

Whole-Cell Biotransformation of 1,12-Dodecanedioic Acid from Coconut Milk Factory Wastewater by Recombinant CYP52A17SS Expressing *Saccharomyces cerevisiae*

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Date Submitted: 2020-12-22

Keywords: *Saccharomyces cerevisiae*, cytochrome P450, hydroxydodecanoic acid, dodecanedioic acid, biotransformation

Abstract:

Biotransformation of fatty acids from renewable wastewater as feedstock to value-added chemicals is a fascinating commercial opportunity. ω,ω' -Dicarboxylic acids (DCAs) are building blocks in many industries, such as polymers, cosmetic intermediates, and pharmaceuticals, and can be obtained by chemical synthesis under extreme conditions. However, biological synthesis can replace the traditional chemical synthesis using cytochrome P450 enzymes to oxidize fatty acids to DCAs. *Saccharomyces cerevisiae* BY(2R)/pYeDP60-CYP52A17SS (BCM), a transgenic strain expressing the galactose-inducible CYP52A17SS cytochrome P450 enzyme, was able to grow in a coconut milk factory wastewater (CCW) medium and produced 12-hydroxydodecanoic acid (HDDA) and 1,12-dodecanedioic acid (DDA). The supplementation of CCW with 10 g/L yeast extract and 20 g/L peptone (YPCCW) markedly increased the yeast growth rate and the yields of 12-HDDA and 1,12-DDA, with the highest levels of approximately 60 and 38 $\mu\text{g/L}$, respectively, obtained at 30 °C and pH 5. The incubation temperature and medium pH strongly influenced the yeast growth and 1,12-DDA yield, with the highest 1,12-DDA formation at 30 °C and pH 5.5. Hence, the *S. cerevisiae* BCM strain can potentially be used for producing value-added products from CCW.

Record Type: Published Article

Submitted To: LAPSE (Living Archive for Process Systems Engineering)

Citation (overall record, always the latest version):

LAPSE:2020.1244

Citation (this specific file, latest version):

LAPSE:2020.1244-1

Citation (this specific file, this version):

LAPSE:2020.1244-1v1

DOI of Published Version: <https://doi.org/10.3390/pr8080969>

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Article

Whole-Cell Biotransformation of 1,12-Dodecanedioic Acid from Coconut Milk Factory Wastewater by Recombinant CYP52A17^{SS} Expressing *Saccharomyces cerevisiae*

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Received: 24 June 2020; Accepted: 7 August 2020; Published: 11 August 2020



Abstract: Biotransformation of fatty acids from renewable wastewater as feedstock to value-added chemicals is a fascinating commercial opportunity. α,ω -Dicarboxylic acids (DCAs) are building blocks in many industries, such as polymers, cosmetic intermediates, and pharmaceuticals, and can be obtained by chemical synthesis under extreme conditions. However, biological synthesis can replace the traditional chemical synthesis using cytochrome P450 enzymes to oxidize fatty acids to DCAs. *Saccharomyces cerevisiae* BY(2R)/pYeDP60-CYP52A17^{SS} (BCM), a transgenic strain expressing the galactose-inducible CYP52A17^{SS} cytochrome P450 enzyme, was able to grow in a coconut milk factory wastewater (CCW) medium and produced 12-hydroxydodecanoic acid (HDDA) and 1,12-dodecanedioic acid (DDA). The supplementation of CCW with 10 g/L yeast extract and 20 g/L peptone (YPCCW) markedly increased the yeast growth rate and the yields of 12-HDDA and 1,12-DDA, with the highest levels of approximately 60 and 38 $\mu\text{g/L}$, respectively, obtained at 30 °C and pH 5. The incubation temperature and medium pH strongly influenced the yeast growth and 1,12-DDA yield, with the highest 1,12-DDA formation at 30 °C and pH 5–5.5. Hence, the *S. cerevisiae* BCM strain can potentially be used for producing value-added products from CCW.

Keywords: biotransformation; dodecanedioic acid; hydroxydodecanoic acid; cytochrome P450; *Saccharomyces cerevisiae*

1. Introduction

Thailand is the world's ninth largest producer of coconut, with the east and south coastal areas being used for the large-scale production of coconuts [1], which amounted to 856,920 tons in 2018 and is increasing annually. Coconut waste was generated from food industry, and it was reported as a pollutant. Coconut milk extraction results in the production of the waste byproducts, which are comprised of solid fat and wastewater. Currently, 2000–3000 kg of coconut milk are lost every day into wastewater during the extraction process [2,3]. The coconut oil in the wastewater pond can be

changed into solid fat that then floats to the top of the wastewater and can be eliminated from the wastewater pond and used as a substrate for biogas fermentation [4]. However, the residual coconut milk processing wastewater (CCW) can be a severe environmental and health problem, forming an odor and foul air in the neighborhood, clogging, and harboring pathogens [3,5,6].

