## **Objectives of the project:**

Our ambition is to implement a revolutionary solution for hydrogen production by harnessing the exceptional properties of microalgae. By aligning our research with the latest scientific advancements, we aim to develop a process that offers significant advantages in terms of sustainability and energy efficiency.

This initiative holds crucial importance in the realm of scientific research for several compelling reasons. Firstly, by adopting a biomass-based approach, our project will contribute to mitigating greenhouse gas emissions, thus aiding in the fight against climate change. Secondly, by targeting green hydrogen production, we address the growing demand for a clean and renewable energy source.

Scientific literature highlights the interest in hydrogen production from microalgae, necessitating a comprehensive approach to overcome obstacles and leverage global progress. However, much of this research remains qualitative, lacking concrete quantification. Moreover, there is a notable lack of in-depth comparative studies on various strains of microalgae and the impact of operational conditions (such as temperature, pH, light influence, and nutrient input) on hydrogen production. Additionally, the transition from laboratory research to industrial applications, including precise quantification of material and energy balances and cost estimation, remains inadequately addressed in current literature. This project aims to address these gaps by integrating two doctoral students dedicated entirely to these challenges, hoping to provide clear answers to these inquiries.

## This PhD proposal

The novelty of hydrogen production from lactose by freshwater microalgae lies in its innovative carbon source. Unlike other microalgae hydrogen production processes that use CO2 or simple sugars, this technology utilizes lactose as a carbon source, a mode of culture known as mixotrophic. Furthermore, lactose is a sugar abundant in whey, a by-product of cheese production. However, the use of lactose allows for the valorization of a polluting waste and reduces dependence on fossil carbon sources. Additionally, our process utilizes freshwater microalgae, which are easier to cultivate and less sensitive to pH and temperature variations than marine microalgae. In addition to scientific and technical originality, this process also presents innovation and sustainability from a broader perspective. Hydrogen production by microalgae is a clean and renewable process. It does not produce greenhouse gases and can contribute to carbon footprint reduction. It is a promising technology that could contribute to energy transition and the creation of a greener economy.

Key strengths to consider include waste valorization, a renewable carbon source, easier cultivation of freshwater microalgae, a clean and sustainable process, and multiple applications such as electricity production.

Several strains of microalgae will be studied and compared: Scenedesmus ecornis, Scenedesmus arcuatus, Scenedesmus bijugatus, Scenedesmus obliquus, Monoraphidium circinale, Monoraphidium contortum, Raphidocelis subcapitata, Ankistrodesmus falcatus, Chlamydomonas debaryana, Chloromonas ulla, Chlorella sp, considering that the strains Chlorella sp and Chlamydomonas sp will serve as our reference or control cultures as they have been well studied in the literature under different conditions.

Project Description :

To carry out this project, we propose three missions:

Mission 1: Optimization of hydrogen production from various strains of microalgae

This mission begins with:

Identification of high-performing freshwater microalgae strains: Selecting the most suitable strains for hydrogen production, focusing on their ability to accumulate energy-rich compounds. This selection process absolutely involves a study of the kinetics of growth and development of microalgae strains. This microscopic and kinetic identification may potentially be confirmed through molecular phylogenetic identification.

Optimization of growth conditions: Investigating and adjusting environmental conditions such as light, temperature, and nutrients to maximize microalgae growth and hydrogen production, while utilizing whey (a byproduct of the dairy industry) or polluted water as a culture medium instead of conventional media such as BG11.

Development of conversion processes: Developing efficient conversion methods to ensure a circular economy. We propose a study of pyrolysis of microalgae biomass to evaluate the possibility of producing biofuel and/or electricity on-site necessary for the operation of the facility, or their use as biofertilizer detailed in Mission 2.

Mission 2: Improvement of the quality of essential oils from an aromatic and medicinal plant through foliar fertilization with microalgae extracts

Exploring solutions to valorize liquid residues and microalgae biomass generated during the hydrogen production process by transforming them into useful byproducts such as fertilizers (biofertilizers).

