



HAL
open science

Environmental impact assessment of biohydrogen production from orange peel waste by lab-scale dark and photofermentation processes

Brenda Nelly López-Hernández, Carlos Escamilla-Alvarado, María Guadalupe Paredes, Alonso Albalate-Ramírez, Santiago Rodríguez-Valderrama, Jean-Pierre Magnin, Pasiano Rivas-García

► To cite this version:

Brenda Nelly López-Hernández, Carlos Escamilla-Alvarado, María Guadalupe Paredes, Alonso Albalate-Ramírez, Santiago Rodríguez-Valderrama, et al.. Environmental impact assessment of biohydrogen production from orange peel waste by lab-scale dark and photofermentation processes. *Revista Internacional de Contaminacion Ambiental*, 2024, 40, pp.27-40. 10.20937/RICA.55063 . hal-04729643

HAL Id: hal-04729643

<https://hal.univ-grenoble-alpes.fr/hal-04729643v1>

Submitted on 10 Oct 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

ENVIRONMENTAL IMPACT ASSESSMENT OF BIOHYDROGEN PRODUCTION FROM ORANGE PEEL WASTE BY LAB-SCALE DARK AND PHOTOFERMENTATION PROCESSES

Evaluación de impacto ambiental de la producción de biohidrógeno a partir de bagazo y cáscara de naranja por procesos de fermentación oscura y luminosa a escala laboratorio

Brenda Nelly LÓPEZ-HERNÁNDEZ¹, Carlos ESCAMILLA-ALVARADO^{1*}, María Guadalupe PAREDES², Alonso ALBALATE-RAMÍREZ¹, Santiago RODRÍGUEZ-VALDERRAMA², Jean-Pierre MAGNIN³, and Pasiano RIVAS-GARCÍA¹

¹ Universidad Autónoma de Nuevo León, Centro de Investigación en Biotecnología y Nanotecnología (CIByN), Faculty of Chemical Sciences, Parque de Investigación e Innovación Tecnológica, km 10 Autopista al Aeropuerto Internacional Mariano Escobedo, Apodaca, 66629, Nuevo León, México.

² Escuela de Ingeniería, Universidad de Monterrey, San Pedro Garza García, 66238, Nuevo León, México.

³ Laboratoire de Electrochimie et Physico-chimie des Matériaux et des Interfaces, Institute d'Ingénierie et de Management, Université de Grenoble Alpes, 38000 Grenoble, France.

*Author for correspondence: carlos.escamillalv@uanl.edu.mx

(Received: July 2023; accepted: February 2024)

Key words: bioenergy, bioprocesses, life cycle assessment, organic waste, purple non-sulfur bacteria.

ABSTRACT

Orange peel waste (OPW), as a relevant and recoverable residue, is composed of peel, internal tissue, pulp, and seeds. This organic residue is rich in carbohydrates useful for biofuel production processes. Biohydrogen is obtained from dark fermentation (DF) and photofermentation (PF), which are complementary in the bioprocessing chain. The objective of this work was to compare the environmental impact assessment of a sequential dark-photofermentation process (Scenario DF-PF) and the individual fermentation processes: dark fermentation (Scenario DF) and photofermentation (Scenario PF). The assessment was performed at a laboratory scale, and the functional unit (FU) was defined as the production of 1 kg H₂. Scenario DF showed the lowest environmental impacts regarding global warming and fossil resource scarcity indicators, with 375.5 kg CO₂-eq/FU and 108.8 kg oil-eq/FU, respectively, followed by Scenario DF-PF and Scenario PF. Scenario DF-PF presented the lowest impact regarding the water consumption indicator (7.6 m³/FU), followed by Scenario DF, which had 46.6% more impact. Additionally, Scenario DF-PF required 3.3 times less substrate than Scenario DF due to more efficient substrate conversion. The high share of fossil fuels in the Mexican electricity mix directly impacts the processes that require electric energy, such as the PF process. Therefore, eco-friendly light sources should be used to minimize effects. Valorization of OPW could follow two routes depending on whether the aim is to reduce a specific environmental impact indicator or biowaste conversion efficiency.

Palabras clave: análisis de ciclo de vida, bacterias púrpuras no del azufre, bioenergía, bioprocesos, residuos orgánicos.

RESUMEN

El bagazo y cáscara de naranja (BCN) son un residuo importante y valorizable en México. Se compone principalmente de cáscara, tejido interno, pulpa y semillas. El BCN es un residuo orgánico rico en carbohidratos útiles en la producción de biocombustibles, como el biohidrógeno, que se puede obtener de procesos como la fermentación oscura (DF) y fotofermentación (PF). El objetivo de este trabajo fue comparar la evaluación del impacto ambiental de un proceso secuencial de fermentación oscura–fotofermentación (Escenario DF-PF) y los procesos individuales: fermentación oscura (Escenario DF) y fotofermentación (Escenario PF). La evaluación se realizó a escala laboratorio, y la unidad funcional (UF) se definió como la producción de 1 kg de H₂. El Escenario DF mostró los impactos ambientales más bajos en cuanto a los indicadores de calentamiento global y escasez de recursos fósiles, con 375.5 kg CO₂-eq/UF y 108.8 kg crudo-eq/UF, respectivamente, seguidos por el Escenario DF-PF y el Escenario PF. El Escenario DF-PF presentó el impacto más bajo en términos del indicador de consumo de agua (7.6 m³/UF), seguido del Escenario DF, que tuvo un 46.6 % más de impacto. Además, el Escenario DF-PF requirió 3.3 veces menos sustrato que el Escenario DF debido a una conversión de sustrato más eficiente. La alta proporción de combustibles fósiles en la matriz eléctrica mexicana impacta directamente en los procesos que requieren energía eléctrica, como el proceso PF, lo cual indica que se deben usar fuentes de luz más amigables con el medio ambiente para minimizar sus efectos. La valorización del BCN podría seguir dos vías dependiendo de si se busca la reducción de un indicador de impacto ambiental específico o la eficiencia de conversión de biomasa.

INTRODUCTION

Oranges are the most consumed and produced citrus fruits in the world. They are about 60% of the world's citrus (Hilali et al. 2019). In 2021, 75.6 Mt of oranges were produced worldwide, and Mexico was the fourth largest producer of this citrus fruit (FAO 2023). In the same year, orange was Mexico's most productive fruit crop, producing nearly 4.6 Mt (SIAP 2022).

Approximately 70% of the oranges produced are primarily used in the food industry (Martín et al. 2010). Orange juice is the main product of this citrus processing, of which 50–60%w of these processed citrus fruits are residues, denoted as orange peel waste (OPW) (Santi et al. 2014, Santiago et al. 2020). OPW is composed of peel (albedo and citrus peel), pulp (vesicles), nucleus, membrane, and seeds (Satari and Karimi 2018, Zema et al. 2018).

OPW is an organic residue with acidic pH, high water content, rich organic matter, and essential oils constitution (Bicas et al. 2008, Calabrò et al. 2015). Due to these characteristics, its final disposal in landfills is banned according to Directive 2008/98/EC of the European Parliament (EP and EU Council 2008); besides, it has the potential to yield value-added products. Despite this, in Mexico, most OPW is disposed of in landfills, which emits approximately 860 kg CO₂-eq/t (Tsydenova et al. 2019). Therefore, improving this waste management is a must that will allow greenhouse

gas (GHG) emissions reduction and migration to sustainable systems and circular economies.

