Rentrée 2019

PROPOSITION DE SUJET DE THESE

Formulaire demande de financement : ARED - ISblue - ETABLISSEMENTS - …

pour dépôt sur le serveur https://theses.u-bretagneloire.fr/sml au format PDF

Identification du projet

Acronyme du projet *(8 caractères maximum)* **: BB**

Intitulé du projet *en langue française* : Approche respectueuse de l'environnement pour la **B**iorestauration des sols contaminés et la production de **B**iostimulant : Contribution potentielle des algues marines côtières européennes à l'amélioration de l'environnement.

Intitulé du projet en langue anglaise: An ecofriendly approach for **B**ioremediation of contaminated soil environment and production of **B**iostimulant: Potential contribution of European coastal seaweed to environmental improvement.

Domaine d'innovation stratégique (DIS) du projet

Cocher le DIS prioritaire au sein duquel le projet de thèse s'intègre.

□ DIS 1 : Innovations sociales et citoyennes pour une société ouverte et créative

□ DIS 2 : Chaîne alimentaire durable pour des aliments de qualité

☑ DIS 3 : Activités maritimes pour une croissance bleue

□ DIS 4 : Technologies pour la société numérique

□ DIS 5 : Santé et bien-être pour une meilleure qualité de vie

□ DIS 6 : Technologies de pointe pour les applications industrielles

□ DIS 7 : Observation et ingénieries écologique et énergétique au service de l'environnement

Si aucun DIS ne correspond, cocher « Projet Blanc ».
□ « Projet Blanc »

Préciser le sous-domaine correspondant : 3B

DIS secondaire si nécessaire :

Présentation de l'établissement porteur (bénéficiaire de l'aide régionale)

Établissement porteur du projet : Université Bretagne Sud

Ecole Doctorale : Sciences de la Mer et du Littoral

Identification du-de la responsable du projet (futur-e directeur-trice de thèse)

Nom du laboratoire d'accueil : Laboratoire de Biotechnologie et Chimie Marines

Code du laboratoire (U/UMR/USR/EA/JE/…) : EA

Directrice du Laboratoire : Nathalie Bourgougnon

Nombre HDR dans le laboratoire : 8

Nombre de thèses en cours : 10

Nombre de post-docs en cours : 3

Nom et prénom du directeur de thèse (HDR), porteur du projet : Professeure Nathalie Bourgougnon

- e-mail : nathalie.bourgougnon@univ-ubs.fr

- Téléphone : 02 97 01 71 55

- Publications récentes du directeur-trice de thèse *(nb total et 5 références max au cours des 5 dernières années)* **:**

> 90 publications - 36 depuis 2014

https://scholar.google.fr/citations?user=0_LDGF4AAAAJ&hl=fr

Kevin Hardouin, Gilles Bedoux, Anne-Sophie Burlot, Pi Nyvall-Collen, **Nathalie Bourgougnon** (2014). Enzymatic recovery of metabolites from seaweeds: potential applications. *Advance Botanical research,* Volume 71, Pages 279-320

Kulshreshtha G., Burlot A.S., Marty C., Critchley A., Hafting J., Bedoux G., **Bourgougnon N.**, Prithiviraj B. (2015). Enzyme-assisted extraction of bioactive material from *Chondrus crispus* and *Codium fragile* and its effect on *herpes simplex* virus (HSV-1). Marine Drugs 13, 558-580

Kevin Hardouin Gilles Bedoux, Anne-Sophie Burlot, Claire Donnay-Moreno, Jean-Pascal Bergé, Pi Nyvall-Collén, **Nathalie Bourgougnon** (2016). Enzyme-assisted extraction (EAE) for the production of antiviral and antioxidant extracts from the green seaweed *Ulva armoricana* (Ulvales, Ulvophyceae). Algal Research 16 (2016) 233–239

Burlot Anne-Sophie, Bedoux Gilles and **Bourgougnon Nathalie** (2016). Response Surface Methodology for Enzyme-Assisted Extraction of Water-Soluble Antiviral Compounds from the Proliferative Macroalga *Solieria chordalis*. Enzyme Engineering. 5:2

