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Wheat straw and lipids: UV-mutagenized *Yarrowia lipolytica* for the conversion of wheat straw hydrolysate into lipids

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Abstract. Due to the rising energy demand of our modern society and the finite amounts of petroleum-based fuels, renewable forms of energy have become extremely important. Bio-based fuels like bioethanol and biodiesel represent an already applied and accepted alternative. Biodiesel is currently mainly produced from plant oils. A new and promising alternative, which avoids the use of food crops, is the cultivation of the oleaginous yeast *Yarrowia lipolytica*, which possesses the capacity to accumulate up to 40% of its biomass in form of lipids. Moreover, this non-conventional yeast is able to metabolize a broad range of carbon-sources, presenting a sustainable alternative to reutilize a wide spectrum of waste substrates. This makes it an auspicious candidate for the generation of non-edible oils for biodiesel production. In this work, we aimed to generate a *Y. lipolytica* mutant strain with enhanced lipid production when grown on wheat straw hydrolysate as sole carbon source. Therefore, UV mutagenesis was applied and mutants with a high-lipid content were selected by their ability to grow in the presence of the fatty acid inhibitor cerulenin. Further, growth of the mutants on wheat straw hydrolysate was evaluated. The fatty acid composition was analysed by GC-FID and the calculated total lipid content revealed an up to 33% increase compared to the wild type strain. Fermentation optimisation and the combination of various waste substrates as carbon sources are expected to further increase the total lipid yield by the *Y. lipolytica* mutant strain and serve as initial point for its industrial scale evaluation.

Key words: biodiesel, UV-mutagenesis, wheat straw hydrolysate, *Yarrowia lipolytica*.

INTRODUCTION

Fossil fuels are a finite resource unable to cover the rising energy demand of our modern society. Thus, sustainable and renewable energy sources have gained great interest during the last years and are considered key players to combat climate change and other environmental issues. After the first success of converting crops into bio-fuels, like bioethanol or biodiesel, second generation biofuels are currently being investigated due to their environmentally-friendly and sustainable character. Several waste materials are currently used as feed for biorefineries in order to produce different types of bio-based fuels. Normally, biodiesel, which is defined as the alkyl- (normally methyl- or ethyl-) esters of long chain fatty acids, is produced out of plant oil. Alternatively,

production of biodiesel from the fatty acids accumulated by microorganisms like yeast represents a novel possibility in this field.

The oleaginous yeast *Yarrowia lipolytica* is a promising candidate for biodiesel production due to its capacity to accumulate up to 40% of its biomass in form of lipids (Beopoulos et al., 2009; Ageitos et al., 2011; Huang et al., 2013; Groenewald et al., 2014). Additionally, it possesses several advantages compared to plants or microalgae, which are also under investigation for this purpose (Xie, 2017). It grows much faster than microalgae and is not affected by seasonal or climate changes like plants. Furthermore, *Y. lipolytica* is able to metabolize a very broad range of different carbon-sources, which enables them to be cultivated on different waste raw materials (Ageitos et al., 2011; Groenewald et al., 2014). *Y. lipolytica* was stated as 'safe-to-use' by Groenewald et al. (2014) and is, therefore, a good candidate for economic, environmentally friendly and ethically acceptable lipid production and biodiesel generation.

Lignocellulose materials, like wheat straw, corncobs, sugar cane or rice hulls are a widely used feedstock for biorefineries worldwide (Saha, 2003; Maitan-Alfenas et al., 2015; Jönsson & Martín, 2016). Wheat straw represents the major crop residue in Europe and is only used as animal litter to a certain extent (Dias et al., 2010; Mühlenhoff, 2013). In order to utilize wheat straw as a substrate for yeast growth, two steps are required before its use: 1) Pre-treatment and 2) Hydrolysis. A detailed review about these steps is described in Cristobal-Sarramian & Atzmüller (2018).