Due to its high energy content per unit mass (142 MJ/kg), biohydrogen is considered a promising alternative energy carrier. Additionally, its combustion does not contribute to GHG emissions or acid rain (Ghimire et al. 2015). Over the past decades, the biological processes involved in biohydrogen production have been extensively studied (Argun et al. 2017, Mishra et al. 2019, Rodríguez-Valderrama et al. 2020a). Existing biological techniques for producing biohydrogen include dark fermentation (DF) and photofermentation (PF) using carbohydrate-rich substrates as raw materials.

DF is a bioprocess in which hydrogenogenic fermentative bacteria produce biohydrogen from biomass with volatile organic acids (VOA) and carbon dioxide as by-products (Das and Basak 2021). On the other hand, PF is the process by which photosynthetic bacteria, mainly purple non-sulfur bacteria (PNSB), convert monosaccharides and VOA into biohydrogen and carbon dioxide under anaerobic conditions in the presence of light (Argun and Kargi 2011). In PF, sugar and VOA can be used as substrates. However, in this biological process, VOA are more beneficial for biohydrogen production (Tian et al. 2019). Furthermore, dark fermentation liquid effluent (DFE_{liquid}) is rich in VOA; hence, it can be used as a substrate in PF. Moreover, sequential DF-PF processes are more efficient in biohydrogen production than single-step processes (Mishra et al. 2019, Tian et al. 2019).

However, it is crucial to consider these processes from an environmental perspective. Life cycle assessment (LCA) is a scientific study that quantifies environmental impacts by considering all inputs (i.e., energy, materials, and water) and outputs (i.e., products, emissions, and energy) of the system under study. Through LCA, it is possible to identify the hotspots of a system, whereas decision-making is facilitated by its technical and ecological feasibility (Tian et al. 2019). For instance, Joglekar et al. (2019) performed a LCA of the biorefinery approach for valorizing fruit peels to produce biofertilizers, dietary fiber, livestock feed, enzymes, bioactive compounds, and bioenergy. The authors found that the contribution to environmental impacts in the biorefineries of peels and fruit residues is affected differently according to the bioprocess involved and conclude that special attention should be paid to the type of process, as this directly compromises environmental impacts and the development of biorefineries.

Studies have quantified the environmental impacts of sequential DF-PF processes to produce biohydrogen from various organic residues. Ochs et al. (2010) evaluated a DF-PF process through LCA Eco-Indicator 99 methodology from steamed potato peel, where they found that the most significant environmental impacts are due in 53.5% to the use of phosphates as a buffer in the fermentative process. Djomo and Blumberga (2011) assessed a pilot plant DF-PF process using wheat straw, sorghum stalk, and potato husks as substrates through the LCA impact 2002+ method. They reduced GHG emissions by up to 57% using a DF-PF process compared to a methane reforming process.

Indeed, there is no information regarding the environmental impacts of biohydrogen production using OPW by a sequential process DF-PF. Considering the potential of this substrate, this work contributes to the environmental evaluation of the sequential DF-PF and its comparison to individual fermentation processes to broaden the knowledge in biohydrogen production and contribute to the adoption of sustainable recovery schemes for OPW.

Therefore, this study aims to conduct the environmental impact assessment using global warming, water consumption, and fossil resource scarcity indicators within the framework of LCA to produce 1 kg of biohydrogen from OPW by DF and PF, individual and couple processes. Specific areas of concern and hotspots were identified to contribute to using fermentative bioprocesses for biohydrogen production. In the context of circular economy and waste valorization, the proposed scientific study on biohydrogen production from OPW holds significant importance.

MATERIALS AND METHODS

The LCA method was followed according to the ISO 14040 (ISO 2006a) and ISO 14044 (ISO 2006b). Therefore, the following subsections comply with the stages of LCA methodology: 1) goal and scope definition, 2) inventory analysis (LCI), and 3) impact assessment (LCIA). The fourth stage, interpretation of results, is comprised in the Results and Discussion section. For the LCI build-up, experimental data from our research group were considered.

Goal and scope definition

The goal of this study was to perform an environmental impact assessment of the sequential DF-PF process for biohydrogen production from OPW and of the individual fermentation processes DF and PF.

The environmental impact evaluation was performed according to the LCA methodology under an attributional “gate-to-gate” approach that complies with the experimental data and development stage of the technologies. As the scale of the considered processes is at the laboratory level, the functional unit (FU) was established as the production of 1 kg H₂.

This assessment aimed to identify hotspots that could contribute to the application of fermentative bioprocesses for biohydrogen production, generate value-added products, and participate in the solution of OPW management. The results of this study will pave the way for the design of environmentally efficient OPW valorization schemes. They will be an essential pillar in the transition of the agri-food industry toward a circular economy.

System definition and boundaries

The systems compared in this study and their boundaries are shown in **figure 1**. The base scenario is described in **figure 1a**, corresponding to the sequential DF-PF process (Scenario DF-PF). This scenario was selected because this study aims to glimpse the environmental benefits or deficiencies of the sequential process DF-PF, as it has been highlighted as an evolution of single biomass bioprocessing for biohydrogen production (Rao and Basak 2022).

The stages of Scenario DF-PF included grinding, alkaline hydrogen peroxide (AHP) pretreatment, DF, and PF. Grinding of the OPW was performed by an industrial blender (TAPISA[®] T3L, Mexico). Subsequently, the OPW underwent the AHP pretreatment with a solution of 3% H₂O₂ v/v