The CCW is comprised of variable but high levels of biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total solids (TS), and fat, oil, and grease (FOG) (Table 1) [7,8], making it unsuitable for environmental discharge. For example, the FOG content of the CCW of the sample used in this study was almost 400-fold over the coconut wastewater industrial effluent standards for industrial plants and industrial estates in Thailand. The industrial effluent standard of FOG in Thailand legally should not exceed 5 mg/L [9]. However, the wastewater contains many nutrients and minerals that could be used as a medium for growth of microorganisms [10]. Since the CCW with free fatty acids is a readily and plentiful low-cost resource without alternative (competing) applications, it is a sustainable and cheap supply. Lauric acid is the main fatty acid contained in CCW, because it is the major fatty acid in coconut oil at approximately 45–53% [11,12]. This is of interest, since lauric acid can potentially be used as a substrate for 12-hydroxydodecanoic acid (HDDA) and 1,12-dodecanedioic acid (DDA) production.

Table 1. Initial characteristics of the CCW used in this study. Data from [7].

Parameter	Value
pH	5.5 ± 0.1
TS (mg/L)	5836 ± 248
TSS (mg/L)	5270 ± 197
Total Kjeldahl nitrogen (mg/L)	83.6 ± 4.5
VS (mg/L)	5172 ± 258
FOG (mg/L)	1994 ± 165
BOD (mg/L)	3516 ± 100
COD (mg/L)	7982 ± 361
Sulfate (mg/L)	1 ± 0.12
Total phosphorus (mg/L)	1.31 ± 0.1
Copper (mg/L)	0.06 ± 0.0004
Calcium (mg/L)	3.24 ± 0.2
Magnesium (mg/L)	19.3 ± 1.6
Reducing sugar (%)	0.02 ± 0.004
Free fatty acids (%)	0.21 ± 0.017

Data are shown as the mean ± 1SD.

Medium chain α,ω -dicarboxylic acids (α,ω -DCAs) are versatile chemical intermediates of different chain length, usually presented as $\text{HOOC-(R)}_n\text{-COOH}$ and a product of economic interest, because they are widely used as feedstocks for chemical products and intermediates, such as polyamides, nylons, lubricants, and perfumes. Normally, α,ω -DCAs are produced from petrochemical processes via chemical reaction under extremely unpleasant conditions of high pressure and temperature and using a strong acid [13,14]. α,ω -DCAs can be produced in two ways; chemical synthesis and fermentation. The chemical synthesis of α,ω -DCAs leads to environmental problems and pollution. Consequently, microbial production of α,ω -DCAs as a greener and sustainable processes is of interest to replace the chemical processes. Microbial production was developed via metabolic engineering and fermentation processes. Omega oxidation (ω -oxidation) is known as hydroxylation of the terminal alkyl carbon of a fatty acid to an alcohol and then further oxidation to a DCA (Figure 1). ω -Oxidation of fatty acid can generally be divided to three steps. The first step: fatty acid is oxidized into ω -hydroxyfatty acid by cytochrome P450 monooxygenase and a NADPH–cytochrome P450 oxidoreductase complex in the endoplasmic reticulum (ER) membrane [15]. The second step: ω -hydroxyfatty acid is catalyzed to fatty acid aldehyde by fatty alcohol oxidase (FAO). Then, the aldehyde produced in the previous step is further oxidized to DCA by aldehyde dehydrogenase (FAHDH) [16]. While ω -hydroxylating cytochrome P450s can be responsible for oxidizing the initially ω -hydroxy products further, in the

absence of other enzymes, dicarboxylic acids can be converted [15]. Fatty acids are enzymically catalyzed to hydroxyl fatty acids by various cytochrome P450 enzymes in vivo from microbes, fungi, plants, and animals. Moreover, in vitro fatty acid oxidation can be performed in situ by fungi, animals, and plants. Some microbes possess the ability to assimilate n-alkane and fatty acid as a carbon source. *C. tropicalis* ATCC 20336 is capable of oxidizing myristic and oleic acid (saturated and unsaturated fatty acids) to α,ω -DCAs via a ω -oxidation pathway [15]. *C. tropicalis* expressing CYP52A17 can catabolize methyl tetradecanoate to produce hydroxytetradecanoic [17]. Moreover, *C. tropicalis* W10-1, a DCA-producing strain, has ability of converting alkane to DCA13 [18]. An expressing CYP52A21 and CYP52A23 in *E. coli* can oxidize mid-chain fatty acids and longer-chain fatty acids, respectively [19]. While, *E. coli* expressing AlkBGL can convert dodecanoic acid methyl ester to 12-hydroxydodecanoic acid methyl ester [20].

