COLLEGE VEGETAL
DOCTORAL ANIMAL, ALIMENT
PAYS DE LA LOIRE MER, ENVIRONNEMENT

PhD PROPOSAL FOR THE DOCTORAL SCHOOL « Végétal, Animal, Aliment, Mer, Environnement »

GENERAL INFORMATION

Thesis title: Biocompatible extraction of proteins from photosynthetic microorganisms
Acronym of the project: PROSPECTIVEµ
Disciplinary field 1: Biotechnology **Disciplinary field 2:** Biochemistry, Molecular and Cellular Biology
Three keywords: microalga, cyanobacteria, durability
Registration establishment: Le Mans University (LMU)
Research unit: Biologie des Organismes, Stress, Santé Environnement (BiOSSE)
Name of the thesis director HDR (Accreditation to supervise research) required: Prof Benoît SCHOEFS **Email address of the thesis director:** Benoit.schoefs@univ-lemans.fr **Name of the thesis co-supervisor 1 (if applicable):** Dr Justine MARCHAND **Email address of the thesis co-supervisor 1 (if applicable):** Justine.marchand@univ-lemans.fr
Contact(s) (mailing address and E-mail): Benoit.schoefs@univ-lemans.fr/justine.marchand@univ-lemans.fr
☒ **Doctoral school contest** ☐ **Interview** ☐ **Other (specify):**

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SCIENTIFIC DESCRIPTION OF THE PhD PROJECT

Socio-economic and scientific context: (10 lines)

The demand for sustainable protein sources to feed the global population is increasing because the population is anticipated to reach 9.7 billion by 2050. Therefore, a search for alternative protein sources to meat proteins is necessary. A promising alternative is microalgae due to their high protein content while having a reduced environmental impact compared to animals and plant proteins. Beside the challenge constituted by consumer acceptance of these alternative proteins, protein production from microalgae still requires the development of methods that will enable to improve the extraction of proteins. Conventional protein extraction methods, such as enzymatic hydrolysis and biochemical processes, are laborious, time- and energy-consuming, and may require the use of solvents. In addition, extraction methods that use physical treatments are all destructive for the biomass that needs to be produced at the start of each extraction cycle. Alternative nondestructive extraction processes, called biocompatible, have been proposed for rendering extraction more friendly from the environmental and economic point of views. In this frame, Justine MARCHAND and Benoît SCHOEFS develop methodologies aiming at extracting proteins using different means, including biocompatible pulsed electric field (bcPEF). bcPEF are reversible PEF *i.e.* they keep cells alive. The thesis will be part of a larger research program financed by the national research program France2030 and involves several academic and industrial partners.

Assumptions and questions (8 lines)

One of the goals of the work will be to understand how microalgae respond to the repeated extraction of a significant proportion of their internal soluble proteins triggered by repetitive bcPEF. The proposed work lies on 5 hypotheses **: H1-**bcPEF-induced stress is moderate and the microalga recovery is fast; **H2**- the cellular protein quota (Q_{Prot}) is reconstituted rapidly and the extraction yield does not vary significantly between 2 extractions; **H3**-cell division is not severely affected by bcPEF; **H4**: the composition in terms of types of proteins is constant and the extracted proteins are not damaged between 2 extractions and **H5**: biociompatible extraction process established at the laboratory scale can be scaled up. The questions to be answered corresponding to these 5 hypotheses: **Q1**: what is the level of permeability and the microalgal stress induced by bcPEF? **Q2**: how fast is the *de novo* protein accumulation between bcPEF treatment? **Q3**: how bcPEF impact cell division rate? **Q4**: what are the identity and the state of the extracted proteins? and **Q5**: what are the parameters that needs to be compromised for scaling?

The main steps of the thesis and scientific procedure (10-12 lines)

The main steps are (1) establishing the culture of the different taxa considered in the research program (*Arthrospira platensis, Chlorella vulgaris*, and *Tetraselmis chui*) and (2) optimizing the conditions allowing the best protein extraction while preserving cell viability. The team research is equiped with several types of microalga culture facilities and PEF delevering machines. The laboratory is also equiped with a flow cytometer, microscope, and offer facilities for studying microalgal physiology. The answers to the 4 questions mentionned above will be obtained by: **A1**: evaluating the level of permeability and the microalgal stress level induced by bcPEF; **A2**: establishing the kinetics of *de novo* protein accumulation between 2 bcPEF; **A3**: evaluating the impact of bcPEF on cell division rate, **A4**: determining the quality and the identity of the extracted proteins and **A5**: finding the compromise for scaling up.