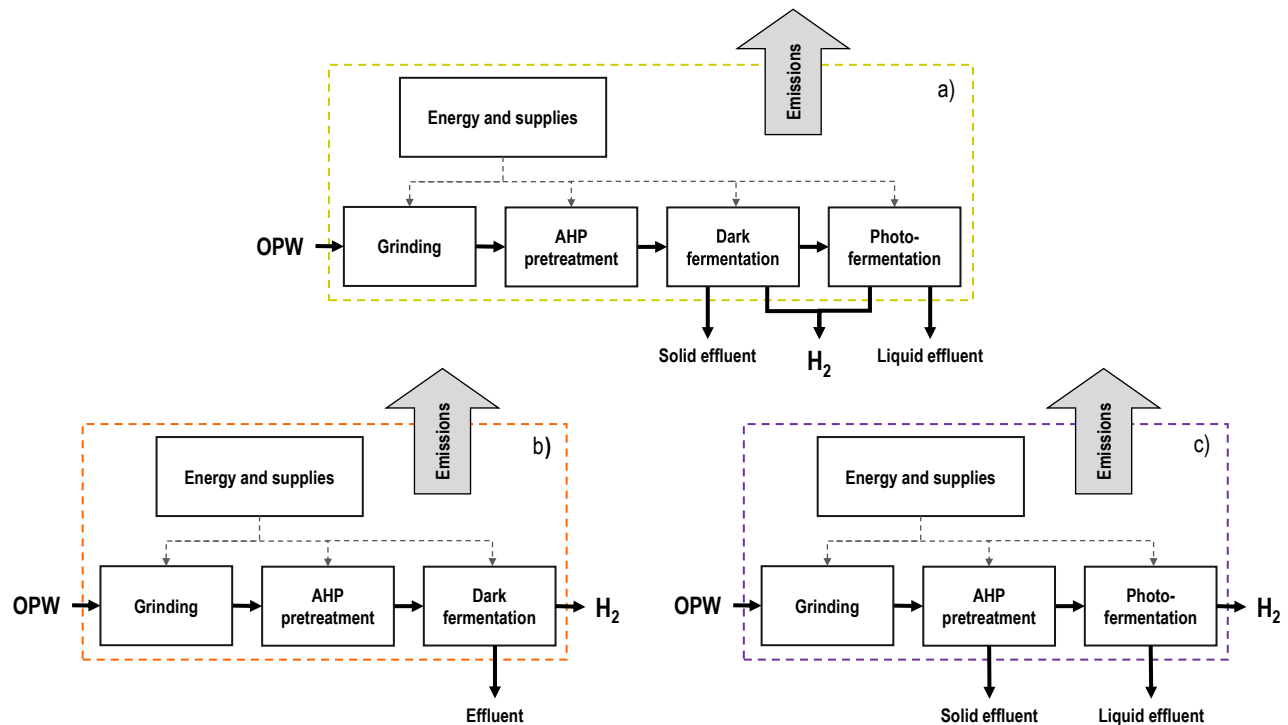


Fig. 1. System boundaries of a) Scenario sequential dark photofermentation process (Scenario DF-PF); b) Scenario individual dark fermentation process (Scenario DF); c) Scenario individual photofermentation process (Scenario PF).

at pH 11.5 and room temperature for 24 hours, maintaining a 40 gVS/L concentration. Afterward, the temperature was increased to 50 °C for 30 min to decompose the remaining H₂O₂ (Michalska and Ledakowicz 2014). The pretreated OPW was fed into the DF laboratory scale batch reactors, loaded at an inoculum-substrate ratio (ISR) of 0.6, and operated at 35 °C for seven days. DFE_{liquid} was recovered after centrifugation and used as the carbon source in the PF. The biohydrogen production by PF was performed at 30 °C using a high-pressure sodium light (Sylvania SHP-TS 150W), considering three days of operation, which corresponded to the exponential phase of PNSB at which biohydrogen production is most efficient (Lu et al. 2020).

In this work, the environmental impacts of our base scenario were compared with individual fermentative biohydrogen production processes: Scenario DF and Scenario PF. The system boundaries for these scenarios are shown in **figures 1b–c**. Scenario DF did not consider the photofermentative stage; hence, it only included grinding, AHP pretreatment, and DF stages. In contrast, Scenario PF omitted the DF stage; therefore, it only comprised grinding, AHP pretreatment, and PF stages.

The operating conditions of the processes within these scenarios were the same as those in the corresponding Scenario DF-PF.

The following are further considerations included in the impact evaluation.

Wet OPW substrate was introduced into the system after being industrially processed to extract the juice and essential oil (d-limonene). Extracting d-limonene in the juice industry is a common practice, as it is a high-value product with a commercial price of 2437 MXN for 1 mL (Sigma Aldrich 2023). In addition, its antibacterial properties have been reported to inhibit fermentation processes such as anaerobic digestion (Ruiz and Flotats 2016). Transportation of OPW from the production source to the processing laboratory was not considered for the system boundaries.

The inoculum for DF biohydrogen production was obtained from an anaerobic digester fed with vegetable and fruit residues. The DF inoculum underwent heat pretreatment in a water bath at 96 °C for 2 hours to inhibit the methanogenic archaea and benefit the presence of hydrogen-producing microorganisms (Rodríguez-Valderrama et al. 2020b). No mineral media, buffers, or initial pH

adjustments were considered for low-impact biohydrogen production.

The biohydrogen production by PF was carried out in an RCV mineral medium (He et al. 2006). Each liter of RCV medium was composed of a phosphate buffer (0.6 g/L KH_2PO_4 and 0.9 g/L K_2HPO_4) to maintain the neutral pH, 50 mL of super salts medium, and 20 mL of trace element solution. Super salts medium (1 L) was composed of 0.236 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.4 g EDTA ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$), 1.5 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 4 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.02 g thiamine (vitamin B1). One liter of trace element solution contained: 2.8 g H_3BO_3 , 1.592 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.04 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.24 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.752 g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$.

This study did not include emissions from DF inoculum formulation and growth of PNSB. The energy and emissions from equipment manufacturing were omitted due to their irrelevance compared to

the overall amount of biohydrogen that can be obtained during their operation lifetime (Djomo and Blumberga 2011). The analysis did not consider emissions due to waste disposal.

Life cycle inventory analysis and impact assessment

The LCI included the supplies and energy requirements of each stage based on the mass and energy balances of the process, as well as its associated impacts obtained from the Ecoinvent 3.7.1 database (Ecoinvent Association 2020). The specific ecoinventories used for each activity are shown in **table I**. The operation conditions for the primary processing stages for both individual and sequential biohydrogen production scenarios are shown in **table II**.

The energy required by the industrial blender and centrifuge was calculated from the information provided by the manufacturer's technical data sheets (**Table II**). For the preparation of the PF

TABLE I. REFERENCE TECHNOLOGIES USED FOR ACTIVITIES IN ENVIRONMENTAL IMPACT ASSESSMENT.

Stages	Process / production	Ecoinvent reference technologies used
Grinding	OPW 22.6% TS grinding	Electricity, low voltage (MX) market for APOS, S
AHP Pretreatment	Pretreatment of OPW with hydrogen peroxide 3%v/v	Water, deionized (RoW) market for water, deionized APOS, S Sodium hydroxide, without water, in 50% solution state (GLO) market for APOS, S Hydrogen peroxide, without water, in 50% solution state (RoW) market for hydrogen peroxide APOS, S Electricity, low voltage (MX) market for APOS, S
Dark fermentation	Biohydrogen and VOA production from pretreated OPW	Electricity, low voltage (MX) market for APOS, S
Separation	Separation of liquid and solid fractions by centrifugation	Electricity, low voltage (MX) market for APOS, S
Photofermentation	Biohydrogen production from $\text{DFE}_{\text{liquid}}$ (Scenario DF-PF) and $\text{PAHPE}_{\text{liquid}}$ (Scenario PF)	Sodium phosphate (RoW) market for APOS, S Water, deionized (RoW) market for water, deionized APOS, S Iron sulphate (RoW) EDTA, ethylenediaminetetraacetic acid (GLO) market for APOS, S Calcium chloride (RoW) market for APOS, S Magnesium sulphate (GLO) market for APOS, S Manganese sulphate (GLO) market for APOS, S Boric acid, anhydrous, powder (GLO) market for APOS, S Copper sulphate (GLO) market for APOS, S Zinc monosulphate (RoW) market for APOS, S Ammonium chloride (GLO) market for APOS, S Electricity, low voltage (MX) market for APOS,

APOS: Allocation at the point of substitution; GLO: global; MX: Mexico; RoW: rest of the world; S: system.

substrate, a separation of the effluents from the previous stages was performed with a centrifuge (ZM 03-ECO 1, Germany) of 1.5 kW. The production of supplies and energy required were contemplated for each process stage. Equations 1-4 (**Table III**) comprise the energy requirements for reaching operation temperatures at each stage of the processes and the heat losses to the environment. To determine the luminosity of the lamp, an approximation of 20 klx \approx 51 W/m² was considered, according to Rodríguez-Valderrama et al. (2022). Our research

group obtained this relationship with two devices: an irradiance meter (VOLTcraft PL-110SM) and a luminosity meter (Steren HER-410). The energy requirements were calculated from experimental and bibliographic data, applying the parameter values from **table IV**.