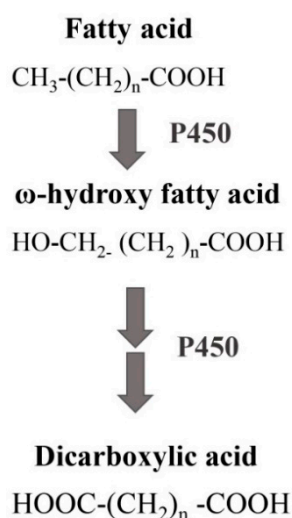


Figure 1. Omega-oxidation of fatty acid in yeast [15].

Saccharomyces cerevisiae is a generally-regarded-as-safe (GRAS) microorganism with genetic engineering advantages and is easy to transfer the technology into industry [14]. There have been several reports on *S. cerevisiae* as a recombinant microorganism producing DCAs [21]. Initially, the *FoCYP539A7* and *FoCYP655C2* cytochrome P450 genes from the fungi *Fusarium oxysporum* were expressed in *S. cerevisiae* to oxidize fatty acids (caprylic, capric, and lauric acids) into ω -hydroxy fatty acids [22]. Furthermore, decanedioic acid and DDA were produced by *S. cerevisiae* expressing CYP94C1 and cytochrome reductase ATR1 using renewable sugars and fatty acids as substrates [14]. Subsequently, *S. cerevisiae* (BCM), which has an extra copy of the BY4741 genetic background harboring the NADPH cytochrome P450 reductase (*CPR*) gene under its own promoter [23] and the *CYP52A17^{SS}* gene under galactose induction, was shown to effectively convert lauric acid into 1,12-DDA [7]. Notably, whole-cell biocatalysts have advantages over isolated enzymes, such as the absence of the high cost and energy for enzyme purification. A whole-cell system is a nonrequirement of cofactor; biotransformation by several enzymes could be continually conducted, which relates to the CYP reaction pathway, so the reaction can be carried out at a near-ambient temperature and ease the process [19]. Moreover, the enzyme environment of a whole cell can protect the protein structure from conformation changes [24]. Thus, this process provides a better alternative for industrial production, which can be applicable to wastewater.

In the study presented here, recombinant *S. cerevisiae* BCM were used as the whole cell in the biotransformation, as previously reported [7]. An efficient biotransformation would be established to produce 1,12-DDA via CCW, or CCW supplemented with 10 g/L yeast extract and 20 g/L peptone (YPCCW) as a medium by the recombinant BCM. Moreover, the effect of the medium pH and temperature of the biotransformation were also determined.

2. Materials and Methods

2.1. Chemicals

The DCA ($\geq 98\%$), 12-HDDA (97%), and 1,12-DDA (99%), which were used as the standards for the gas chromatography (GC) analysis, were purchased from Sigma-Aldrich, St. Louis, MO, USA. Boron trifluoride-methanol solution for forming ester derivative, galactose, and yeast synthetic drop-out medium supplements without histidine using cultivation of recombinant yeast were also obtained from Sigma-Aldrich, MO, USA. Tert-butyl methyl ether for product extraction was purchased from Merck KGaA, Darmstadt, Germany.

2.2. Microorganism and Cultivation Conditions

Saccharomyces cerevisiae BCM expressing CYP52A17 produced the highest 1,12-DDA from lauric acid, as in previous study [7] was used in this study. The formation of the *S. cerevisiae* BCM yeast strain has been described elsewhere [7]. The yeast strain was cultured in YPGE (Yeast extract Peptone Glucose Ethanol) medium [10 g/L yeast extract, 20 g/L peptone, 2% (v/v) ethanol, 5 g/L glucose] or complete supplement mixture (CSM) without histidine (-His) with appropriate amino acids for the selection of the auxotrophs. The CCW and YPCCW media (CCW adding 10 g/L yeast extract and 20 g/L peptone) were used for the biotransformation procedure. The biotransformation was conducted in 500 mL shake flasks and incubated in a shaking incubator at 30 °C, 200 rpm (shaking diameter is 25 mm). An amount of 5 mL of the culture was collected at several time points for product extraction and quantification by GC. The product concentration ($\mu\text{g/L}$) and conversion efficiency (% molar conversion of substrate to 12-HDDA or 1,12-DDA products) were calculated in accordance with the GC data. Yeast growth was monitored as the optical density at 600 nm (OD_{600}).