Ana Peñuela, Daniel Robledo, **Nathalie Bourgougnon**, Gilles Bedoux, Emanuel Hernandez, Yolanda Freile-Pelegrín (2018). Environmentally Friendly Valorization of *Solieria filiformis* Gigartinales, Rhodophyta) from IMTA using a Biorefinery Concept. Marine drugs *in press*

- Expériences d'encadrement et co-encadrement de doctorants (passées et en cours)

En cours

Shareen Nazreen Banu A Abdul Malik (2018-2021). Active substances from *Hamymenia floresii* (Clemente) C. Agardh used as antifouling products in aquaculture. Contrat doctoral Université Bretagne Sud. Co-encadrement 50% Pr. Daniel Robledo CINVESTAV Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (Mexique).

Rexie P. Magdugo (2018-2021). Marine seaweeds with ecological and economic importance in the Philippines: Valorization of 4 species: *Caulerpa sp*., *Ulva sp*. *Sargassum spp*., and *Halymenia spp*. Financement CHED-PhilFrance Scholarships. Co-encadrement 50% Dr. Gilles Bedoux.

Thèses soutenues et devenir

Romain Boulho (2014-2017). Application de procédés écoresponsables pour l'extraction de molécules de la macroalgue *Solieria chordalis*, caractérisations chimiques et étude d'activités biologiques. Financement Région Bretagne-CG56. Co-encadrement 50% Dr. Gilles Bedoux. En poste chez Gilson Inc., Saint-Avé, France depuis 2018.

Maya Puspita (2014-2017). Enzyme Assisted Extraction of phlorotannins from *Sargassum* species (Phaeophyceae, Fucales) and biological activities. Cotutelle avec Université de Diponegoro (Indonesie). Financement indonésien. Co-encadrement à 50% Dr. Ita Widowati. Executive Director of Indonesian Seaweed Association (ARLI) en Indonésie depuis 2017.

Anne-Sophie Burlot (2013-2016). Étude de la macroalgue rouge *Solieria chordalis* : aspects écophysiologiques, production d'extraits et perspectives d'applications. Financement sur le FUI VB2. Co-encadrement 50% Dr. Gilles Bedoux. Créatrice de l'entreprise *Térang*'Algue. CDI chez OLMIX, Bréhan, France depuis octobre 2018.

Kévin Hardouin (2012-2015). Production d'extraits aqueux à partir *d'Ulva sp*., au moyen de procédés d'hydrolyse enzymatique : caractérisation, valorisation et perspectives de développement. CIFRE Société OLMIX. Encadrement 100%. 2,5 de post-doctorat à la station biologique de Roscoff. Actuellement en post-doctorat au LBCM.

Ludovic Tripoteau (2010-2015). Valorisation des coproduits de l'holothurie *Cucumaria frondosa* par l'étude d'extraits bioactifs et approche écotoxicologique des métabolites secondaires relargués en situation de stress. Financement FIA Canadian. Encadrement 50% avec le Dr. Jacques Gagnon, Institut de recherche sur les zones côtières, Shippagan, Nouveau Brunswick. Ingénieur technico-commercial STARLAB depuis 2016.

Amélie Ségarra. Doctorat (2011-novembre 2014). Expression des gènes viraux au cours du cycle de l'herpès virus de l'huître, OsHV-1 chez *Crassostrea gigas*. Relation entre la séquence du variant *µvar* et son phénotype de virulence. Financement IFREMER/Région Poitou-Charente. Encadrement 20% avec Dr. Tristan Renault, IFREMER, La Tremblade. Post-doctorat NY puis Labex Mer. Actuellement en poste chez Adiagène filiale de Bio-X Diagnostics, Saint-Brieuc**.**

Anaelle Tanniou. Doctorat (2010- 2014). Thèse Université de Bretagne Occidentale. Etude de Phlorotannins d'algues brunes modèles par des approches combinées de profilage métabolique et d'écophysiologie. Contrat Doctoral Université Bretagne-Occidentale. Encadrement 30% avec le Dr. Valérie Stiger, LEMAR Laboratoire des Sciences et de l'Environnement Marin, IUEM Institut Universitaire Européen de la Mer, UBO. Enseignante en SVT au Lycée Galilée chez Éducation Nationale à Guérande.