The evaluation of the LCIA was carried out using an attributional approach. The software SimaPro 9.2 (PRé Sustainability 2021) was used to create the analysis model applying the ReCiPe 2016 midpoint (H) V1.05 / World (2010) H methodology

TABLE II. OPERATION CONDITIONS OF THE PROCESSING STAGES WITHIN BIOHYDROGEN PRODUCTION SCENARIOS.

Grinding ^a	AHP pretreatment	DF ^b	PF ^c
P = 0.37 kWh t = 0.5 min/kg OPW	H ₂ O ₂ 3%v/v pH 11.5 T = 25 °C [OPW] = 40 g VS/L [VOA] _{out} = 5.31 g/L	ISR 0.6 T = 35 °C t = 7 days [VOA] _{out} = 9.07 g/L Y _{H₂} = 68.94 mL H ₂ /g VS	T = 30 °C t = 3 days Light intensity = 20 klx ^b C/N ⁺ = 17 VOA 35%v/v Y _{H₂} = 486.83 mL H ₂ /g VOA

^a: industrial blender TAPISA[®] T3L, Mexico.

^b: data from López-Hernández 2023

^c: 20 klx \approx 51 W/m² according to Rodríguez-Valderrama et al. (2022).

C/N⁺: carbon-to-nitrogen ratio; VS: volatile solids; Y_{H₂}: biohydrogen yield.

TABLE III. EQUATIONS USED FOR ENERGY CONSUMPTION ESTIMATIONS.

Variables	Formulae	Units	Eq.
Energy consumption in AHP pretreatment	$E_{AHP} = m_{AHP} C_{pAHP} (T_{op} - T_{amb}) + U_{AHP} A_{AHP} (T_{op} - T_{amb})t$	kW	1
Energy consumption for inoculum heat-pretreatment in DF	$E_{DFi} = m_{DFi} C_{pDFi} (T_{op} - T_{amb}) + U_{DFi} A_{DFi} (T_{op} - T_{amb})t$	kW	2
Energy consumption in DF	$E_{DF} = m_{DF} C_{pDF} (T_{op} - T_{amb}) + U_{DF} A_{DF} (T_{op} - T_{amb})t$	kW	3
Energy consumption in PF	$E_{PF} = m_{PF} C_{pPF} (T_{op} - T_{amb}) + U_{PF} A_{PF} (T_{op} - T_{amb})t$	kW	4
AHP pretreatment, DF, PF	$U = \frac{1}{L\lambda + 1/h}$	kW/m ² k	5

A: heat transfer area; *h*: convection coefficient; λ : conduction coefficient for glass; E_{AHP} : energy consumption in the AHP pretreatment; E_{DFi} : energy consumption in the inoculum heat-pretreatment used in DF; E_{DF} : energy consumption in DF; E_{PF} : energy consumption in PF; L: glass thickness; T_{amb}: ambient temperature; t: time; T_{op}: operation temperature; U: overall heat transfer coefficient of each process.

TABLE IV. PARAMETERS USED IN ENERGY CONSUMPTION ESTIMATIONS.

Parameter	Variable	Value	Units	Reference
Overall heat transfer coefficient for AHP pretreatment	U_{PAHP}	9.71	W/m·K	Own calculation
Heat transfer area for AHP pretreatment	A_{PAHP}	70.55 ^a ; 230.5 ^b ; 250.14 ^c	m ²	Own calculation
Heat capacity at constant pressure for AHP pretreatment (OPW+H ₂ O+ H ₂ O ₂).	C_{PPAHP}	4.16	kJ/kg·K	(Cengel, 2007)
Overall heat transfer coefficient for DF inoculum heat-pretreatment	U_{DFi}	9.71	W/m·K	Own calculation
Heat transfer area for DF inoculum heat-pretreatment	A_{DFi}	29.19 ^a ; 95.34 ^b	m ²	Own calculation
Heat capacity at constant pressure for DF inoculum heat-pretreatment (inoculum)*	C_{PDFi}	4.18	kJ/kg·K	(Cengel, 2007)
Overall heat transfer coefficient for DF	U_{DF}	9.71	W/m·K	Own calculation
Heat transfer area for DF	A_{DF}	99.74 ^a ; 325.88 ^b	m ²	Own calculation
Heat capacity at constant pressure for DF (inoculum+OPW pretrated)	C_{PDF}	4.17	kJ/kg·K	(Cengel, 2007)
Overall heat transfer coefficient for PF	U_{PF}	9.71	W/m·K	Own calculation
Heat transfer area for PF	A_{PF}	656.2 ^a ; 1596.07 ^c	m ²	Own calculation
Heat capacity at constant pressure for PF (RCV medium+DFE)*	C_{PPF}	4.18	kJ/kg·K	(Cengel, 2007)

*Assumed as the C_p of water = 4.18 kJ/kg·K

^a:Scenario DF-PF; ^b: Scenario DF; ^c: Scenario PF.

(Huijbregts et al. 2017). Materials and energy production data for the process supply were obtained from the Ecoinvent 3.7.1 database (Ecoinvent Association 2020). The indicators of global warming (GWI), water consumption (WCI), and fossil resource scarcity (FSI) were selected for the environmental impact assessment due to their high incidence in the process.

RESULTS AND DISCUSSION

The LCI is shown in **tables V-VII**, where a vast difference in energy consumption can be observed between the different scenarios. The highest energy consumption was obtained in Scenario PF (2058.49 kWh/FU), followed by Scenario DF-PF (874.73 kWh/FU)

and Scenario DF (254.95 kWh/FU), mainly due to the energy consumption in the PF process, specifically, electricity used in light irradiation. A similar trend was observed in H₂O₂ consumption at the AHP pretreatment stage, where Scenario PF consumed 7.8 and 72% more H₂O₂ than Scenario DF and Scenario DF-PF, respectively. Furthermore, Scenario PF required 3.1 and 2.7 times more water compared to the DF and DF-PF scenarios because of higher inlet flows in Scenario PF, resulting in more water consumption to maintain a volumetric proportion of 35% VOA (**Table II**).

Figure 2a-c shows the results of the environmental impact assessment for the studied indicators. Scenario PF presented the most significant environmental impacts in the three indicators evaluated: 1642.8 kg CO₂-eq/FU, 493.0 kg oil-eq/FU, and

TABLE V. LIFE CYCLE INVENTORY OF THE SCENARIO SEQUENTIAL DARK-PHOTOFERMENTATION PROCESS.