Steam explosion is a chemical-free and very efficient type of pre-treatment to disrupt the fibre structure of wheat straw. As a result of this, the accessibility of the long chain carbon polymers, like hemicellulose, for enzymes during the hydrolysis step is improved and, thereby, the degradation of those carbon polymers into sugars is increased (mainly: glucose, galactose, xylose and mannose) (Eisenhuber et al., 2013; Marcos et al., 2013). The obtained sugar-rich solution is then further used as a carbon source during the fermentation step.

UV mutagenesis is an easy and effective method to introduce random mutations into a microorganism´s genome. The mechanisms underlying this technique have been previously described and are a suitable way to be used to generate *Y. lipolytica* mutant strains (Ikehata & Ono, 2011; Lindquist et al., 2015). For each organism and application, an optimization step is needed in order to determine the best ratio of UV-intensity and irradiation period. Additionally, the development of an adequate screening method is a critical factor for obtaining the desired strain.

For the screening of strains with enhanced fatty acid content, the fatty acid (FA) biosynthesis inhibitor cerulenin, has been shown to be an effective agent (Omura, 1976; Tapia et al., 2012; Katre et al., 2017). As cerulenin inhibits FA synthesis, it is speculated that only mutants with an enhanced FA synthesis activity will survive a lethal dose of this agent. In line with this hypothesis, Katre et al. (2017) showed, that the colony size of strains correlates with their lipid content when they are plated on cerulenin-containing media plates.

In this study, we combined the agricultural waste wheat straw as a feedstock with the capability of *Y. lipolytica* to grow on a wide range of carbon sources to produce fatty acids for biodiesel generation. Furthermore, we show, that UV mutagenesis can be used to enhance the fatty acid production and generate even more efficient strains for this purpose.

MATERIALS AND METHODS

Strains, media and growth conditions

The *Y. lipolytica* H222 strain (YlWT; *MATa* wild-type) (Barth & Gaillardin, 1996) was used as the parental strain for the UV-mutagenesis to generate the YlUV10 and YlUV12 strains. Yeast cultures were grown in YPD (yeast extract 10 g L^{-1}, peptone 20 g L^{-1}, glucose 20 g L^{-1}) or YNB medium (yeast nitrogen base without amino acids 6.7 g L^{-1}, Sigma, glucose 20 g L^{-1}). Hydrolysate (HL) medium was prepared by adding 20% wheat straw hydrolysate to YNB (6.7 g L^{-1}). Solid media was prepared by adding 2% agar to the corresponding medium. Yeast solid cultures were grown at 28 °C for up to 72 h and stored at 4 °C. For liquid cultures, precultures were prepared in 5 mL YPD and grown overnight at 28 °C on a rotary shaker (170 rpm). Prior to inoculation, precultures were washed with deionized water. Main cultures were performed in shaking flasks containing 50 mL HL-medium, inoculated with a starting OD_{600} of 0.1 and grown for 24–72 h on a rotary shaker (170 rpm). For growth characterization, this was performed three times for each strain. For lipid determination, strains were harvested after 24, 48 and 72 h by centrifugation (3,000 rpm, 10 min), washed twice with water and the cell pellets were stored at -80 °C.

UV mutagenesis and selection of strains with a high lipid content

For the UV mutagenesis, cells were grown overnight in YPD and 10 OD units were harvested, washed and resuspended in deionized water to a final volume of 10 mL. This was irradiated with UV light (254 nm) from a distance of 9 cm for 1.5 min while stirring. The killing rate was determined by harvesting cells every 30–60 s and determining their viable cell count on YPD plates in two independent experiments. Mutants with a high lipid content were selected on HL-medium plates containing 6 µg mL^{-1} cerulenin (Sigma-Aldrich, ≥ 98%). Mutants showing the biggest colonies after 48 h of incubation were further characterized.