Post-doctorats

Hugo Pliego Cortes in LBCM (Prestige European program – SAD Brittany area financial support March 2018-November 2019): Glycoalg: Extraction, Purification and characterization of novel glycoproteins from different marine seaweeds.

Isuru Wijesekara Liyanage 01/09/2015 – 31/08/2016. Extraction, Purification and characterization of novel glycoproteins from *Ulva sp*. Financement projet LITTORALG et Campus France PRESTIGE.

Co-directeur-trice de thèse et co-encadrant scientifique : Izabela Michalak (Assistant Professor at WUST from 12 December 2018)

- Laboratoire de recherche co-encadrant (nom + code U/UMR/USR/EA/JE/...) Wroclaw University of Science and Technology – WUST, Faculty of Chemistry, Department of Advanced Material Technologies, Wroclaw, Poland,

- e-mail : izabela.michalak@pwr.edu.pl

- Téléphone : +48 713202434

Izabela Michalak (https://scholar.google.fr/citations?user=ONsds1AAAAAJ&hl=fr) from Wroclaw University of Science and Technology (Poland) has good skills in the testing of utilitarian properties of algal extracts in germination test, pot experiments, as well as in field trials. She has also experience in the utilization of the algal biomass as a biosorbent of metal ions. By combining our respective skills, we would like to carry out a laboratory scale preparation of algae extracts from Poland and French biomass (France) and an analysis of biostimulant properties of algal extracts and biosorption properties of the examined biomass (Poland). Notably, she is known for two famous publications:

Michalak, I., & Chojnacka, K. (2015). Algae as production systems of bioactive compounds. *Engineering in Life Sciences*, 15(2), 160-176.

Michalak, I., Tuhy, Ł., & Chojnacka, K. (2015). Seaweed extract by microwave assisted extraction as plant growth biostimulant. *Open Chemistry*, 13(1).

Expériences d'encadrement et co-encadrement de doctorants (passées et en cours)

Auxiliary supervisor of PhD thesis of MSc Katarzyna Godlewska entitled: The effect of extracts from higher plants on the growth, yielding and chemical composition of selected vegetable species, Wrocław University of Environmental and Life Sciences, from October 2018

Supervisor of master's theses – WUST

1. **Monika Dajlidko**, Production of seaweed extracts by composting and the evaluation of utilitarian values, **2013**
2. **Iga Kostuj**, Production of algal extracts by chemical hydrolysis using potassium hydroxide and evaluation of their utilitarian properties, **2013**
3. **Ewelina Sługocka**, Production of algal extracts by chemical hydrolysis using phosphoric acid (V) and and evaluation of their utilitarian properties, **2013**
4. **Katarzyna Godlewska**, Seaweed extracts as biostimulants for plants, **2014**
5. **Renata Kowalczyk**, The production of seaweed extracts with the application of microwaves, **2014**
6. **Urszula Miller**, The application and potential odour impact assessment of the compost produced from Baltic seaweeds in plant nutrition, **2014**
7. **Paula Pilarska-Skoruch**, The production of seaweed extracts with the application of organic solvents, **2014**
8. **Magdalena Pierzchała**, Algal extracts applied into soil, foliarly and into seeds: the effect on plant growth, **2015**
9. **Malwina Miller**, The production of algal extracts and analysis of their utilitarian properties, **2015**
10. **Elżbieta Demusz**, Algal extracts obtained by enzymatic method, **2016 (in English)**
11. **Katarzyna Kopka**, The analysis of the properties of biologically active compounds isolated from algae, **2016**
12. **Paulina Plesner**, Plant biomass enriched with microelements via biosorption process as a feed component, **2016**
13. **Anna Dziurbas**, Algae as a biosorption material - potential applications, **2017**
14. **Anna Kafka**, Biosorption of microelement ions by chosen biosorbents, **2017**
15. **Małgorzata Piecha**, The technology of the production of feed additives on the basis of algal biomass and analysis of their chemical composition, **2018**