Stage	Substance	Inputs	Outputs	Unit
Grinding	OPW	247.79	247.79	kg/FU
	EE	0.76	-	kWh/FU
AHP Pretreatment	H ₂ O ₂ 30%v/v	36.17	-	L/FU
	H ₂ O	1302.22	-	L/FU
	OPW	56.02	-	kg TS/FU
	NaOH 40%w/v	27.03	-	L/FU
	E _{PAHP}	27.97	-	kWh/FU
	PAHPE	-	1421.44	L/FU
	V _{PAHPE}	1421.44	-	L/FU
DF	V _{inoculum}	588.18	-	L/FU
	V _{reactor}	2009.62	-	L/FU
	E _{DF}	6.27	-	kWh/FU
	EE _{separation}	23.01	-	kWh/FU
	E _{DFi}	42.38	-	kWh/FU
	H ₂	-	3686.11	L/FU
	DFE _{liquid}	-	1915.37	L/FU
	DFE _{solid}	-	94.25	kg/FU
	<i>Rhodobacter capsulatus</i> B10	164.17	164.17	L/FU
	DFE _{liquid}	1915.37	1915.37	L/FU
PF	DFE _{liquid}	7.70	7.70	kg/FU
	C ₅ H ₈ NO ₄ Na	3.28	3.28	kg/FU
	KH ₂ PO ₄	4.93	4.93	kg/FU
	K ₂ HPO ₄	3557.11	3557.11	L/FU
	H ₂ O	64.58	64.58	g/FU
	FeSO ₄ ·7H ₂ O	109.45	109.45	g/FU
	C ₁₀ H ₁₄ N ₂ Na ₂ O ₈ ·2H ₂ O	410.44	410.44	g/FU
	CaCl ₂ ·2H ₂ O (7.5 %)	1094.50	1094.50	g/FU
	MgSO ₄ ·7H ₂ O (20 %)	5.47	5.47	g/FU
	Thiamine-HCl (0.1%)	15.32	15.32	g/FU
	H ₃ BO ₃	8.71	8.71	g/FU
	MnSO ₄ ·H ₂ O	0.22	0.22	g/FU
	CuSO ₄ ·5H ₂ O	1.31	1.31	g/FU
	ZnSO ₄ ·7H ₂ O	4.12	4.12	g/FU
	Na ₂ MoO ₄ ·2H ₂ O	36.35	-	kWh/FU
	E _{PF}	757.98	-	kWh/FU
	E _{lamp}	-	8457.47	L/FU
H ₂	-	-	-	
Total energy		874.73		kWh/FU
Water consumption		4859.33	-	L/FU

*DFE_{liquid}: dark fermentation liquid effluent; DFE_{solid}: dark fermentation solid effluent; EE: electric energy; E_{PAHP}: energy consumption in the AHP pretreatment; E_{DF}: energy consumption in DF; E_{DFi}: energy consumption in the inoculum heat-pretreatment used in DF; E_{PF}: energy consumption in PF; E_{lamp}: energy consumption by the lamp; PAHPE: AHP pretreatment effluent.

TABLE VI. LIFE CYCLE INVENTORY OF THE SCENARIO INDIVIDUAL DARK FERMENTATION PROCESS.

Stage	Substance	Inputs	Outputs	Unit
Grinding	OPW	816.31	816.31	kg/FU
	EE	2.52	-	kWh/FU
AHP Pretreatment	H ₂ O ₂ 30%v/v	119.14	-	L/FU
	H ₂ O	4290.07	-	L/FU
	OPW	184.54	-	kg TS/FU
	NaOH 40%w/v	89.06	-	L/FU
	E _{PAHP}	92.14	-	kWh/FU
	PAHPE	-	4682.81	L/FU
	V _{PAHPE}	4682.81	-	L/FU
DF	V _{inoculum}	1937.71	-	L/FU
	V _{reactor}	6620.52	-	L/FU
	E _{DF}	20.66	-	kWh/FU
	E _{Eseparation}	-	-	kWh/FU
	E _{DFi}	139.62	-	kWh/FU
	H ₂	-	12143.59	L/FU
	DFE _{liquid}	-	6310.02	L/FU
	DFE _{solid}	-	310.50	kg/FU
Total energy		254.95		kWh/FU
Water consumption		4290.07	-	L/FU

DFE_{liquid}: dark fermentation liquid effluent; DFE_{solid}: dark fermentation solid effluent; EE: electric energy; E_{PAHP}: energy consumption in the AHP pretreatment; E_{DF}: energy consumption in DF; E_{DFi}: energy consumption in the inoculum heat-pretreatment used in DF; PAHPE: AHP pretreatment effluent.

22.3 m³/FU for GWI, FSI, and WCI, respectively. These results were due to the high contribution of the PF stage and AHP pretreatment of the OPW. Scenario DF had 43.5 and 45.7% less impacts than Scenario DF-PF in GWI and FSI, respectively. The low impacts in Scenario DF were partly because of the lack of mineral medium in the process. Ochs et al. (2010) found that about 53.5% of the environmental impacts are ascribed to phosphates in the mineral buffer in fermentative processes.

GWI and FSI presented a similar trend, where the highest environmental impacts were derived from the energy use in the PF process. Scenario PF obtained 147.1% more GWI impacts than Scenario DF-PF, mainly owing to the PF stage, since 92% of its impacts were related to the electricity consumed by the lamp. These high environmental impacts arise from the 0.68 kg CO₂-eq emitted per kWh of energy produced in Mexico (Ecoinvent Association 2020) since over 64% of the total primary energy produced in this country comes from fossil sources (SENER 2020). The impact of electricity consumption in the biorefinery could be reduced using lower-impact

electricity, such as solar photobioreactors (Androga et al. 2011). Nevertheless, such environmental improvements to an analog biorefinery must be analyzed in future studies.

The second stage with the highest impact was AHP pretreatment, which contributed more than 40% to the analyzed impact categories caused by H₂O₂. In terms of GWI, this high contribution resulted from the conventional H₂O₂ production process (anthraquinone auto-oxidation process or AO process), which emits 1.6 kg CO₂-eq per kg of 50% H₂O₂ solution (Ecoinvent Association 2020). In the scientific literature, using H₂O₂ has been stated as a green alternative because its decomposition produces water and oxygen (Ho et al. 2019). Nevertheless, such studies do not consider the background environmental impacts of H₂O₂ production.

Scenario DF had 46.6% more impact in WCI than Scenario DF-PF, a contrasting trend compared to GWI and FSI. This difference was mainly associated with the high amount of OPW needed to produce 1 kg of H₂ (Tables V-VI), entailing a higher amount

TABLE VII. LIFE CYCLE INVENTORY OF THE SCENARIO INDIVIDUAL PHOTOFERMENTATION PROCESS.