2.3. Biotransformation of CCW-Based Media to DDA and Its pH and Temperature Optimization

2.3.1. Role of the Recombinant CYP52A17^{SS} and CCW-Based Media

Saccharomyces cerevisiae BCM were precultured in CSM-His medium (YNB without amino acids 6.7 g/L, glucose 20 g/L, and drop-out supplements without histidine 1.92 g/L) and were then inoculated into YPCCW or CCW to an OD_{600} of 0.4. The cultures were cultivated at 30 °C, 200 rpm (shaking diameter is 25 mm) until at an OD_{600} of 1, where upon 20 g/L of galactose was added into the cultures to induce CYP52A17^{SS} expression. Then 5 mL of sample was taken every 12 h for analysis of yeast growth or every 24 h for product formation analysis over 120 h. The non-transformed parental *S. cerevisiae* BY(2R) strain was cultured in the same manner in parallel as a reference control.

2.3.2. Optimal Temperature

The BCM cells were precultured overnight in 25 mL CSM-His medium at 30 °C with agitation at 200 rpm. The overnight grown cells were then cultured in YPCCW (natural pH 5.5) to an OD_{600} of 0.4 before addition of 20 g/L galactose and then cultured at 20, 25, 30, 35, 40, or 45 °C for 120 h with shaking at 200 rpm (shaking diameter is 25 mm). Samples were harvested after 120 h for determination of the product formation and yeast growth. The sample was acidified by addition of 6 M HCl and then tert-butyl methyl ether was added in the sample for extraction. The extracted sample was analyzed by GC. Yeast growth was determined as cell dry weight of which 1 mL of the sample was centrifuged ($10,000\times g$, 5 min, room temperature) to collect the cell pellet and completely dried at 65 °C for 24 h. Then, the dried cell was cooled and weighed afterwards.

2.3.3. Optimal Initial pH of the Medium

The BCM cells were cultured in 25 mL CSM-His medium. The cells were precultured and inoculated and grown in YPCCW medium with galactose as above except the YPCCW was set to an initial pH of 3, 4, 5, 6, 7, or 8. The medium pH was initially adjusted to acidic pH by the addition

of 3 M acetic acid, whereas the alkaline pH was adjusted by adding 3 M NaOH. Then the cultures were incubated at 30 °C for 120 h with shaking at 200 rpm. Samples were harvested after 120 h for yeast growth and acidified to pH 1 by addition of 6 M HCl, extracted into tert-butyl methyl ether, and analyzed by GC for determination of the product formation. Cell growth was monitored by measuring the cell dry weight. A 1 mL of sample was centrifuged (10,000× g, 5 min, room temperature), the pellet was dried at 65 °C for 24 h, and then weighed. Calculation of the difference in weight was performed.

2.4. Product Extraction

For product analysis, 1 mL of 6 M HCl (QREC, Auckland, New Zealand) and 5 mL of tert-butyl methyl ether (Merck KGaA, Darmstadt, Germany) was added to 5 mL of the culture sample, vortexed, and shaken at 30 °C for at least 1 h. The mixture was then left for 5–10 min to separate the hydrophilic and organic phases. The upper phase was dried under nitrogen gas, and the dried sample was then derivatized with 14% (*w/v*) boron trifluoride in methanol (Sigma-Aldrich, MO, USA) and extracted with 1 mL of hexane (Fisher Scientific, Seoul, Korea). The upper phase was analyzed by GC (Section 2.5).

2.5. Product Analysis

The concentration of each product was determined by GC (GC-2010A Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and DB-wax capillary column (30 m × 0.530 mm × 0.25 μm film thickness) (Agilent, Santa Clara, CA, USA). The column's temperature limit is 20–23 °C. Helium was used as the carrier gas at a flow rate of 1 mL/min. The temperature of column, injector, and detector was 45, 210, and 220 °C, respectively. The sample concentrations were determined by comparison of sample peak retention times with the standards.

2.6. Statistical Analysis

All experiments were performed in triplicate, and the data are presented as the mean ± one standard deviation (SD).

