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Impact of sunlight irradiation on CvFAP photodecarboxylation

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A visible-light-driven photocatalytic decarboxylation of palmitic acid and related fatty acids is described in this study. Remarkable decarboxylation rates have been observed with full conversion in less than 20 min. In this study, we have demonstrated that sunlight irradiation, even on cloudy days, can deliver similar results to traditional LED lamps while using much less energy and minimizing environmental impact. The findings indicate that the process of enzymatic decarboxylation could be useful for the production of different biofuels in the future.

KEYWORDS

photodecarboxylation, biocatalysis, green chemistry, sunlight, CvFAP

1 Introduction

Ten years have passed since the 100th anniversary of the article published by Giacomo Ciamician in which he challenges chemists, especially organic chemists, to imagine a chemical industry that could mimic plants by using a more sustainable and renewable process to synthesize organic molecules (Ciamician, 1912). In the past decades, chemists have made remarkable advances regarding the use of light to perform chemical reactions and the renaissance of organic photochemistry is underway (Akita and Koike, 2015). The biocatalysis field has also been part of this development with an increasing number of publications on photobiocatalysis where enzymes or co-factors could be activated or regenerated by light (Peng et al., 2021). Some examples of natural photobiotransformations include chlorophyll biosynthesis (Heyes et al., 2021), the repair of light-damaged DNA (Weber, 2005) and the photodecarboxylation of long-chain acids to the corresponding alkane/alkene via fatty acid photodecarboxylase (FAP) (Huijbers et al., 2018; Sorigué et al., 2018; Xu et al., 2019; Lakavath et al., 2020). Advances in photochemistry, specifically in the development of LED technology, have allowed a significant improvement in the investigation of new light-dependent reactions, transforming photochemistry into a powerful tool for organic chemistry and catalysis (Xie et al., 2020). Interestingly, the majority of photochemical studies do not utilize real sunlight, the ultimate green source of energy, but rather focus on artificial illumination (Esser et al., 19194). From a Green Chemistry perspective, the Sun represents a virtually inexhaustible energy source (Sambigi and Noël, 2020). Therefore, we became interested in exploring the possibility of applying the experience of our group (Benincá et al., 2022) to further develop the field of sunlight decarboxylation mediated by *Chlorella variabilis* FAP (CvFAP).

2 Materials and methods

2.1 Strain, vector, and materials

Escherichia coli DH5 α was used for DNA manipulation. The plasmid containing the CvFAP gene (short length; comprising residues 62-654) has been reported previously (Huijbers et al., 2018). Competent *E. coli* BL21 (DE3) cells were transformed with the plasmid for recombinant enzyme production. Kanamycin was purchased from Fluka. Palmitic acid was purchased from Sigma-Aldrich.

2.2 Preparation of the enzymatic extract

Enzyme expression was performed as described by Huijbers et al. (2018). After enzyme expression, cells were harvested by centrifugation (11,000 g at 4°C for 10 min), washed twice with washing buffer (50 mM Tris-HCl buffer, pH 8, containing 100 mM NaCl), resuspended in the same buffer with the addition of 1 mM PMSF and 20% glycerol, and stored at -80°C. The cells were prepared for lysis by centrifugation and resuspension in lysis buffer (100 mM Tris-HCl buffer, pH 8.5, containing 1 mM PMSF and 5% glycerol). Cell lysis was performed by VIBRA-CELL VCX 500 Sonics (United States) instrument using the following settings: 30% amplitude; 15 min sonication; ON/OFF pulses of 10 s on and 30 s off. The obtained extract was frozen with liquid nitrogen and stored at -80°C to be used in the photocatalytic reactions. The total protein concentration of the extracts was determined and standardized by Smith (1985) assays (Smith et al., 1985).

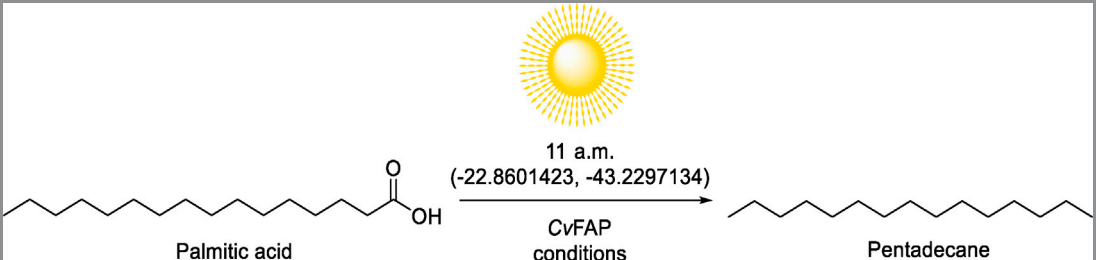
2.3 Photocatalytic reactions in batch with solar light