Stage	Substance	Inputs	Outputs	Unit
Grinding	OPW	885.85	885.85	kg/FU
	EE	2.73	-	kWh/FU
AHP Pretreatment	H ₂ O ₂ 30%v/v	129.29	-	L/FU
	H ₂ O	4655.53	-	L/FU
	OPW	200.26	-	kg TS/FU
	NaOH 40%w/v	96.64	-	L/FU
	E _{PAHP}	99.99	-	kWh/FU
	E _{separation}	7.62	-	kWh/FU
	PAHPE _{solid}	-	384.18	kg/FU
	PAHPE _{liquid}	-	4697.55	L/FU
	<i>Rhodobacter capsulatus</i> B10	402.65	402.65	L/FU
PAHPE _{liquid}	4697.55	4697.55	L/FU	
C ₅ H ₈ NO ₄ Na	18.89	18.89	kg/FU	
KH ₂ PO ₄	8.05	8.05	kg/FU	
K ₂ HPO ₄	12.08	12.08	kg/FU	
H ₂ O	8724.02	8724.02	L/FU	
FeSO ₄ ·7H ₂ O	0.16	0.16	kg/FU	
C ₁₀ H ₁₄ N ₂ Na ₂ O ₈ ·2H ₂ O	0.27	0.27	kg/FU	
CaCl ₂ ·2H ₂ O (7.5 %)	1.01	1.01	kg/FU	
MgSO ₄ ·7H ₂ O (20 %)	2.68	2.68	kg/FU	
Thiamine-HCl (0.1%)	0.01	0.01	kg/FU	
H ₃ BO ₃	37.58	37.58	g/FU	
MnSO ₄ ·H ₂ O	21.37	21.37	g/FU	
CuSO ₄ ·5H ₂ O	0.54	0.54	g/FU	
ZnSO ₄ ·7H ₂ O	3.22	3.22	g/FU	
Na ₂ MoO ₄ ·2H ₂ O	10.09	10.09	g/FU	
E _{PF}	89.15	-	kWh/FU	
E _{lamp}	1858.99	-	kWh/FU	
H ₂	-	12143.59	L/FU	
Total energy		2058.49		kWh/FU
Water consumption		13379.55	-	L/FU

EE: electric energy; E_{PAHP}: energy consumption in the AHP pretreatment; E_{PF}: energy consumption in PF; E_{lamp}: energy consumption by the lamp; PAHPE_{liquid}: AHP pretreatment liquid effluent; PAHPE_{solid}: AHP pretreatment solid effluent.

of water needed in AHP pretreatment, causing a more significant amount of H₂O₂ used to carry out the process, leading the WCI to be harmed. Our results showed that this pretreatment has high environmental impacts due to its origin. It highlights the necessity to evaluate any effort towards developing sustainable processes through a holistic methodology, such as LCA.

To our knowledge, no studies have reported the environmental impacts of H₂O₂ as a pretreating agent for OPW through LCA. Nevertheless, the effects on the environmental profile of other pretreatment methods of lignocellulosic wastes have been studied, and some found that the pretreatment was not so impacting. For instance, Albalade-Ramírez

et al. (2022) analyzed the GHG emissions of cow manure NaOH pretreatment. The authors found that their pretreatment accounted for less than 8.5% of the total GHG emissions; however, they did not use OPW as the primary substrate, nor H₂O₂ as a pretreatment agent, and the operating conditions were substantially different (T > 90 °C). The use of H₂O₂ for the pretreatment should not be taken lightly as it strongly affects the environmental profile of the process. Therefore, other pretreatment agents for OPW delignification should be considered to decrease the environmental impacts of the process.

Tables V-VII show a high energy consumption, which is a hotspot to solve in any production

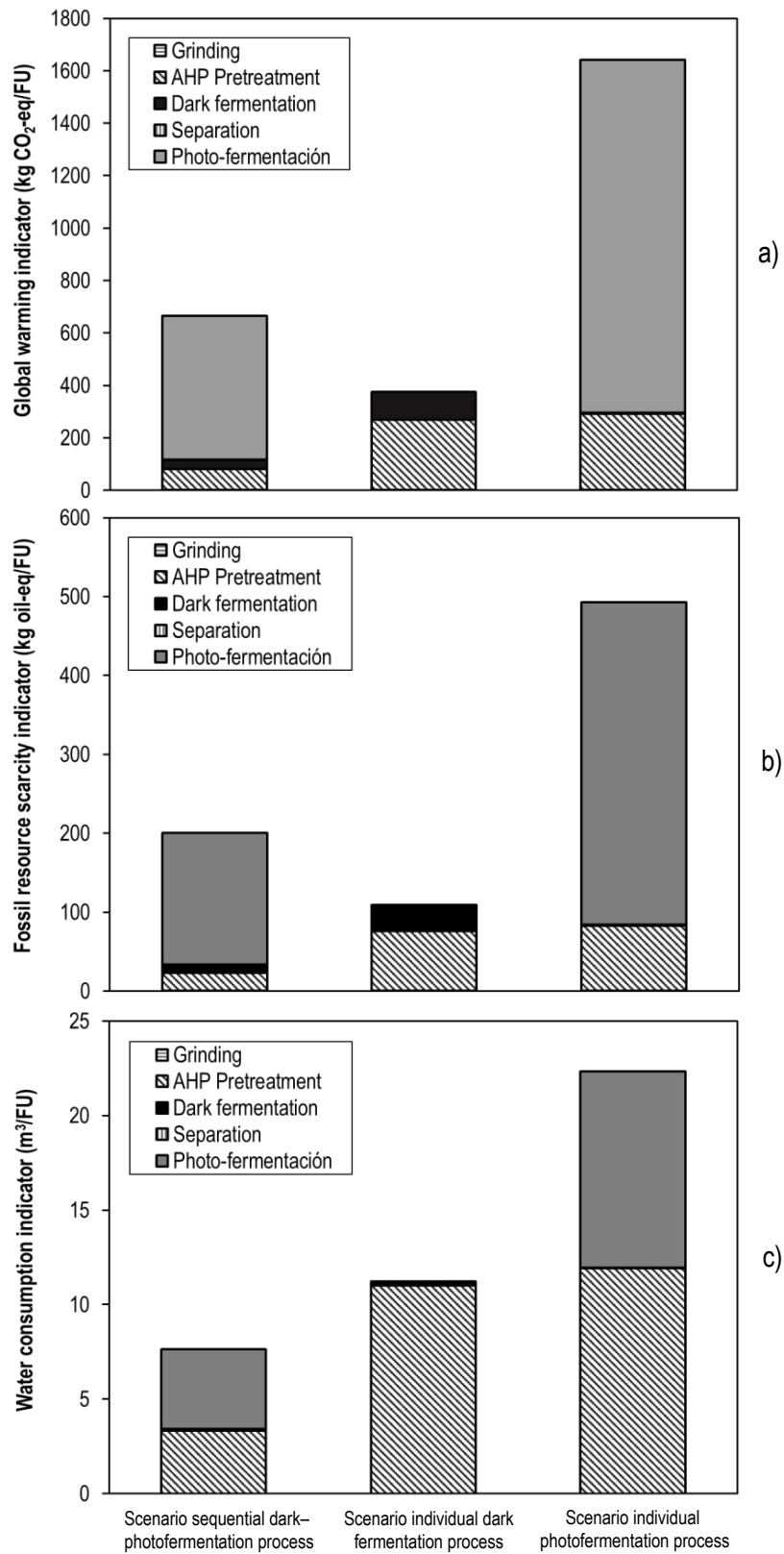


Fig. 2. Environmental impact assessment for the selected categories: a) Global warming indicator, b) Fossil resource scarcity indicator, c) Water consumption indicator.

research (Hinkley et al. 2022). Still, hydrogen is a remarkably useable commodity that is desirable to be produced. The amount of OPW required by the scenarios to produce 1 kg H₂ was 247.8 kg (Scenario DF-PF), 816.3 kg (Scenario DF), and 885.8 kg (Scenario PF) (**Tables V-VII**). Scenario DF-PF needed less substrate to produce the same amount of biohydrogen as the other scenarios. Although Scenario DF had 43.5 and 45.7% less impacts than Scenario DF-PF on the GWI and FSI, respectively, it required 3.3 times more OPW to produce 1 kg H₂. If a transport stage is included in the environmental impact assessment, Scenario DF would probably receive more impacts than Scenario DF-PF due to the high substrate requirement in the process.