Preparation and characterisation of wheat straw hydrolysate

Dried wheat straw was chopped to a particle size of 2–3 cm and further disintegrated by steam explosion as previously described (Eisenhuber et al., 2013). Briefly, straw was mixed with water in a 1:1 ratio and steam explosion was performed at 200 °C for 10 min. The dry weight of the steam exploded wheat straw was detected using an IR-scale (Ohaus MB45), which was immediately used for enzymatic hydrolysis. For this, wheat straw was mixed in a 1:10 (dryweight : buffer) ratio with 0.1 M citric acid buffer (pH 5.0) and 0.3 mL Accellerase 1500 (Genercor) per g dry weight. After incubation at 50 °C, 120 rpm for 72 h, big particles were removed by filtration and the liquid hydrolysate was sterile filtered and stored at 4 °C.

Saccharides, organic acids and furans from the sterile-filtered hydrolysate were quantified by HPLC, using a Jasco HPLC (2000 plus series) with an Aminex hpx 87 h column at 65 °C. H_2SO_4 (c = 5 mmol L^{-1}) as an eluent and an isocratic flow rate of 0.8 mL min^{-1} was used. Data acquisition was performed with a refractive index detection and UV-detection at 210 nm from three independently prepared samples. Data were analysed with CromPass (Version 1.8.6.1).

Fatty acid determination

Prior to analytical quantification of the fatty acid content, the washed yeast-pellets were lyophilized. Therefore a BenchTop Pro with Omnitronics (SP Scientific) freeze dryer at -50 °C (± 1) was used to dry pre-deepfreezed samples (-80 °C) for 24 h.Two times 5 mg of lyophilized yeast were resuspended and methylated separately with 5 ml of methanol : acetyl chloride (ChemLab, Sigma-Aldrich, both p.a. ≥ 99%) with a volumetric ratio of 50:2 for 8 h at 60 °C in a drying cabinet. Afterwards, the reactions were stopped by slowly adding 2.5 mL of potassium carbonate solution (VWR, ≥ 98%) with a concentration of 60 g L^{-1}. The resulting fatty acid methyl esters were extracted by adding 2 mL of hexane (Honeywell, HPLC grade) and vortexing for 1 minute. 1 mL of the supernatant phase, containing the methyl esters, was transferred in a 1.5 mL vial with a crimp cap and stored at -18 °C until measurement.

The hexane - extract was injected in a Thermo Trace 1300GC, equipped with an autosampler AS 1310 and an SSL injector. The detection was carried out by FID. The chromatographic conditions were as follows: The injection volume was 1 µL, the injector temperature was set at 240 °C. Helium was used as a carrier (constant flow) with 1.5 mL min^{-1} and a split flow at 30 mL min^{-1}. An Agilent J&W capillary column DB23 60 m, 0.25 mm ID and 0.25 µm film thickness was used for analytical separation. The oven temperature gradient was: 0–3 min 130 °C; 6.5 °C min^{-1} to 170 °C. 2.8 °C min^{-1} to 214 °C held 12 minutes. 3 °C min^{-1} to 240 °C held for 15 minutes. The FID was set at a temperature of 280 °C, 450 mL min^{-1} air flow, 45 mL min^{-1} hydrogen flow and nitrogen as make up gas at 40 mL min^{-1} were the torch conditions. Data analysis was performed with Chromeleon (Version 7.2). For calibration, the external standard method was used.

RESULTS AND DISCUSSION

Generation and selection of *Y. lipolytica* H222 mutant strains

In order to introduce mutations that lead to an improved accumulation of lipids, *Y. lipolytica* was irradiated with UV light. Prior to this mutagenesis, the optimal duration and distance for the irradiation treatment was determined. When using a lamp with a wavelength of 254 nm and a distance of 9 cm between the lamp and the cells, an exposure of 3.5 min or more lead to a kill rate greater than 90%. Sampling at 0.5, 1.5 and 2.5 min revealed kill rates of 22%, 54% and 78%, respectively (Fig. 1). Therefore, *Y. lipolytica* cultures were irradiated with UV light for 1.5 minutes to generate mutant strains.