3. Results and Discussion

3.1. Cell Growth of *S. cerevisiae* BCM in CCW and YPCCW with and without Galactose Induction of CYP52A17 Expression

Conversion of lauric acid from CCW to added-value products is a fascinating commercial opportunity and also reduces the cost of the medium. The *S. cerevisiae* BCM and the control untransformed BY(2R) strains were grown in CCW or YPCCW. The recombinant BCM strain presented good growth in the YPCCW medium above that of the control BY(2R) strain, and this was dramatically increased by the induction of CYP52A17^{SS} expression by galactose (Figure 2), where the growth of the recombinant yeast gradually increased up to 60 h and then remained stable (stationary phase). Without galactose, the recombinant yeast initially grew slightly slower in the YPCCW medium but reached a much higher maximal growth and stationary phase at 36 and 42 h, respectively. On the other hand, this recombinant strain grew equally well in the CCW medium with or without galactose, but their growth was still lower than in either YPCCW media. In contrast, the BY(2R) grew poorly in the YPCCW and especially the CCW media.

Thus, the absence of yeast extract and peptone addition to the CCW resulted in a lower cell growth, since they are nitrogen sources and also contain necessary supplements, such as vitamins and amino acids, required for optimal yeast growth [25]. The recombinant yeast could grow in the CCW without any required dilution even though the wastewater (coconut washing line) contained high COD, BOD, and FOG values that exceeded the industrial effluent standards for industrial plants and industrial estates in Thailand [9]. Moreover, the pH of the CCW was 5.5, which is the optimal pH for *S. cerevisiae* growth. Indeed, maintaining the *S. cerevisiae* growth medium at pH 5.5 was reported to

give a high cell concentration and product formation [17], while adjusting the medium pH to 5–5.5 in the methyl laurate fermentation by the yeast *Wickerhamiella sorbophila* UHP4 promoted a high cell growth [26].

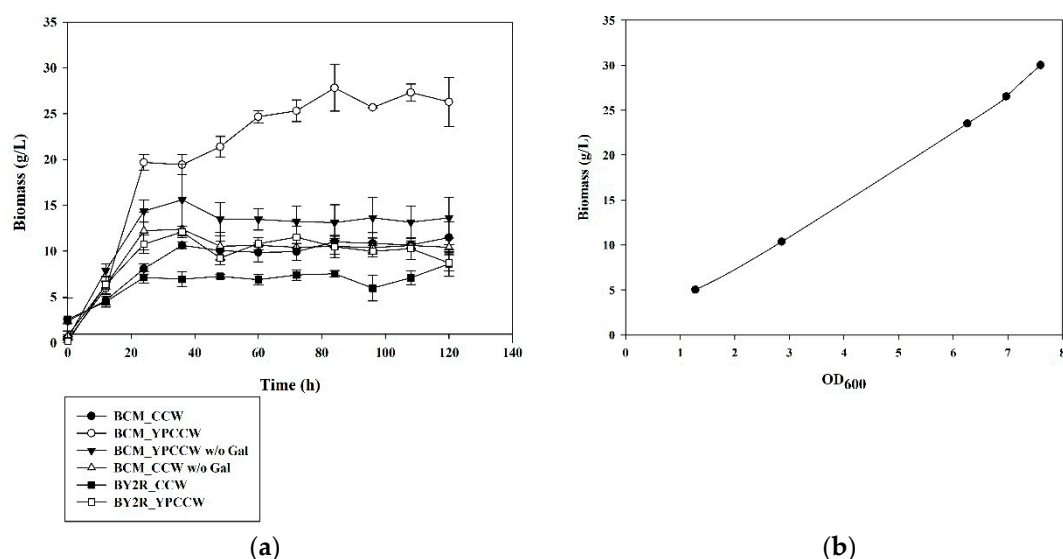


Figure 2. Growth (a) at 30 °C of the *S. cerevisiae* BCM and BY(2R) strains in YPCCW and CCW medium with or without (*w/o*) galactose induction of *CYP52A17*^{SS} expression. Data are shown as the mean \pm 1SD from three independent repeats. (b) Calibration curve for yeast culture optical density at the wavelength of 600 nm.

It is likely that, in this study, the recombinant yeast consumed substrates, such as sugars and fatty acids, from the CCW as alternative carbon sources for its growth. Dodecane and methyl laurate were shown to be able to be assimilated by *Candida tropicalis* for growth, because *C. tropicalis* expresses the cytochrome P450 *CYP52* gene [27]. The recombinant *S. cerevisiae* BCM used in this study not only has the galactose-inducible *CYP52A17*^{SS} cytochrome P450 gene but also a supplementary copy of the *CPR* gene under its own promoter as described in previous study [7]. The *CYP52A17* gene in combination with a redox partner, such as *CPR*, can oxidize fatty acids to α,ω -DCAs [15]. Therefore, the recombinant yeast had the ability to assimilate fatty acids in the CCW for growth and DCA production.