Methodological and technical approaches considered (4-6 lines)

Axenic culture of photosynthetic microorganisms will be tested: *Arthrospira platensis, Chlorella vulgaris*, and *Tetraselmis chui*. The project is organized in 3 WPs: **(1) WP1-Electroporation and cell responses after 1 PEF**: permeability measurements (flow cytometry, fluorescence microscopy), quantification and identification of the released proteins (performed with the help of the **PROTEOTOUL platform**), stress level and impact of the protein unbalance on metabolic changes (transcriptomics, proteomics, spectroscopies, oxidative stress measurements) → bcPEF operating conditions and relaxation time for bcPEF treatment, biomass/protein production modelling and

small scale cultivation adjustments (nutrients, dilution and lighting) considering the extracted proteins (**A1**); **(2) WP2–Repetitive extraction:** morphologic changes, division rate, identification, quantitative and qualitative analyses of the released proteins by mass spectrometry (**PROTEOTOUL)**, viability → repetitive extraction operating conditions, biomass/protein production modelling and small scale cultivation adjustments (nutrients, dilution and lighting) (**A2-A4**); **(3) WP3-Scaling**: morphologic changes, division rate, quantitative and qualitative analyses of the released proteins by mass spectrometry (**PROTEOTOUL)**, viability → repetitive extraction operating conditions, biomass/protein production modelling and small scale cultivation adjustments (nutrients, dilution and lighting) (**A5**).

Scientific and technical skills required by the candidate

The project is multidisciplinary. Knowledge in algal culture, physiology, biochemistry, biophysics, electroextraction, flow cytometry, bioinformatics, bioprocess will be good point. Autonomy, knowledge of English, mobility are mandatory.

THESIS SUPERVISION

Unit name: BiOSSE	**Team name:** MIMMA
Unit director name: MOUGET	**Team director name:** ULMANN/SCHOEFS
Mailing address of the unit director: Jean-luc.mouget@univ-lemans.fr	**Mailing address of the team director:** Lionel.ulmann@univ-lemans.fr/benoit.schoefs@univ-lemans.fr

Thesis director

Surname, first name: SCHOEFS, Benoît

Position: Professor

Obtained date of the HDR (Accreditation to supervise research): 2001

Employer: LMU

Doctoral school affiliation: VAAME

Rate of thesis supervision in the present project (%): 50

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 140

Number of current thesis supervisions/co-supervisions: 3

Thesis co-supervisor 1 (if applicable)

Surname, first name: MARCHAND, Justine

Position: Assistant professor

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Accreditation to supervise research ☐ yes ☒ no If yes, date diploma received:

Employer: LMU

Doctoral school affiliation: VAAME

Rate of thesis supervision in the present project (%): 50

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 130

Number of current thesis supervisions/co-supervisions: 3

Professional status of previous PhD students supervised by both director and co-supervisors (from 5 years)

List of publications from the thesis work (in bold, the name of the supervisors):

1. Tanguy G, Legat A, Goncalvez O, Marchal L & **Schoefs B** (2021) Selection of culture conditions and cell morphology for biocompatible extraction of β-carotene from *Dunaliella salina*. *Marine Drugs* **19**, 648 (**6,085; Q1**)
2. Thomas, C-T, Manisekaran R, Santoyo-Salazar J, **Schoefs B**, Velumani S, Castaneda H & Jantrania A (2021). Graphene oxide decorated TiO_2 and $BiVO_4$ nanocatalysts for enhanced visible-light-driven photocatalytic bacterial inactivation. *Journal of Photochemistry and Photobiology A: Chemistry* **418**, 113374 (**5,141, Q2**)
3. Scarsini M, Thurotte A, Veidl B, Amiard F, Niepceron F, Badawi M, Lagarde F, **Schoefs B** & **Marchand J** (2021) Metabolite quantification by Fourier Transform Infra-Red spectroscopy: applications to diatoms. *Frontiers in Plant Science* **12**, 2146 (**6,627; Q1**)
4. Scarsini M, Thiriet-Rupert S, Veidl B, Mondeguer F, Hu H, **Marchand J** & **Schoefs B** (2022) The transition toward nitrogen deprivation in diatoms requires chloroplast stand-by and deep metabolic reshuffling. *Frontiers in Plant Science* **12**,760516 (**5,6; Q1; 2022**)
5. Murison V, Hérault J, Schoefs B, **Marchand J** & Ulmann L (2023) Bioinformatics-based screening approach for the identification and characterization of lipolytic enzymes from the marine diatom *Phaeodactylum tricornutum. Marine Drugs* 21, 125 (**5,4; Q1; 2022**)
6. Murison V, Hérault J, Côme M, Guinio S, Lebon A, Chamot C, Bénard M, Galas L, Schoefs B, **Marchand J**, Bardor M & Ulmann L (2023). Comparison of two *Phaeodactylum tricornutum* ecotypes under nitrogen starvation and resupply reveals distinct lipid accumulation strategies but a common degradation process. *Frontiers in Plant Science* **14**: 1257500 (**5,6; Q1; 2022**)

