

EVALUATION OF REACTION SYSTEMS FOR PHOTODECARBOXYLATION OF FATTY ACIDS MEDIATED BY CvFAP

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ABSTRACT

The photoenzymatic decarboxylation of fatty acids to alkanes has been studied as an alternative for the synthesis of biofuels through biocatalytic processes. The present project used the recently discovered fatty acid photodecarboxylase from *Chlorella variabilis* NC64A (CvFAP), capable of irreversibly converting fatty acids (C_n) into their respective hydrocarbons (C_{n-1}) in the presence of blue light. The enzyme was expressed in *Escherichia coli*, and the cell was lysed to obtain the crude enzymatic extract. The reaction conditions were optimized, aiming for better reaction times and more efficient light sources for the evaluated process. The influence of the wavelength of the light source was evaluated using 300 W blue LED and 50 W violet LED lamps, applied in photocatalytic systems with different fatty acids as substrates. The transformation of the evaluated acid substrates to hydrocarbons was observed with conversions greater than 99% for stearic acid, palmitic acid and arachidic acid in 30 and 60 minutes of reaction using blue light, and in significantly shorter times, with only 10 minutes in the presence of violet light.

Keywords: CvFAP, Fatty Acid, Photodecarboxylase, LED Light, Decarboxylation.

1 INTRODUCTION

Photoenzymes are biocatalysts whose activity depends on constant light excitation. A notable example is the group of fatty acid photodecarboxylases (FAP), whose activity is mediated by the coenzyme flavin adenine dinucleotide (Aleksenko, 2020). FAP from the single-celled green microalga *Chlorella variabilis* NC64A (CvFAP) has been recognized as a potential alternative strategy for biodiesel synthesis (Sorigué, 2017). This flavoenzyme demonstrates high affinity for long-chain fatty acids and catalyzes decarboxylation to produce the corresponding hydrocarbons (alkanes or alkenes), as illustrated in Figure 1. High-power blue LED light (300 W) was confirmed as an effective light source for the photodecarboxylation of palmitic acid, as evidenced by previous studies carried out by our research group (Benincá, 2022). The present study proposes the development of a new protocol for photocatalytic reactions mediated by CvFAP, using different fatty acids under 300 W blue LED light and 50 W violet light at shorter reaction times.

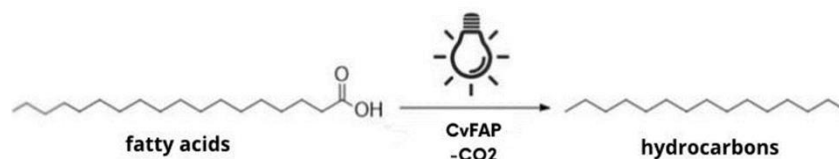


Figure 1. Representation of CvFAP-mediated decarboxylation.

2 MATERIAL & METHODS

Enzyme extract

Lyse with the help of a cutting-edge sonicator

Photocatalytic reactions

Substrate [13 mM]
CvFAP (5.1 mg.mL⁻¹)
Tris-HCl Buffer
DMSO 30%
Blue LED 300W (Time: 30 min and 60 min)
Violet LED 50W (Time: 10 min and 60 min)

Gas chromatography

Analysis of results by gas chromatography coupled to mass spectrometry

Figure 2. Project steps scheme. Cell lysis, photocatalytic reactions and gas chromatography analysis were performed as described by Benincá et al.³

3 RESULTS & DISCUSSION

Table 1. Conversion of the photoenzymatic decarboxylation reaction using blue light (300W).

Substrate	Conversion (%)	
	60 minutes	30 minutes
Myristic Acid	>99	>99
Palmitic Acid	>99	>97
Stearic Acid	>99	>99
Arachidic Acid	>99	>99

Reaction conditions: Substrate (13 mM), CvFAP (5.1 mg.mL⁻¹), Tris-HCl buffer (pH 8.5), 30% DMSO, illumination with blue LED light (300W) for 30 and 60 min. Myristic acid (C₁₄H₂₈O₂) MM= 228.53g/mol; Palmitic acid (C₁₆H₃₂O₂) MM= 256.4 g/mol; Stearic acid (C₁₈H₃₆O₂) MM= 284.48 g/mol; Arachidic acid (C₂₀H₄₀O₂) MM= 312.5304 g/mol.

This work carried out a study to optimize reaction conditions, such as LED power and reaction time, accelerating the formation of alkanes, going from 60 min to 10 minutes. Analyzing Table 2, in 60 minutes, conversion greater than 99% is observed in all acids used with chains above 16 carbons. However, it can be observed that, within 10 min, with the exception of myristic acid, all reactions reached a level close to complete conversion. The resistance of myristic acid to violet light confirms the preference of CvFAP for long-chain acids, as already mentioned in the literature by HUIJBERS et. al, 2018, and leads us to think about the need for greater light power for the conversion to occur.

Table 2. Conversion of the photoenzymatic decarboxylation reaction using violet light (50W).

Substrate	Conversion (%)	
	60 minutes	10 minutes
Palmitic Acid	>99	>99
Stearic Acid	>99	>99
Arachidic Acid	>99	>99

Reaction conditions: Substrate (13 mM), CvFAP (5.1 mg.mL⁻¹), Tris-HCl buffer (pH 8.5), 30% DMSO, illumination with violet light (50W) for 10 and 60 min. Palmitic acid (C₁₆H₃₂O₂) MM= 256.4 g/mol; Stearic acid (C₁₈H₃₆O₂) MM= 284.48 g/mol; Arachidic acid (C₂₀H₄₀O₂) MM= 312.5304 g/mol.

4 CONCLUSION

The study provides high conversions in a short time for photodecarboxylation of different fatty acids in the presence of an alkane synthase, CvFAP, establishing a new protocol for the formation of these hydrocarbons, using alternative LEDs with different substrates. Two reaction times were used and photoenzymatic decarboxylation proved to be efficient both on acidic substrates with a 14-carbon chain and acids with a chain composed of 20 carbons, expanding the range of substrates that can be used in these photodecarboxylation reactions. Compared with results previously reported in the literature, this methodology demonstrates the efficiency of CvFAP in catalytic processes, pointing out new methods for converting different acidic substrates. The use of higher substrate concentrations should be considered for the next steps, overcoming the challenges to obtain the broader use of this family of enzymes for biotechnological applications.

5 REFERENCES

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5 ACKNOWLEDGEMENTS