3.2. Production of DDA and HDDA from YPCCW (with Galactose) Using *S. cerevisiae* BCM

The *S. cerevisiae* BCM strain is known to be able to produce 12-HDDA and 1,12-DDA from lauric acid [7]. To determine the DDA production in CCW or YPCCW medium, the recombinant *S. cerevisiae* BCM was cultured in the CCW or YPCCW media at 30 °C and natural pH (pH 5.5). Galactose was added to the cultures for inducing the expression of *CYP52A17* and starting the whole-cell biotransformation. In both the CCW and YPCCW media containing the initial lauric of 20.3 mg/L, DDA and HDDA were both formed, but with different kinetics and yields. In YPCCW, 12-HDDA was detected from 24 h and increased to a maximum level of 60 μ g/L (10.56% conversion) in YPCCW 96 h, before falling at 120 h. Its oxidation to DDA was mainly evident as the sharp rise in DDA levels between 48 and 72 h (30 μ g/L) with 6.13% conversion and then rose slowly to a maximum level of ca. 38 μ g/L at 120 h. In CCW, HDDA was not detected until 72 h and increased steadily to a maximum of 41 μ g/L at 120 h (5.37% conversion). Its oxidation to DDA was firstly detected at 96 h and remained at a level of around 20 μ g/L up to 120 h (3.28% conversion) (Figure 3). However, no production of either 12-HDDA or 1,12-DDA from the CCW or YPCCW was detected in the control BY(2R) cultivations (data not shown). Moreover, the value of COD and BOD in CCW after the biotransformation separating the biomass were 7186 and 1500 mg/L, respectively. The result indicated that the BOD level after the biotransformation was 57% lower, while the COD value was slightly decrease from the initial level. On the other hand,

the biotransformation with biomass led to the higher COD and BOD level (48,706 and 13,470 mg/L), because the yeast cell was produced during the process, and yeast biomass was measured as BOD in the sample [28].

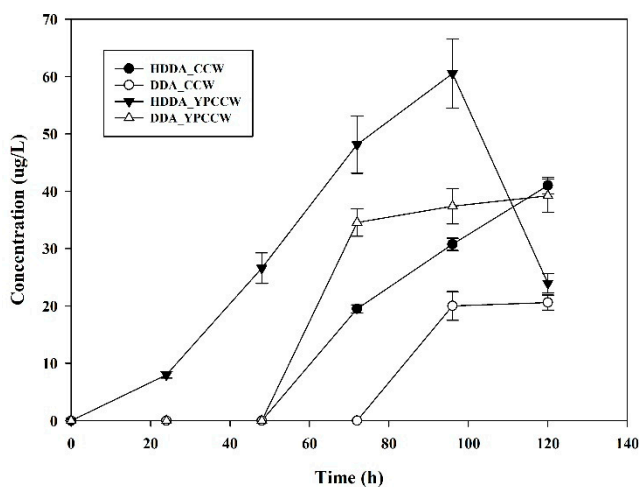


Figure 3. The production of DDA and HDDA from CCW or YPCCW media (both with galactose) using *S. cerevisiae* BCM at 30 °C and pH 5.5. Data are shown as the mean \pm 1SD from three independent repeats.

The yeast cell density (as OD₆₀₀) in the YPCCW medium was approximately two-fold greater than in the CCW, accounting for the approximately two-fold higher levels of product formation. Thus, the recombinant BCM yeast is able to oxidize fatty acids to DCAs via ω -oxidation, and the increased cell growth would contribute to the enhanced cytochrome P450 enzyme level and, hence, the greater product formation. The initial step of biotransformation is oxidation of fatty acids, which is carried out by a hydroxylase complex, cytochrome P450 monooxygenase with NADPH-CYP oxidoreductase, to oxidize the fatty acid into the ω -hydroxyfatty acid and the corresponding DCA in the ER [15] (Figure 1). The endogenous fatty acid synthetic pathway of *S. cerevisiae* can combine with the recombinant cytochrome P450 to generate the main products of HDDA and DDA in recombinant *S. cerevisiae* [14].