Supervisor of engineering projects – WUST

1. **Oskar Pławszewski**, The application of seaweed extracts in animal feeding, **2013**
2. **Agnieszka Raczkowska**, The application of seaweed extracts in fertilizers, **2013**
3. **Anna Powideł**, Production of seaweed extracts with the application of enzymes, **2014**
4. **Joanna Nowak**, Production of components of the fertilizers by biosorption, **2016**
5. **Paulina Wojnowiak**, The application of XRF and ICP-OES techniques in the identification of multielemental composition of the biomass, **2016**
6. **Klaudia Pajek**, Biosorption with the use of algal biomass as a method of removal metal ions from wastewaters, **2017**
7. **Aleksandra Trzynoga**, The application of the biosorption process in the production of the natural diet supplements, **2017**
8. **Olaya Rodriguez**, Removal of toxic metals from wastewater using waste biomass as adsorbents, **2017 (in English)**
9. **Paulina Gierus**, Technology of the production of algal extracts by solvent extraction, **2017**
10. **Aleksandra Kacperczyk**, The application of biochar produced from freshwater macroalgae in the plant cultivation, **2018**
11. **Żaneta Małecka**, The application of biochar produced from freshwater macroalgae in removal of inorganic pollutants, **2018**

Présentation du projet

Résumé du projet *(4000 caractères maxi espaces compris) :*

Over the last 20 years, agricultural strategies all around the world have been adapted to the changing environmental conditions by modifying and diversifying its production system by using chemical fertilizers, pesticides, and water management. However, recent data of soil qualities and forecasted agro-hydrological change threaten the availability of these farming and social systems and subsequently food security in lot of countries. There are significant constraints that limit the ability of the farmer to adapt the suitable cultivars to certain specific conditions. These constraints include soil nutrient management, lack of knowledge of potential threats from acid or saline soil, emission of greenhouse gases from different types of culture field in aerobic or anaerobic condition etc.. Improvements made in agriculture usually lead to a larger production capacity which can therefore increase the environmental impact. For example, pollution due to toxic metals and its implications for public health and the environment have led to the increased interest in the developing of environmental biotechnology approaches. In a food challenge context of increased competition on raw materials, environmental challenges and public health shared globally, the **strategic character of agriculture**, also carries geopolitical dimensions. The agriculture resonates worldwide with growing food demand and increased competition in emerging countries. The research, development and innovation are the main drivers of competitiveness of companies in this sector in Europe.

Currently, there is a **tendency to search for a new generation of agro-products manufactured on the basis of raw materials of biological origin**. Among the marine flora encountered in Europe, some macroalgae can become invasive or proliferative and have profound adverse ecological impacts. Due to unique composition of algae, this underexploited biomass can be used to manufacture agricultural products that are safe for consumers and natural environments (no ecotoxicological, toxicological hazard, residues on agricultural products). **Algal products (e.g., extracts)** that improve the uptake of minerals from the soil and increase the resistance of plants to biotic and abiotic stress can contribute to the **reduction of chemicals used in agriculture**. Combined with more precise dosing of mineral fertilizers and synthetic plant protection products, they will have a very positive impact on the natural environment.

Thus, **the development of innovative products and processes, but also the valorization of by-products,** may allow them to differentiate by improving their image, the quality and their margins. However, the management of environmental and health issues is a key element for competitiveness and sustainability. To this respect it is important to introduce the **biorefinery** concept that integrates biomass conversion processes in value-added chemicals from biomass. Nowadays, the concept of biorefinery involves the development of eco-friendly processes for sequential recovery of molecules, especially bioactive molecules and **while preserving most of the intrinsic qualities of raw material**. We propose to choice a Green Chemistry approach combining selectivity, cost-effectiveness, and an eco-friendly process. To maximize the added value of the biomass by allowing the co-extraction of valuable components, a strategy of biorefinery will be developed at lab and industrial levels.

The aim of the project is to develop an innovative biorefinery concept with invasive European seaweeds to produce a sustainable liquid biostimulant of plant growth that will improve crop yield and quality. Unexploited algal biomass collected from the seashore, as well as post-extraction residues from the isolation of biologically active compounds from seaweeds can be also used in the bioremediation of polluted water (with the use of biosorption process) and soil.