The hypothesis of this mission is that foliar fertilization with microalgae extracts can improve the quality of essential oils by increasing the concentration of bioactive compounds. The methodology of this mission consists of 4 steps:

Selection of the aromatic and medicinal plant

The first step involves selecting the aromatic and medicinal plant on which the mission will be conducted. The selection criteria are as follows:

The plant must be cultivated in Morocco and France.

The plant must produce essential oils with medicinal properties.

The plant must be easy to cultivate and harvest (e.g., sage).

Experimental study under moderate and severe saline stress conditions.

Conducting real comparative tests of physiological parameters (e.g., plant growth, leaf surface area, internode distance) and biochemical parameters (e.g., contents of phenolic compounds, photosynthetic pigment contents, lipid contents) (from late November to early April). Foliar fertilization

The second step involves fertilizing plants with extracts from the microalgae of Mission 1. Microalgae extracts can be applied through foliar spraying.

Extraction of essential oils and characterization of their quality

The third step involves extracting and analyzing the quality of essential oils from the chosen plant. Extraction can be carried out by steam distillation or hydrodistillation.

Valorization of residues from aromatic and medicinal plants (AMP) after extraction of essential oils

A study of pyrolysis of AMP residues to evaluate the possibility of producing biofuel and/or electricity on-site, accompanied by a profitability study to assess the industrial potential of this application of biofertilizers.

To carry out this mission, the Moroccan doctoral student will certainly need several analyses and characterization of microalgae extracts and also leaves after fertilization, analyses of essential oils, and analyses of biofuel from AMP residues.

Mission 3: Scaling up process and economic profitability study

The main objective of this section is to evaluate the economic viability of hydrogen production from microalgae. This evaluation will take into account various factors such as production costs, potential market opportunities, and available financial incentives. Once technical feasibility is confirmed, particularly in terms of chemical reactions, yield, and operating conditions, it becomes imperative to proceed with a techno-economic analysis. This analysis aims to determine the economic feasibility of the project and to accurately estimate the production cost. The following steps are crucial to successfully conduct this analysis: This mission is considered cross-cutting due to its close interaction with the two previous missions. It is anticipated that this mission will proceed as follows:

Step 1: Mathematical model: To develop a mathematical model describing the mechanism of hydrogen (H2) production from microalgae, in-depth experimental study is required. This project involved detailed analyses of the liquid and gas phases over the course of microalgae development, thus enabling the design of a sophisticated mathematical model. This model is designed to integrate a variety of parameters, an approach highlighted in previously published work (see articles [19], [20]). Indeed, a series of tools have been developed specifically to refine the parameters of this mathematical model. The aim is to make the model robust,

flexible, and experimentally validated enough to be integrated into large-scale simulations, such as those performed with Aspen Plus software, thus facilitating the analysis and optimization of hydrogen production processes by microalgae.

Step 2: Development of a process flowsheet: This step will be carried out using specialized software such as Aspen Plus, the license for which has been acquired by INSA Rouen. The objective is to design a comprehensive process diagram that will identify all material and energy flows involved in the installation. This visual representation will facilitate understanding of the process and serve as a basis for establishing an overall material balance. In addition to hydrogen production, microalgae processes can generate H2S, a toxic and malodorous gas. The latter comes from the metabolism of bacteria naturally present in the culture medium. The presence of H2S can pose environmental and safety issues, and can also affect the quality of the produced biofuel or hydrogen. Installing a scrubbing column allows capturing H2S before its release into the atmosphere. This technology is effective and significantly reduces the environmental impact of microalgae processes.

Step 3: In-depth market study: A market analysis will be undertaken, initially focused on France and Morocco, to understand the dynamics of the hydrogen market in these countries. If time permits, an extension of this study to other countries, especially those in the European Union, will enrich the project understanding and provide a broader perspective on potential hydrogen selling prices.

Step 4: Estimation of production costs: To assess the project's profitability, it is crucial to conduct a detailed study of investment costs (CAPEX) and operational costs (OPEX). This analysis will begin by estimating the cost of acquiring the installation and necessary equipment, then evaluate the operational expenses required for the optimal operation of the production unit. The analysis of CAPEX and OPEX will determine if the company's financial resources are adequate to support the project.

This approach aims to ensure a comprehensive evaluation of the economic feasibility of the project for hydrogen production from microalgae, taking into account all technical and economic aspects.