It is remarkable that the biotransformation of DCAs has recently been successful. The engineered *C. tropicalis*, with 16 genes eliminated, such as the native CYP52 enzymes, alcohol oxidases, and alcohol dehydrogenases, was not capable to produce ω -hydroxy tetradecanoic acid from tetradecanoic acid methyl ester. Whereas the strain carrying the P450s CYP52A17 obtained 174 g/L of 14-hydroxytetradecanoic acid at 148 h of bioconversion [17]. The elimination of 16 genes would lead to the lower efficiency of conversion of dodecanoic acid. Therefore, it is quite difficult to apply these modifications for DDA production in *S. cerevisiae*. However, *Candida* strains were identified as an opportunistic pathogen in humans that make it hard to generate to the industry [29]. Hence, it is interesting to use a GRAS strain such as the recombinant *S. cerevisiae* strain used in this study. *S. cerevisiae* BCM was higher grown in YPCCW and produced more HDDA and DDA than in CCW, so YPCCW was selected to the further study.

3.3. Effect of Temperature on HDDA and DDA Production

The effect of temperature on the DDA formation (bioconversion) was evaluated by culturing the recombinant yeast in YPCCW (pH natural = 5.5) at various temperatures (20, 25, 30, 35, 40, and 45 °C) using the initial inoculum prepared at 30 °C. The temperature had a similar effect on the growth of the recombinant yeast as on HDDA and DDA production (Figure 4). The optimum temperature for growth was 30 °C, in terms of the highest biomass, when temperature was increased, the biomass declined four-fold at 30 °C when compared to at 45 °C. The highest concentration of HDDA and DDA at 25.6 and 30 µg/L, respectively, were also found at 30 °C, decreasing slightly at 35 °C and

rapidly to a four-fold lower level at 40 °C with no products being detected at 45 °C. Growth and carboxylic production by yeast can be influenced by many factors. Temperature is one of the most important physical parameters. Most laboratory and industrial yeasts generally grow at a wide range of temperatures and are more available for cell reproduction at 25–30 °C [30]. Especially, *S. cerevisiae* can be grown at a higher temperature (35 °C). The normal growth and metabolic activity would be affected by a lower temperature, whereas the growth of yeast would be suppressed by a higher temperature and carry out its autolysis. Furthermore, microbial activity and fermentation processes are regulated by enzymes, which are sensitive to high temperatures. High temperatures would lead to inactive of enzymes and the denaturation of its tertiary structure. Therefore, temperature is a key factor in biotransformation, which must be carefully regulated through the process [31].

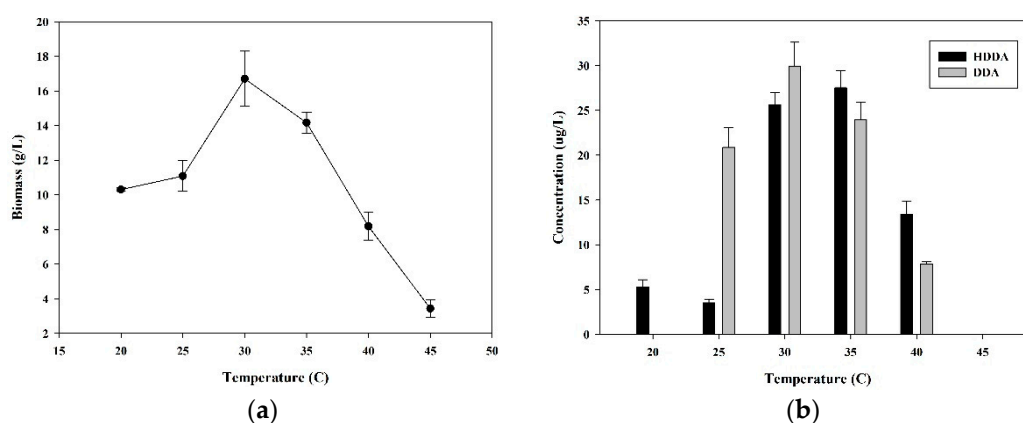


Figure 4. The (a) growth (as weight biomass) of *S. cerevisiae* BCM and (b) yield of HDDA and DDA when cultured in YPCCW (pH natural = 5.5) at various temperatures for 120 h. Data are shown as the mean \pm 1SD derived from three independent repeats.