Présentation détaillée du projet :

1 - Hypothèse et questions posées, identification des points de blocages scientifiques

Hypothesis 1. Seaweed extracts will act as biostimulants of plant growth: Plant growth and development relies on the availability of a favorable growing environment, which includes healthy soils, availability of nutrients, aswell as protection against pests and other stresses. These conditions can be met naturally or provided artificially. The main problem is an inappropriate soil quality which therefore leads to limited food production. Amongst various methods used to improve crop growth and development, the use of plant biostimulants has become more and more significant in the recent years. Biostimulants are materials, other than fertilizers or plant nutrients, which are able to stimulate plant growth and development when applied in small quantities. They are also referred to as 'metabolic enhancers'. Amongst a wide variety of benefits, biostimulants may enhance fertilizer use efficiency, enhance tolerance to nutrients, water and salinity stress (Sci. Hort. 2015, 196, 3–14). The main aim of this project will be the production of algal extracts from different species of seaweeds, using mainly novel extraction techniques and analysis of the utilitarian properties of these extracts in germination tests and pot experiments. The best candidate/candidates as a

biostimulant of plant growth will be selected.

Hypothesis 2. Seaweeds have very good biosorption properties and can be used in wastewater treatment and soil bioremediation: Pollution with toxic metals and its implications for public health and the environment has led to the increased interest in developing environmental biotechnology approaches. For this reason, a biosorption process can be used. This process relies on a passive cation binding by dead biomass (it's not metabolically controlled) and is based on the physicochemical interactions between metal ions in an aqueous solution and functional groups of the biosorbent's cell wall. This process is cheap and very efficient and can offer an alternative to the conventional methods used for the environment decontamination. Moreover, marine seaweeds are known to have very good biosorption properties and they can efficiently remove toxic metals from polluted soil and water. The main aim of this step will be the selection of different species of green, brown and red seaweeds and examination of their biosorption properties. The best candidate/candidates as a sorbent of toxic metal ions will be selected.

Principal scientific bottlenecks: The main obstacle for industrial markets is the access to large quantities of active molecules from available biomass. However, marine algae from the French Brittany and Poland coasts constitute a significant and diversified natural vegetable production. As a result, nearly 700 species of algae are currently listed on the Breton littoral zone and 40 species on the Polish littoral zones. Among the flora encountered in Brittany and in Mexico, native or introduced macroalgae can become **invasive or proliferative** and have profound adverse ecological impacts including the alteration of the ecosystem structure, the reduction of indigenous biodiversity, and economic losses. **In France**, strandings of *Solieria chordalis* (Gigartinales, *Solieriaceae*), *Ulva* sp. (Chlorophyta, *Ulvaceae)* and *Sargassum muticum* (Phaeophyta, Fucales) appear along the shallow sandy bays. Monthly monitoring of the (bio)chemical composition of *Ulva*, *Solieria chordalis* and *Sargassum muticum* has been conducted over the last 4 years. Several biochemical parameters were regularly measured bringing enhanced knowledge of the composition of this biomass and the breakdown into its primary metabolites. **In Poland**, green macroalgae dominate in the floral composition, especially *Enteromorpha ahlneriana*, *E. intestinalis*, *Cladophora albida*, *Cl. glomerata*, *Cl. rupestris*, *Cl. sericea*, *Cl. vagabunda*. Among brown algae – *Pilayella litoralis*, *Fucus vesiculosus* are distinguished. *Polysiphonia violacea*, *Ceramium diaphanum* are the main red algae and *Zostera marina* is a main vascular plant.