This study did not consider a valorization strategy for the liquid effluents. The scope of this work was to compare the environmental impacts of the biohydrogen production strategies and not to propose an entire OPW valorization scheme. Valorization strategies for different effluents could be applied, such as solid biofertilizer production and soil improvers following the anaerobic digestion, pigment extraction from PF bacterial biomass, dietary fiber, and enzymes from DF solid effluents, among others (Joglekar et al. 2019).

CONCLUSION

The environmental impacts from the production of 1 kg H₂ under three scenarios (sequential DF-PF and single processes: DF and PF) exhibited contrasting results amidst the categories evaluated. Scenario DF showed the lowest environmental impacts in global warming and fossil resource scarcity indicators, with 375.5 kg CO₂-eq/FU and 108.8 kg oil-eq/FU, respectively, followed by Scenario DF-PF and Scenario PF. Scenario DF-PF presented the lowest impact regarding the water consumption indicator (7.6 m³/FU), followed by Scenario DF, which had 46.6% more impact. Additionally, Scenario DF-PF required 3.3 times less substrate than Scenario DF due to more efficient substrate conversion.

Notably, PF was the most energy-intensive process due to its high electrical consumption, which is correlated with the GHG emissions associated with the primary electricity production in Mexico. Thus, the need to replace or modify the energy source becomes evident, leaning towards natural solar or derived from photocells.

Our findings demonstrated the need to evaluate any effort towards developing sustainable processes

through holistic methodologies such as LCA to provide a solid basis for considering a transition to a circular economy focused on resource efficiency and minimizing environmental impacts on biohydrogen production.

ACKNOWLEDGMENTS

Brenda Nelly López Hernández thanks to *Consejo Nacional de Humanidades, Ciencias y Tecnologías* (Conahcyt) for the scholarship No. 1080936.

REFERENCES

- Albalate-Ramírez A., Alcalá-Rodríguez M.M., Miramontes-Martínez L.R., Padilla-Rivera A., Estrada-Baltazar A., López-Hernández B.N. and Rivas-García P. (2022). Energy production from cattle manure within a life cycle assessment framework: statistical optimization of co-digestion, pretreatment, and thermal conditions. *Sustainability* 14 (24), 16945. <https://doi.org/10.3390/su142416945>
- Androga D.D., Ozgur E., Gunduz U., Yucel M. and Eroglu I. (2011). Factors affecting the longterm stability of biomass and hydrogen productivity in outdoor photofermentation. *International Journal of Hydrogen Energy* 36 (17), 11369-11378. <https://doi.org/10.1016/j.ijhydene.2010.12.054>
- Argun H., Gokfiliz P. and Karapinar I. (2017). Biohydrogen production potential of different biomass sources. In: *Biohydrogen production: sustainability of current technology and future perspective*. (A. Singh and D. Rathore, Eds.). Springer, India, pp. 11-48. https://doi.org/10.1007/978-81-322-3577-4_2
- Argun H. and Kargi F. (2011). Bio-hydrogen production by different operational modes of dark and photo-fermentation: an overview. *International Journal of Hydrogen Energy* 36 (13), 7443-7459. <https://doi.org/10.1016/j.ijhydene.2011.03.116>
- Bicas J.L., Fontanille P., Pastore G.M. and Larroche C. (2008). Characterization of monoterpene biotransformation in two pseudomonads. *Journal of Applied Microbiology* 105 (6), 1991-2001. <https://doi.org/10.1111/j.1365-2672.2008.03923.x>
- Calabrò P.S., Pontoni L., Porqueddu I., Greco R., Pirozzi F. and Malpei F. (2015). Effect of the concentration of essential oil on orange peel waste

- biomethanization: preliminary batch results. *Waste Management* 48, 440-447. <https://doi.org/10.1016/j.wasman.2015.10.032>
- Cengel Y.A. (2007). *Heat and mass transfer: a practical approach*. 3rd ed. Mc Graw-Hill, New York, NY, USA, 901 pp.
- Das S.R. and Basak N. (2021). Molecular biohydrogen production by dark and photo fermentation from wastes containing starch: recent advancement and future perspective. *Bioprocess and Biosystems Engineering* 44 (1), 1-25. <https://doi.org/10.1007/s00449-020-02422-5>
- Djomo N.S. and Blumberga D. (2011). Comparative life cycle assessment of three biohydrogen pathways. *Bioresource Technology* 102 (3), 2684-2694. <https://doi.org/10.1016/j.biortech.2010.10.139>
- Ecoinvent Association. (2020). Ecoinvent 3.7.1 (3.7.1). <https://ecoinvent.org/>
- EP and EU Council (2008). Directive 2008/98/EC of the European parliament and of the council of 19 November 2008 on waste and repealing certain directives. The European Parliament and the Council of the European Union. Official Journal of the European Union.
- FAO (2023). Crops and livestock products. Food and Agriculture Organization of the United Nations [online]. <https://www.fao.org/faostat/en/#data/QCL/12/12/2023>.
- Ghimire A., Valentino S., Frunzo L., Trably E., Escudié R., Pirozzi F., Lens P.N.L. and Esposito G. (2015). Biohydrogen production from food waste by coupling semi-continuous dark-photofermentation and residue post-treatment to anaerobic digestion: a synergy for energy recovery. *International Journal of Hydrogen Energy* 40 (46), 16045–16055. <https://doi.org/10.1016/j.ijhydene.2015.09.117>
- He D., Bultel Y., Magnin J.P. and Willison J.C. (2006). Kinetic analysis of photosynthetic growth and photohydrogen production of two strains of *Rhodospirillum rubrum*. *Enzyme and Microbial Technology* 38 (1–2), 253-259. <https://doi.org/10.1016/j.enzmictec.2005.06.012>
- Hilali S., Fabiano-Tixier A.S., Ruiz K., Hejjaj A., Ait Nouh F., Idlimam A., Bily A., Mandi L. and Chemat F. (2019). Green extraction of essential oils, polyphenols, and pectins from orange peel employing solar energy: toward a zero-waste biorefinery. *ACS Sustainable Chemistry and Engineering* 7 (13), 11815–11822. <https://doi.org/10.1021/acssuschemeng.9b02281>
- Hinkley J.T., Heenan A.R., Low A.C. and Watson M. (2022). Hydrogen as an export commodity—capital expenditure and energy evaluation of hydrogen carriers. *International Journal of Hydrogen Energy* 47 (85), 35959-35975. <https://doi.org/10.1016/j.ijhydene.2022.08.192>
- Ho M.C., Ong V.Z. and Wu T.Y. (2019). Potential use of alkaline hydrogen peroxide in lignocellulosic biomass pretreatment and valorization – a review. *Renewable and Sustainable Energy Reviews* 112, 75-86. <https://doi.org/10.1016/j.rser.2019.04.082>
- Huijbregts M.A.J., Steinmann Z.J.N., Elshout P.M.F., Stam G., Verones F., Vieira M., Zijp M., Hollander A. and van Zelm R. (2017). ReCiPe2016: a harmonised life cycle impact assessment method at midpoint and endpoint level. *International Journal of Life Cycle Assessment* 22, 138-147. <https://doi.org/10.1007/s11367-016-1246-y>
- ISO (2006a). ISO 14040:2006 Environmental management. Life cycle assessment. Principles and framework. International Organisation for Standardisation. Geneva, Switzerland.
- ISO (2006b). ISO 14044:2006 Environmental management. Life cycle assessment. Requirements and guidelines. International Organisation for Standardisation. Geneva, Switzerland.
- Joglekar S.N., Pathak P.D., Mandavgane S.A. and Kulkarni B.D. (2019). Process of fruit peel waste biorefinery: a case study of citrus waste biorefinery, its environmental impacts and recommendations. *Environmental Science and Pollution Research* 26, 34713-34722. <https://doi.org/10.1007/s11356-019-04196-0>
- López-Hernández B.N. (2023). Producción fotofermentativa de hidrógeno a partir de efluentes de pretratamientos y bioprocesos: pruebas de concepto y evaluación de impacto ambiental. M.Sc. Thesis. Facultad de Ciencias Químicas, Universidad Autónoma de Nuevo León. Monterrey, Nuevo León, México, 73 pp.
- Lu C., Jing Y., Zhang H., Lee D., Tahir N., Zhang Q., Li W., Wang Y., Liang X., Wang J., Jin P. and Zhang X. (2020). Biohydrogen production through active saccharification and photofermentation from alfalfa. *Bioresource Technology* 304, 123007. <https://doi.org/10.1016/j.biortech.2020.123007>
- Martín M.A., Siles J.A., Chica A.F. and Martín A. (2010). Biomethanization of orange peel waste.