2016-2022	G. TANGUY	**Co-direction:** L MARCHAL (U Nantes, UN), **B SCHOEFS** **Co-supervisor**: O CONGALVEZ (UN) **Job position: Engineer ZENI Company (Nantes, France)**
2017-2021	M. SCARSINI	**Direction: B SCHOEFS** **Co-supervisor**: **J MARCHAND** **Job position: Postdoctorate (Ecole Normale Supérieur, Paris)**
2017-2021	CT. THOMAS	**Co-direction:** V SUBRAMANIAN (CINVESTAV, Mexico), **B SCHOEFS** **Job position: Unknown**
2020-2023	V. MURISON	**Direction**: L ULMANN (LMU) **Co-supervisors** : J HERAULT, **J MARCHAND** **Job position: in search**

Five main recent publications of the supervisors on thesis subject

Coustets M, Joubert-Durigneux V, Hérault J, **Schoefs B**, Blanckaert V, Garnier J-P & Teissié J (2015) Optimization of proteins electroextraction from microalgae by a flow process. *Bioelectrochemistry* 103, 74-81 ; DOI: 10.1016/j.bioelechem.2014.08.022 (3,356 ; Q2)

Vinayak V, Manoylov KM, Gateau H, Blanckaert V, Pencreac'h G, Hérault J, **Marchand J**, Gordon R & **Schoefs B** (2015) Diatom milking: a review and new approaches. *Marine Drugs* 13, 2629-2665; DOI : 10.3390/md13052629 (3,345; Q1)
Gateau H, Moreau B, Blanckaert V, **Marchand J** & **Schoefs B** (2018) Procédé d'extraction de molécules hydrosolubles intracellulaires essentiellement non destructif de microalgues unicellulaires. Brevet FR1762818
Tanguy G, Legat A, Goncalvez O, Marchal L & **Schoefs B** (2021) Selection of culture conditions and cell morphology for biocompatible extraction of β-carotene from *Dunaliella salina*. *Marine Drugs* 19, 648 (6,085; Q1)
Gateau H, Blanckaert V, Veidl B, Schiltz O, Pichereaux C, **Marchand J** & **Schoefs B** (2021) Application of pulsed electric fields for the biocompatible extraction of proteins from the microalga *Haematococcus pluvialis*. *Bioelectrochemistry* 137, 107588 (5,760; Q2)
Ahirwar A, Khan MJ, Sirotiya V, Mourya, M, Rai A, **Schoefs B**, **Marchand J**, Varjani S & Vinayak V (2023) Pulse electric field assisted cell permeabilization of microalgae *Haematococcus pluvialis* for milking of value-added compounds: Constraints and perspectives. *Bioenergy Research* 16, 311–324 (3,852; Q3, 2021)

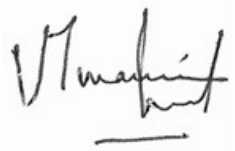
THESIS FUNDING

Origin(s) of the thesis funding: CDE
Gross monthly salary: 2100 euros
Thesis funding state: Acquired
Funding beginning date/duration of the thesis funding: 2024 10 01, 3 years

Date: 2024 02 25

Name, signature of unit director: MOUGET

Name, signature of team director: ULMANN



Name, signature of thesis project director: SCHOEFS