Unfortunately, there is a remarkable lack of detailed data about the processes of algal extraction technologies for agricultural purposes, mostly because the manufacturing methods are rarely published and held as proprietary information. In fact, several extraction procedures have been adopted for agricultural biostimulant production from marine macroalgae. In most cases, extracts are made by processes using water, alkalis or acids, or physically by disrupting the seaweed by low temperature milling to give a micronized suspension of fine particles. For seaweed utilization as fertilizer and biostimulant components, water extraction seems to be the most cost-effective and practicable tool for better release of micro- and macro-elements from the biomass. Thus, several reports described the use of water or alkaline extracts from algae as plant growth biostimulants (tomato, *Arabidopsis*, spinach, *Vigna sinensis*, etc.) under normal and stressed environments. In almost all cases, beneficial effects on growth of cereals, pulses, and flowering plants have been reported (Plant Soil, 2014, 383, 3–41; J. Appl. Phycol. 2014, 26, 465–490; J. Plant Growth Regul. 2009, 28, 386–399). Algal fertilizers are usually prepared with a chemical method where potash lyse and high temperature are applied. However, such conditions are quite severe and cause decomposition of most of the biologically active compounds. Thus, the primary challenge when extracting algal bioactive compounds will be to find a compromise between the cost of production of sufficient quantities and quality of compounds in the shortest timeframe, finding the optimum processing condition and meeting the principles of green chemistry and green technology (J. of Agric. Food Chem., 2013, 61: 4667–4675). In this context, the effort will be put on cell wall structure disruption in order to enhance the liquefaction of the seaweeds, the release of their internal components and their partial conversion. The aim of this project is to investigate technical possibilities of using the marine macroalgae accumulating on the coasts and beaches to produce bioactive fractions for agricultural applications. Using combinations of innovative extraction processes, i.e. Enzyme Assisted Extraction (EAE), Ultrasounds Assisted Extraction (UAE), Pulsed Electric Fields (PEF), Microwave Assisted Extraction (MAE) alone or in combination for maximizing the liquefaction of the algal biomass. These processes could be combining with mechanic processes (extrusion, ball milling). The production of bioactive products or extracts at an industrial level is targeted within the concept of biorefinery. This bio-based technology also assumes the utilization of wasted seaweed biomass and post-extraction residues as sorbents of metal ions.

2 - Approche méthodologique et techniques envisagées

1. *Production of seaweed extracts using* **combinations of innovative extraction processes** *i.e.* Enzyme Assisted Extraction (EAE) *with commercial enzymes* and/or water extraction will be evaluated in small reactors (from 1 to 6l). The resulting two phases (soluble & insoluble) will be quantified and qualified with a particular focus on biological activities into the soluble one. The soluble phase will be tested as a biostimulant. The insoluble phase can be resubmitted to additional treatment in order to check its biosorption properties. We can compare them with

the raw biomass before extraction. For each case, the influence of key parameters on the extraction yield will be investigated such as the nature of enzyme(s) and different enzyme combinations. The PEF will be studied in collaboration with IRDL (UBS), MAE (time, temperature parameters) and UAE in WUST combining with EAE and mechanic processes (extrusion, ball milling).

2. *Analysis of extracts:* Resulting extracts will be biochemically characterized: dry matter content, mineral content, nitrogen content (Kjeldhal), peptide molecular size (HPLC-MS/MS), amino acids profile (HPLC-UVDAD), total sugars, uronic acids, sulfate groups, monosaccharides (HPAEC) and oligosaccharides molecular size profile (HPSEC–RI).

3. *Biostimulant properties of seaweed extracts:* The produced seaweed extracts will be tested in germination tests according to the International Seed Testing Association (ISTA). Germination tests will be evaluated using Jacobsen germinator on Petri dishes with model plants such as for example garden cress and/or radish. Different doses of seaweed extracts will be tested. Seaweed extracts are usually most efficient (stimulation of plant growth) in low concentrations. Algal extracts can be applied in three different methods:

- to the seeds (soaking of seeds in extract),
- foliar application (when seeds germinated),
- soil application (extract added to the Petri dish before seeds sowing).