3.4. Effect of pH on DDA Production

In addition to the microorganism strain and substrate, the cultivation condition also has an important effect on the DDA production rate and yield. To evaluate the influence of the initial medium pH on DDA production, the initial pH of the YPCCW medium was adjusted to various levels (pH 3, 4, 5, 6, 7, or 8). As seen in Figure 5a, the culture exhibited the highest growth (biomass of 14 g/L) at pH 5 and then gradually (almost linearly) decreased with increasing pH up to pH 8. However, the recombinant *S. cerevisiae* cells were very sensitive to acidic media with very low and essentially no growth at pH 4 and 3, respectively. From the tested pH values (integer values), the biotransformation was optimal at pH 5 (in terms of the highest DDA yield) reaching 32 $\mu\text{g/L}$ for DDA at pH 5, as seen in Figure 5b. There was no HDDA and DDA at pH 3 and only a very small amount of HDDA at pH 4. The quantitative analysis of HDDA and DDA did not differ between pH 6 to 8 but were markedly lower than at pH 5.0. The influence of the media pH on the conversion of lauric acid to HDDA and DDA seen in this study shows a similar pH optimum to that reported for recombinant *S. cerevisiae* harboring CYP539A7, CYP655C2 [17], and *Candida cloacae* [32]. Moreover, *C. cloacae* cells can produce more DDA between pH 4 and 5, but at pH 3 very few products were detected [32]. However, in *C. tropicalis*, a higher pH (pH 8) culture medium in the production phase showed a low productivity [33]. The medium pH plays a key role in the DCA production process by microorganisms, where it enhances DCA secretion and maintains its solubility [34,35]. This biotransformation provides a potential alternative for industry that can be applied to factory wastewater. Wastewater is another renewable resource that can be used for biotransformation to produce added-value products. To enhance it, the biotransformation can be developed by increasing the high density of cell biomass in the growth phase, and the production can be improved by adjusting the appropriate parameters [36].

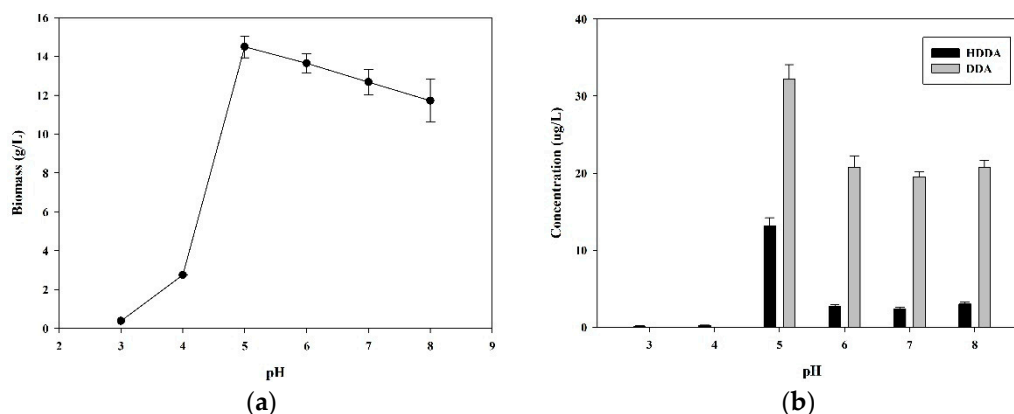


Figure 5. The (a) growth (as weight biomass) of *S. cerevisiae* BCM and (b) HDDA and DDA yields when cultured in YPCCW medium at 30 °C for 120 h at different initial medium pH. Data are shown as the mean \pm 1SD derived from three independent repeats.

4. Conclusions

This is the first report on the whole-cell biotransformation of 1,12-DDA from CCW by *S. cerevisiae* BCM using CCW as the base medium. The obtained data indicated that *S. cerevisiae* BCM is a suitable strain for cultivation in CCW and especially in YPCCW (both with galactose for induction of CYP52A17^{SS} expression) for DDA production. The optimum temperature and pH on the biotransformation were found to be 30 °C and pH 5–5.5. That the recombinant *S. cerevisiae* was able to grow in the CCW-based media and also produce HDDA and DDA makes this strain potentially useful for a variety of biotechnological applications, such as producing value-added products from wastewater.

Author Contributions: Conceptualization and methodology, P.B., N.B., G.T., and W.C.; investigation, P.B.; writing—original draft preparation, P.B. and W.C.; writing—review and editing, G.T. and W.C.; funding acquisition, W.C. and N.B. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Thailand Research Fund (TRF). The authors acknowledge the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0147/2553) and the government of France for financial support. The authors thank PTT (Plc.) and LISBP/INSA, Toulouse, France.

Conflicts of Interest: The authors declare no conflict of interest.

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