimal culture media for lipid production from *Yarrowia lipolytica* DSM3286. The basal minimal culture medium (S2) designed yielded lipid content up to 35% of the microbial dry cell weight. A set of fermentation strategies based on this minimal medium was developed and the lipid content was raised to 51%. The scale-up under different fermentation conditions based on S2 medium led to a maximum lipid content of 65%. The produced microbial oils displayed interesting properties to be used as a feedstock for high quality biodiesel production. The minimal media and operable cultivation strategies devised in this study, in association with the works done so far by other authors, could enable fast, massive, viable and more economical production of single cell oils and smooth biodiesel manufacture.

1. Introduction

Yarrowia lipolytica, the archetypical oleaginous yeast of the *Yarrowia* clade owing to comprehensive information provided by genomic, systems biology, genetic engineering and transcriptomic data (Beopoulos et al., 2009; Michely et al., 2013) achieved these recent years, is talented in the buildup of valuable lipids (Beopoulos et al., 2009; Fontanille et al., 2012; Michely et al., 2013; Papanikolaou and Aggelis, 2002; Tsigie et al., 2011) and is considered as a promising sustainable biocommodity production scheme for biodiesel due to the "vegetable oils-like" profile of fatty acids accumulated (Katre et al., 2012). Conversely, for a high-performance substantiation of *Y. lipolytica* for industrial production of biodiesel, a high production rate of lipids able to generate fatty acids leading to high-quality biodiesel is compulsory. Different cultivation modes and conditions developed so far have revealed that engineered or the wild-type strains of *Y. lipolytica* can only accumulate lipids as up to 62% of its dry mass. The ability of *Y. lipolytica* to accumulate lipids is intrinsically related to its physiology and biochemistry and occurs via the *de novo* synthesis or the *ex novo* accumulation mechanisms. These mechanisms differently affect the amount and the fatty acids profile of lipids produced and subsequently reverberate on the performance of biodiesel fuels. In the *de novo* synthesis, wild-type cells can only accumulate up to 20% of their dry cell weight as lipids within a period of 3–10 days. Several studies regarding the enhanced oil production from *Y. lipolytica* either at the microbial system level or by the

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optimization of the fermentation medium composition, cultural conditions and the implementation of an efficient operative fermentation process were envisaged. More recently, the *in silico* model of *Y. lipolytica* iYL619_PCP was reconstructed in our research center to survey the would-be improved lipid production from the said species and the odds of employing minimal culture media for cell growth were foretold (Pan and Hua, 2012), suggesting the possibility of making available minimal media for lipid production. The data of its metabolic features also motivated numerous scientists to engineer metabolic pathways of *Y. lipolytica* to increase oil and biofuels productivity from this species (Beopoulos et al., 2008). More recently, through genotypic and phenotypic optimization, *Y. lipolytica*'s native metabolism was rewired for superior *de novo* lipogenesis leading to a lipid content of 90% (Blazeck et al., 2014). Nevertheless, genetic engineering tools encompass a number of shortcomings that are inherent in the fact that these approaches are overpriced and the amounts of oils yielded rarely attain the expected values since the improvement rate is generally too low. In view of these, further studies must be enthused for developing new improvement strategies of the bioprocess routes and substantial attempts have been made to this purpose. For instance, a two-phases fed-batch cultivation of *Y. lipolytica* for lipid production has been developed with the advantage of providing the possibility of nutrient adjunction in sequential combination and fermentation process control (Fontanille et al., 2012). But still, the development of more cost-effective, efficient and easily operable fermentation strategies needs to be settled. More importantly, components constituting the culture medium need to be screened and filtered in order to eliminate undesirable compounds and subsequently reduce the cost of the fermentation process. Generally, the culture medium components based improvement of lipid accumulation in oleaginous microorganism is carried out by limiting some nutrients such as nitrogen, phosphate and sulfate in the medium, with a carbon source in excess (Beopoulos et al., 2009; Wu et al., 2010).

With the ceaselessly increasing development of the mechanization, the rarefaction of natural resources, the more and more increasing demand in term of transport and energy needs, it becomes very essential to set up an effective fermentation platform that will be ready to meet these needs in a permanent and long-lasting way with more productive outcome trough sustainable innovation.

Accordingly, the aspiration of this survey is the development of very minimal culture media for an efficacious and reasonably priced production of single cell oils (SCOs) with biodiesel properties from *Y. lipolytica* DSM3286. Besides, we aim at developing and scaling-up pure fermentation schemes under precise culture conditions for a maximal production of microbial lipids.