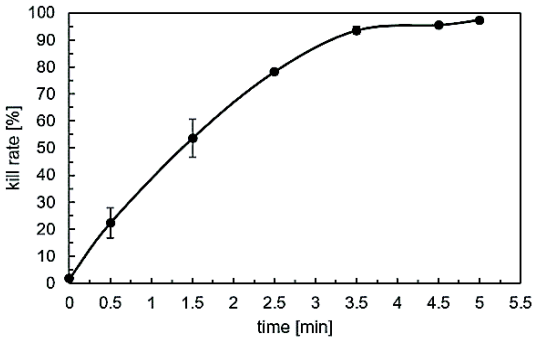

Figure 1. Determination of the kill-rate of *Y. lipolytica* H222 with UV radiation at 254 nm from a distance of 9 cm, (n = 2).

The fatty acid inhibitor cerulenin has previously been shown to be an effective selective agent to isolate yeast strains with an increased lipid content (Katre et al., 2017). Specifically, cerulenin inhibits the FA synthesis and the strains with an increased FA

content tolerate a higher concentration of this agent. In order to find the appropriate cerulenin concentration for the selection of the mutants after UV treatment, growth on solid media with different cerulenin concentrations was investigated. YlWT revealed clearly inhibited growth at concentrations of 4 µg mL^{-1} and higher (Fig. 2, A). To ensure a clear selection of mutants with higher lipid content, the cells were spread onto solid HL-media containing 6 µg mL^{-1} cerulenin. The colonies with the biggest diameter were picked from the plate after 48 h incubation and transferred to a new cerulenin selection plate. After a second selection step on the same media, the mutants YlUV10 and YlUV12 were chosen as potential candidates with an increased lipid content, based on their growth and colony morphology (Fig. 2, B). Usually, UV-mutagenesis experiments are associated with the development of so-called *petite* phenotypes, which are linked to mitochondrial dysfunction and delayed growth (Chanet & Heude, 1974). Thus, in order to avoid the selection of strains with possible growth defects, which would result in a decrease fermentation productivity, we assumed that the strains with the biggest colony sizes would not have a growth defect and were further characterized in this study.

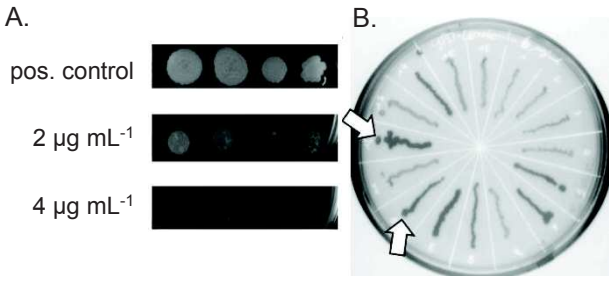

Figure 2. A: Evaluation of the toxic concentration of cerulenin for YlWT after 48 h, pos. control = YPD; B: Second selection step of *Y. lipolytica* mutants after UV mutagenesis on HL-media plates containing 6 µg mL^{-1} cerulenin, selected mutants (YlUV10 and YlUV12) are highlighted with arrows.

Characterisation of wheat straw hydrolysate and growth evaluation

In order to use wheat straw as carbon source for *Y. lipolytica* cultivation, a pre-treatment step of this raw material is required to obtain a fermentable sugar solution. This was achieved by steam-explosion and enzymatic hydrolysis. Several steam-explosion parameters were tested in order to achieve the highest sugar content after enzymatic hydrolysis (unpublished data). HPLC analysis of the wheat straw hydrolysate revealed that steam explosion performed at 200 °C for 10 minutes resulted in the highest sugar concentration (Table 1). Although there are growth inhibitors released during this process, their concentrations were not sufficient to impair *Y. lipolytica* growth (unpublished data), which is also comparable with the findings of Niehus et al. (2018).