The photoenzymatic decarboxylation reactions using solar light irradiation were carried out in an open glass jacketed reactor at 35°C. The reactions were performed in Tris-HCl buffer (pH 8.5, 100 mM) containing 30 vol% DMSO as cosolvent and 13 mM of palmitic acid as substrate. The enzymatic extracts were used in a 5.6 mg \times ml⁻¹ total protein corresponding to a CvFAP concentration of approx. 6.2 μ M final concentration or as indicated. The reactor was exposed to sunlight during the summer season under constant and gentle magnetic stirring starting at 11 a.m. (in Rio de Janeiro, GPS coordinates: -22.8601423 and -43.2297134) on 6 April 2022. Aliquots (20 μ l) were withdrawn, and the substrates and products were extracted with 1 ml of ethyl acetate. The remaining organic phase was analyzed by gas chromatography.

2.4 Gas chromatography analysis

Samples were prepared by extracting 20 μ l of reaction crude in 1 ml of ethyl acetate. Conversion percentages were analyzed by chromatogram areas using the Shimadzu GC2014 GC-FID-Cpsil 5 CB column (50 m \times 0.53 mm \times 1.0 μ m). Injection temperature 260°C, injection split ratio 20.0, carrier gas was N₂, pressure 89.0 kPa, column flow 4.86 ml min⁻¹. The oven temperature setting was: 110°C, heated at 25°C min⁻¹ to 190°C for 3 min, and remained heating at 25°C min⁻¹ to 280°C. Conversion percentages were analyzed by chromatogram area based on a calibration curve.

TABLE 1 Initial evaluation of solar light irradiation on photodecarboxylation reaction of palmitic acid mediated by CvFAP.



Entry	Time (min)	Conv. (%)	CvFAP concentration [μ M]	Light source	References
1	12	>99	6.2 ^a	Sun	This work ^a
2	50	>99	6.2 ^a	Blue LED (300 W)	Benincá et al. (2022)
3	60	>99		White LED (300 W)	
4	180	>99	6	Blue LED	Huijbers et al. (2018) ^b
5	20	>99		Blue LED (WLE)	Duong et al. (2021) ^c

Reaction conditions

^aTo a transparent jacketed reactor (total volume 25 ml) 3 ml DMSO, palmitic acid (13 mM), and 7 ml Tris-HCl buffer (pH 8.5, 100 mM) containing the cell extract (5.6 mg \times ml⁻¹ of total protein) were added. The reactions were carried out at 37°C using 300 W LED (blue or white) or Sun light source at 11 a.m. (22/04/06, -22.8601423, -43.2297134; 127.4 \times 10³ lux). The conversions were measured by gas chromatography; estimated from densimetric analysis of the cell extract.

^bThe reaction was performed with palmitic acid 13 mM in Tris-HCl buffer (pH 8.5, 100 mM) at 37°C using blue light illumination (intensity = 13.7 μ E L⁻¹ s⁻¹).

^cThe reaction was performed in a cylindrical glass reactor (100 ml, 56 mm diameter, 105 mm height) using internal illumination wireless light emitters (WLE) with 451 nm peak emission blue LED.

TABLE 2 Influence of higher palmitic acid concentration on the performance of the CvFAP-catalyzed photodecarboxylation under solar light irradiation.

Entry	Palmitic acid (mM)	Reaction time (min)	Conversion (%)
1	13	20	>99
2	19.5	60	96
3	26	60	49
4	34.5	60	36

*Reaction conditions: To a transparent jacketed reactor (total volume 25 ml) 3 ml DMSO, palmitic acid and 7 ml Tris-HCl buffer (pH 8.5, 100 mM) containing the cell extract ($2.8 \text{ mg} \times \text{m}^{-1}$ of total protein, $[(\text{CvFAP}) \approx 3.1 \text{ } \mu\text{M}]$ were added. The reactions were carried out at 11 a.m. (22/04/13, -22.8601423, -43.2297134) and exposed to the sunlight ($118.1 \times 10^3 \text{ lux}$) at 37°C. Conversions were measured by gas chromatography.