2. Methods

2.1. Media designing

Different series of culture media were developed using a stock of chemical compounds consisted of KH_2PO_4, Na_2HPO_4, $MgSO_4 \cdot 7H_2O$, Glucose, $(NH_4)_2SO_4$, Yeast extract, $CaCl_2 \cdot 2H_2O$, $FeCl_3 \cdot 6H_2O$, $ZnSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$ and $CoCl_2 \cdot 6H_2O$ in view of those habitually used in the literature. All designed media were indicated in Table 1. In specific cases, glucose was replaced by other carbon sources such fructose, glycerol, xylose, maltose, trehalose, L-malate, citrate and sucrose in order to explore the effect of these carbon sources.

2.2. Strain and cultivation conditions

A wild type strain, *Y. lipolytica* DSM3286 purchased from the culture collection of the DSMZ (Germany), was used in this study. The pre-culture was obtained by inoculating a separate colony in YPD medium containing (g/L) glucose 20, peptone 20 and yeast extract 10 and incubating it at 30 °C for 24 h prior to cultivation. The lipid production experiments were performed in duplicate, aerobically, in 250 mL Erlenmeyer flasks containing 100 mL of each of the designed media and inoculated with the pre-culture (initial OD600 = 0.01) and incubated at 30 °C in a rotary shaker incubator under agitation conditions (220 rpm).

2.3. Bioreactor scale up and improvement of fermentation conditions

The S2 medium was used for improving the aerobic fermentation conditions through the monitoring of dissolved oxygen (DO). Cultivations were operated in batch mode in a 1.5 L stirred-tank fermenter (Shanghai Biotech, Shanghai, China) with a working volume of 1 L at 30 °C without pH control. The aeration rate was fixed at 1 vvm (volume air per volume per minute) and the agitation rate was set automatically in function of dissolved oxygen (DO) concentration desired. Sterile air filters with 0.2 μm pores were used for air transfer into bioreactor. The dissolved oxygen concentration (DO) in the culture broth was measured using a pO2 electrode. Silicone was periodically added as an antifoam agent.

The aeration and agitation conditions that gave best productivity were used in fed-batch mode which was carried out in conformity

Table 1
Composition and characteristics of designed culture media (g/L).

	KH_2PO_4	Na_2HPO_4	$MgSO_4 \cdot 7H_2O$	Glucose	$(NH_4)_2SO_4$	Yeast extract	$CaCl_2 \cdot 2H_2O$	$FeCl_3 \cdot 6H_2O$	$ZnSO_4 \cdot 7H_2O$	$MnSO_4 \cdot H_2O$	$CoCl_2$ $6H_2O$	pH without glucose
K1	7	2.5	1.5	30	0.5	0.5	0.15	0.15	0.02	0.06	0.0001	5.78
K2	1.75	2.5	1.5	30	0.5	0.5	0.15	0.15	0.02	0.06	0.0001	6.37
K3	0.007	2.5	1.5	30	0.5	0.5	0.15	0.15	0.02	0.06	0.0001	7.32
K4	0.0007	2.5	1.5	30	0.5	0.5	0.15	0.15	0.02	0.06	0.0001	7.30
E1	7	0	1.5	30	0.5	0.5	0.15	0.15	0.02	0.06	0.0001	4.14
E2	3.5	0	1.5	30	0.5	0.5	0.15	0.15	0.02	0.06	0.0001	4.11
E3	1.75	0	1.5	30	0.5	0.5	0.15	0.15	0.02	0.06	0.0001	4.09
E4	0.875	0	1.5	30	0.5	0.5	0.15	0.15	0.02	0.06	0.0001	4.10
E5	0.07	0	1.5	30	0.5	0.5	0.15	0.15	0.02	0.06	0.0001	4.10
E6	0.007	0	1.5	30	0.5	0.5	0.15	0.15	0.02	0.06	0.0001	4.09
E7	0.0007	0	1.5	30	0.5	0.5	0.15	0.15	0.02	0.06	0.0001	4.09
E8	0	0	1.5	30	0.5	0.5	0.15	0.15	0.02	0.06	0.0001	4.37
S1	0	0	15	30	0.5	0.5	0	0	0	0	0	6.05
S2	0	0	1.5	30	0.5	0.5	0	0	0	0	0	6.24
S3	0	0	0.1	30	0.5	0.5	0	0	0	0	0	6.42
S4	0	0	0	30	0.5	0.5	0	0	0	0	0	6.75