Table 1. Sugar and inhibitor concentrations of wheat straw hydrolysate obtained after steam explosion (200 °C, 10 mins) and enzymatic hydrolysis, (n = 3)

Sugars	Concentration, g L^{-1}	Inhibitors	Concentration, g L^{-1}
Cellobiose	1.82 (± 0.27)	Formic acid	4.28 (± 0.16)
Glucose	32.48 (± 0.59)	Acetic acid	4.09 (± 0.21)
Xylose/ Mannose	13.02 (± 1.25)	Furfural	0.27 (± 0.27)

Growth of the mutants was evaluated in HL-medium and compared with the YlWT strain to ensure that the UV treatment did not affect the strain's ability to grow on wheat straw hydrolysate as the sole carbon source. The growth evaluation shows a similar growth behaviour in all three strains (Fig. 3). This indicates that wheat straw is a suitable raw material to use as a media to grow *Y. lipolytica*, and the introduced mutations did not compromise any essential pathway affecting yeast growth. Therefore, the mutants were further characterized for FA production in HL-medium.

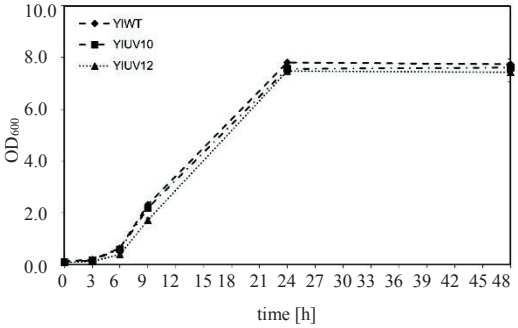

Figure 3. Growth evaluation of YlWT (◆) and the two mutants YlUV10 (■) and YlUV12 (▲) in HL-media, (n = 3).

Fatty acid production

For the comparison of the FA production of the generated mutant strains, YlUV10 and YlUV12, with YlWT, all strains were grown in HL-media and harvested after 24 h, 48 h and 72 h. Samples were freeze dried and fatty acid methylation was performed prior to GC analysis. Interestingly, the total FA content of all three analysed strains was clearly different (Fig. 4). On the one hand, YLUV10, in comparison with YlWT, showed lower values after 24 h and 72 h (-16% and -8%, respectively), but an about 33% higher content after 48 h. On the other hand, YlUV12 showed the opposite behaviour, here after 24 h and 72 h the total FA content is slightly higher (9% each) than for YlWT, but lower (-9%) after 48 h.

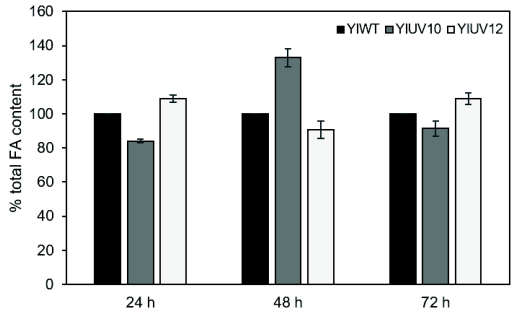

Figure 4. Fatty acid content of YlUV10 and YlUV12 after 24 h, 48 h and 72 h cultivation in shaking flasks with HL-media compared to the YlWT (set to 100%), (n = 2).

The fact that the YlUV10 strain showed an increased FA-content after 48 h, which then decreases after 72 h, indicates that the activity of the FA synthesis enzymes is higher during the first 48 h, which correlates with the enhanced cerulenin resistance of this strain. In case of the YlUV12, the FA content is slightly increased after 72 h in comparison to YlWT. Interestingly, this strain did not show any growth defect in comparison to YlWT. Thus, one may speculate that the differences within this time frame are caused by intensive lipid remodelling events, which are a major event influencing overall yeast physiology (Henry et al., 2012; Renne et al., 2015; Sarria et al., 2017). Another possibility could be associated to an altered sugar uptake or utilization in the mutants, which even if a direct impact on cellular growth was not observed, could have an influence on the total lipid content of these strains (Hapeta et al., 2017; Tanimura et al., 2018). Further investigation

exploring these hypotheses, as well as other possible molecular mechanism behind the lipid accumulation in these mutants strains will be conducted.