TABLE 3 Palmitic acid photodecarboxylation reaction mediated by CvFAP on cloudy conditions.

Entry	Time	Concentration ($\text{mg} \cdot \text{ml}^{-1}$)	Conversion (%)
1	20	2.8	69
2		5.6	87
3	60	2.8	>99
4		5.6	>99

Reaction conditions: To a transparent jacketed reactor (total volume 10 ml) palmitic acid (13 mM) and 2 ml Tris-HCl buffer (pH 8.5, 100 mM) 30% DMSO, containing the cell extract (2.8 or 5.6 mg ml^{-1} of total protein) were added. The reaction was carried out at 11 a.m. (22/04/27, -22.8601423, -43.2297134) and exposed to solar rays ($41 \times 10^3 \text{ lux}$) at 37°C for 20 or 60 min. The conversions were measured by gas chromatography.

3 Results

Following our initial results using high power blue LEDs (300 W) to reduce the reaction time for full conversion, we decided to investigate the batch decarboxylation reaction of palmitic acid catalyzed by CvFAP under sunlight irradiation. The first set of experiments was conducted using conditions previously developed by our research group (13 mM of palmitic acid in DMSO/ $5.6 \text{ mg} \times \text{ml}^{-1}$ of cell extract at 37°C in Tris-HCl buffer) (Benincá et al., 2022) and evaluated how our reaction set-up would perform in terms of product yield (Table 1).

A remarkable response has been found for CvFAP when the Sun was used as the light source, with conversion rates that were comparable to those found with LED. The results revealed that reductions on reaction time are possible, allowing to achieve excellent conversions in just 12 min of reaction using sunlight (Entry 1, Table 1). Following these results, the total protein concentrations were reduced to $2.8 \text{ mg} \times \text{ml}^{-1}$ $[(\text{CvFAP}) \approx 3.1 \text{ } \mu\text{M}]$, and higher palmitic acid concentrations (13–34.5 mM) were evaluated, limiting the reaction time to 1 h. The palmitic acid concentration is an important parameter in process

optimizations while we aim to maximize the space-time-yield of the desired reaction system (Table 2).

As observed the reduction of total protein to $2.8 \text{ mg} \times \text{ml}^{-1}$ allowed us to obtain a full conversion of a 13 mM solution of palmitic acid in only 20 min (Entry 1, Table 2). The reaction carried out under higher concentrations (Entry 2, Table 3) proved to be effective in up to 19.6 mM, with a slight decrease in reaction conversion (96%). However, palmitic acid values higher than 26 mM (Entries 3 and 4, Table 3) demonstrate an abrupt decrease in reaction conversion. The turnover number of CvFAP in these experiments was always around 4,100 indicating that either the reaction time was too short to enable higher conversions or that the biocatalyst was photoinactivated.

Previous studies from the literature have shown that the increase in light intensity is efficient for enhancing the productivity of CvFAP (Sorigué et al., 2018; Duong et al., 2021; Benincá et al., 2022), although the present approach shows that the broader wavelength range provided by sunlight including more energetic wavelengths (<420 nm) with lower intensity light source (Loh et al., 2021) still can provide good conversions. In our experiments (Table 3), we observed a reduction in the efficiency

of our process which can be attributed to the lower sunlight intensity. However, with these experiments, we were able to prove that even under low sunlight availability or considering different parts of the world where sunlight intensity is lower, the photodecarboxylation works, thus motivating us to investigate the reaction reproducibility under less intense luminosity conditions. The natural lower light condition used for the development of the present experiment are detailed described in the [Supplementary Material \(Supplementary Section S4.2.6\)](#) and their respective results are presented in [Table 3](#).

As can be observed (Entries 3 and 4, [Table 3](#)) full conversions were achieved after 60 min, thus revealing that even under unfavorable natural light conditions, the reaction still leads to the desired product. At standard conditions (20 min) a slight decrease could be observed but maintaining conversion value close to the process described at intense lighting using high-power LEDs (Benincá et al., 2022).

4 Conclusion

The results have shown that photodecarboxylation reaction is possible to be performed under sunlight irradiation with very fast reaction times even in cloudy days. The contribution of <400 nm range, a wavelength range shorter than the blue light widely used to date, as a component of sunlight could justify the yields in the sunlight reactions using lower light intensity in comparison to blue LED lamps.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#), further inquiries can be directed to the corresponding author.

Author contributions

AF, KD, GB, and RA performed the experimental work and analysed the data.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fctls.2023.1165079/full#supplementary-material>

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