- Bioresource Technology 101 (23), 8993–8999. <https://doi.org/10.1016/j.biortech.2010.06.133>
- Michalska K. and Ledakowicz S. (2014). Alkaline hydrogen peroxide pretreatment of energy crops for biogas production. *Chemical Papers* 68 (7), 913–922. <https://doi.org/10.2478/s11696-013-0531-5>
- Mishra P., Krishnan S., Rana S., Singh L., Sakinah M. and Wahid Z.A. (2019). Outlook of fermentative hydrogen production techniques: an overview of dark, photo and integrated dark-photo fermentative approach to biomass. *Energy Strategy Reviews* 24, 27–37. <https://doi.org/10.1016/j.esr.2019.01.001>
- Ochs D., Wukovits W. and Ahrer W. (2010). Life cycle inventory analysis of biological hydrogen production by thermophilic and photo fermentation of potato steam peels (PSP). *Journal of Cleaner Production* 18, S88–S94. <https://doi.org/10.1016/j.jclepro.2010.05.018>
- PRé Sustainability. (2021). SimaPro 9.2. <https://simapro.com/>
- Rao R. and Basak N. (2022). Sequential dark-photo batch fermentation and kinetic modelling for biohydrogen production using cheese whey as a feedstock. *Applied Biochemistry and Biotechnology* 194 (9), 3930–3960. <https://doi.org/10.1007/s12010-022-03958-w>
- Rodríguez-Valderrama S., Escamilla-Alvarado C., Magnin J.P., Rivas-García P., Amézquita-García H.J. and Cano-Gómez J.J. (2022). Photo-fermentative hydrogen production from organic acids mixtures evaluated through predictive models for *Rhodobacter capsulatus* strains. *Revista Internacional de Contaminación Ambiental* 38, 93–110. <https://doi.org/10.20937/RICA.54356>
- Rodríguez-Valderrama S., Escamilla-Alvarado C., Magnin J.P., Rivas-García P., Valdez-Vazquez I. and Ríos-Leal E. (2020a). Batch biohydrogen production from dilute acid hydrolyzates of fruits-and-vegetables wastes and corn stover as co-substrates. *Biomass and Bioenergy* 140, 105666. <https://doi.org/10.1016/j.biombioe.2020.105666>
- Rodríguez-Valderrama S., Escamilla-Alvarado C., Rivas-García P., Magnin J.P., Alcalá-Rodríguez M.M. and García-Reyes R.B. (2020b). Biorefinery concept comprising acid hydrolysis, dark fermentation, and anaerobic digestion for co-processing of fruit and vegetable wastes and corn stover. *Environmental Science and Pollution Research* 27 (23), 28585–28596. <https://doi.org/10.1007/s11356-020-08580-z>
- Ruiz B. and Flotats X. (2016). Effect of limonene on batch anaerobic digestion of citrus peel waste. *Biochemical Engineering Journal* 109, 9–18. <https://doi.org/10.1016/J.BEJ.2015.12.011>
- Santi G., Crognale S., D’Annibale A., Petruccioli M., Ruzzi M., Valentini R. and Moresi M. (2014). Orange peel pretreatment in a novel lab-scale direct steam-injection apparatus for ethanol production. *Biomass and Bioenergy* 61, 146–156. <https://doi.org/10.1016/j.biombioe.2013.12.007>
- Santiago B., Moreira M.T., Feijoo G. and González-García S. (2020). Identification of environmental aspects of citrus waste valorization into d-limonene from a biorefinery approach. *Biomass and Bioenergy* 143, 105844. <https://doi.org/10.1016/j.biombioe.2020.105844>
- Satari B. and Karimi K. (2018). Citrus processing wastes: environmental impacts, recent advances, and future perspectives in total valorization. *Resources, Conservation and Recycling* 129, 153–167. <https://doi.org/10.1016/j.resconrec.2017.10.032>
- SENER (2020). Programa de desarrollo del sistema eléctrico nacional 2020-2034. Secretaría de Energía. Plan. Mexico City, Mexico, 219 pp.
- SIAP (2022). Sistema de información agroalimentaria de consulta (SIACON). Servicio de Información Agroalimentaria y Pesquera [online]. https://www.gob.mx/siap/documentos/siacon-ng-161430_12/12/2023
- Sigma Aldrich (2023). Limonene solution [online]. https://www.sigmaaldrich.com/MX/es/product/supelco/crm40448_12/12/2023
- Tian H., Li J., Yan M., Tong Y.W., Wang C.H. and Wang X. (2019). Organic waste to biohydrogen: a critical review from technological development and environmental impact analysis perspective. *Applied Energy* 256, 113961. <https://doi.org/10.1016/j.apenergy.2019.113961>
- Tsydenova N., Morillas A.V., Hernández Á.M., Soria D.R., Wilches C. and Pehlken A. (2019). Feasibility and barriers for anaerobic digestion in Mexico City. *Sustainability* 11 (15), 4114. <https://doi.org/10.3390/su11154114>
- Zema D.A., Calabrò P.S., Folino A., Tamburino V., Zappia G. and Zimbone S.M. (2018). Valorisation of citrus processing waste: a review. *Waste Management* 80, 252–273. <https://doi.org/10.1016/j.wasman.2018.09.024>