CONCLUSIONS

In the present study, we generated *Y. lipolytica* strains with an enhanced lipid content when grown on wheat straw hydrolysate. This was achieved by combining UV-mutagenesis, cerulenin screening and adaptation to wheat straw hydrolysate experiments. Nowadays, metabolic engineering is a widely accepted technology to increase *Y. lipolytica* lipid content (Bhutada et al., 2017). Thus, it will be interesting to apply this approach to further increase the lipid content of the Yl10UV and Yl12UV strains, for instance by modulation the activity of the FA synthesis and neutral lipid hydrolysing enzymes. Furthermore, the ability of *Y. lipolytica* to metabolize different carbon sources provides a great opportunity to use this yeast in biorefineries (Kavšček et al., 2015). Further growth characterization of this yeast on substrates commonly used in biorefineries as well as the optimization of the fermentation parameters will serve as a starting point to evaluate the use of these strains in an industrial scale level.

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REFERENCES

Ageitos, J.M., Vallejo, J.A., Veiga-Crespo, P. & Villa, T.G. 2011. Oily yeasts as oleaginous cell factories. *Appl. Microbiol. Biotechnol.* **90**(4), 1219–1227.

Barth, G. & Gaillardin, C. 1996. Yarrowia lipolytica. In *Nonconv. Yeasts* (pp. 313–88). Springer Berlin Heidelberg.

Beopoulos, A., Cescut, J., Haddouche, R., Uribelarrea, J.-L., Molina-Jouve, C. & Nicaud, J.-M. 2009. Yarrowia lipolytica as a model for bio-oil production. *Prog. Lipid Res.* **48**(6), 375–387.

Bhutada, G., Kavšček, M., Ledesma-Amaro, R., Thomas, S., Rechberger, G.N., Nicaud, J.-M. & Natter, K. 2017. Sugar versus fat: Elimination of glycogen storage improves lipid accumulation in Yarrowia lipolytica. *FEMS Yeast Res.* **17**(3), 1–10.

Chanet, R. & Heude, M. 1974. Variations in UV induced lethality and "petite" mutagenesis in synchronous culture of Saccharomyces cerevisiae. *Mol. Gen. Genet.* **131**(1), 21–26.

Cristobal-Sarramian, A. & Atzmüller, D. 2018. Yeast as a production platform in bioreflneries: Conversion of agricultural residues into value-added products. *Agron. Res.* **16**(2), 377–388.

Dias, A.A., Freitas, G.S., Marques, G.S.M., Sampaio, A., Fraga, I.S., Rodrigues, M.A.M., … Bezerra, R.M.F. 2010. Enzymatic saccharification of biologically pre-treated wheat straw with white-rot fungi. *Bioresour. Technol.* **101**(15), 6045–6050.

Eisenhuber, K., Krennhuber, K., Steinmüller, V. & Jäger, A. 2013. Comparison of different pre-treatment methods for separating hemicellulose from straw during lignocellulose bioethanol production. *Energy Procedia* **40**, 172–181.

Groenewald, M., Boekhout, T., Neuvéglise, C., Gaillardin, C., Van Dijck, P.W.M. & Wyss, M. 2014. Yarrowia lipolytica: Safety assessment of an oleaginous yeast with a great industrial potential. *Crit. Rev. Microbiol*. **40**(3), 187–206.

Hapeta, P., Rakicka, M., Dulermo, R., Gamboa-Meléndez, H., Cruz-Le Coq, A.-M., Nicaud, J.-M. & Lazar, Z. 2017. Transforming sugars into fat - lipid biosynthesis using different sugars in Yarrowia lipolytica. *Yeast* **34**(April), 1–25.

Henry, S.A., Kohlwein, S.D. & Carman, G.M. 2012. Metabolism and regulation of glycerolipids in the yeast Saccharomyces cerevisiae. *Genetics* **190**(2), 317–349.

Huang, C., Chen, X., Xiong, L., Chen, X., Ma, L. & Chen, Y. 2013. Single cell oil production from low-cost substrates: The possibility and potential of its industrialization. *Biotechnol. Adv.* **31**(2), 129–139.

Ikehata, H. & Ono, T. 2011. The Mechanisms of UV Mutagenesis. *J. Radiat. Res.* **52**(2), 115–125.

Jönsson, L.J. & Martín, C. 2016. Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresour. Technol.* **199**, 103–112.

Katre, G., Ajmera, N., Zinjarde, S. & RaviKumar, A. 2017. Mutants of Yarrowia lipolytica NCIM 3589 grown on waste cooking oil as a biofactory for biodiesel production. *Microb. Cell Fact.* **16**(1), 176.

Kavšček, M., Stražar, M., Curk, T., Natter, K. & Petrovič, U. 2015. Yeast as a cell factory: Current state and perspectives. *Microb. Cell Fact.* **14**(1), 1–10.

Lindquist, M.R., López-Núñez, J.C., Jones, M.A., Cox, E.J., Pinkelman, R.J., Bang, S.S., … Hughes, S.R. 2015. Irradiation of Yarrowia lipolytica NRRL YB-567 creating novel strains with enhanced ammonia and oil production on protein and carbohydrate substrates. *Appl. Microbiol. Biotechnol.* **99**(22), 9723–9743.

Maitan-Alfenas, G.P., Visser, E.M. & Guimarães, V.M. 2015. Enzymatic hydrolysis of lignocellulosic biomass: Converting food waste in valuable products. *Curr. Opin. Food Sci.* **1**(1), 44–49.

Marcos, M., García-Cubero, M.T., González-Benito, G., Coca, M., Bolado, S. & Lucas, S. 2013. Optimization of the enzymatic hydrolysis conditions of steam-exploded wheat straw for maximum glucose and xylose recovery. *J. Chem. Technol. Biotechnol.* **88**(2), 237–246.

Mühlenhoff, J. 2013. Reststoffe für Bioenergie nutzen - Potenziale, Mobilisierung und Umweltbilanz. *Renews Spez.* **64**(64), 1–46.

Niehus, X., Crutz-Le Coq, A.M., Sandoval, G., Nicaud, J.M. & Ledesma-Amaro, R. 2018. Engineering Yarrowia lipolytica to enhance lipid production from lignocellulosic materials. *Biotechnol. Biofuels* **11**(1), 1–10.

Omura, S. 1976. The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor of fatty acid synthesis. *Bacteriol. Rev.* **40**(3), 681–697.

Renne, M.F., Bao, X., De Smet, C.H. & de Kroon, A.I.P.M. 2015. Lipid acyl chain remodeling in yeast. *Lipid Insights* **8**, 33–40.

Saha, B.C. 2003. Hemicellulose bioconversion. *J. Ind. Microbiol. Biotechnol.* **30**(5), 279–291.

Sarria, S., Kruyer, N.S. & Peralta-Yahya, P. 2017. Microbial synthesis of medium-chain chemicals from renewables. *Nat. Biotechnol.* **35**, 1158.

Tanimura, A., Sugita, T., Endoh, R., Ohkuma, M., Kishino, S., Ogawa, J., … Takashima, M. 2018. Lipid production via simultaneous conversion of glucose and xylose by a novel yeast, Cystobasidium iriomotense. *PLoS One* **13**(9), 1–13.

Tapia, E.V, Anschau, A., Coradini, A.L., T Franco, T. & Deckmann, A. 2012. Optimization of lipid production by the oleaginous yeast Lipomyces starkeyi by random mutagenesis coupled to cerulenin screening. *AMB Express* **2**(1), 64.

Xie, D. 2017. Integrating Cellular and Bioprocess Engineering in the Non-Conventional Yeast Yarrowia lipolytica for Biodiesel Production: A Review. *Front. Bioeng. Biotechnol.* **5**(October).