In this study, **germination energy** (speed at which the seeds germinate, sometimes expressed as a percentage of the seeds germinated within the first week of analysis with respect to overall germination) and **germination percentage** (after the end of the experiment) will be evaluated. The effect of extracts on plant height, fresh and dry mass, chlorophyll content (SPAD equipment and spectrophotometric method) and root length will be examined. Experiments will be performed in four replications in Petri dishes, under standardized conditions – isolated box with adjustable lighting and temperature (temperature fluctuations ± 4°C) – Jacobsen apparatus. Each dish will be watered with the appropriate volume of algal extract, and the control group will be watered with the same volume of distilled water. The experiment will last for 12 to 14 days. The best concentration of algal extract can be later tested in pot experiments. The same parameters of plants, as in germination tests will be examined. It will last also about 12-14 days. The obtained results will be elaborated statistically, using STATISTICA software. Descriptive statistics (average, standard deviations) for all experimental groups will be performed. The normality of the distribution of experimental results will be assessed by the Shapiro–Wilk test and the homogeneity of variances by the Brown & Forsythe's test. On this basis, the statistical test used to investigate the significance of differences between the tested groups will be selected. The differences between the two groups will be investigated with a *t*-test and between several groups with the one-way analysis of variance (ANOVA) using the Tukey multiple comparison test (for normal distribution and the homogeneity of variances). In the case of the lack of the normal distribution, the Mann-Whitney test will be used (for two groups) and the Kruskal–Wallis test (for more than two groups). The results will be considered significantly different when $p<0.05$.

4. *Utilization of a raw biomass of seaweeds as a biosorbent of toxic metals*: The biomass of red/brown and green seaweeds, as well as post-extraction residue from for example anenzyme/water extraction will be used as a sorbent for soil and/or water bioremediation. Biosorption properties of collected seaweeds will be tested on Cr(III) ions – chosen as a model ion (determination of the concentration in aqueous solutions by spectrophotometric method). The experiments will involve the choice of the best experimental conditions for the biosorption process – e.g., biosorbent size (sieve analysis, e.g., 500, 200 μm), pH (3, 4, 5), initial metal ion concentration (e.g., 100, 200 and 300 mg/l), biomass content in the solution (e.g., 1g/l; 2 g/l, 5 g/l). For the biosorption process, kinetic and equilibrium experiments will be performed for the biomasses and post-extraction residue from extraction of seaweeds. These studies will be carried out in Erlenmeyer flasks, which contain solutions with chromium ions prepared by dissolving in deionized water respective weighed amounts of inorganic salts ($Cr(NO_3)_3 \cdot 3H_2O$). The entire system will be thermostated and stirred with shaking at a constant stirring rate. During kinetic studies, the samples of the biomass in solution with Cr(III) ions will be collected at appropriate time intervals in order to determine the concentration of a metal ion during the biosorption process and to establish the time necessary to reach the state of equilibrium. It is necessary to conduct a study of equilibrium of biosorption process. The obtained results will be described by appropriate models. Mechanism of this process will be studied. The biomass of seaweeds before and after biosorption process will be examined using FTIR technique, in order to establish which functional groups on the surface of the algal cell wall participated in the biosorption process.

3 - Positionnement et environnement scientifique dans le contexte régional, national et international :

Regional: In Brittany, three structures (Station Biologique de Roscoff – Paris Sorbonne; UBO LEMAR and UBS LBCM) are focusing their research on Seaweed. However, the LBCM is the only one to associate innovative ecofriendly extraction processes (EAE) with invasive seaweeds and biological compounds (cosmetic domain and health) and has good recognition in this field. In collaboration with different industrial and academic international partners, the LBCM has developed different extraction processes (CO_2 super critic, PEF and Microwave Assisted Extraction - MAE).

National: Research has been done by Mer Molecules Santé (Nantes University) by combining EAE and Pigment in 2016.

International: Several teams have developed biorefining of marine macroalgal biomass for the production of biofuel in India, Korea, Japan and in China. No association with agricultural objectives. The department of Environmental Sciences and the Faculty of Agriculture at Dalhousie University have a good recognition associating seaweed and agriculture fields but they don't approach ecofriendly extractions. The department of Environmental Protection Technologies at Harran University (Turkey) has a good recognition in phytoremediation in water with whole seaweed.

European perspectives: Currently Wroclaw University of Science and Technology has carried out various kinds of research and collaboration with local universities by forming scientific consortiums in special fields. However, to improve the cooperation, breakthroughs are needed for capacity building. One of the examples is the research collaboration with foreign partners. The output of this activity will provide capacity building to the academic community**. At the laboratory level** between our two institutions: to develop a culture of teaching and researching with partners from abroad for improving capacity building, to transfer knowledge and competences and to contribute to production of publications at international levels and co-supervise PhD students (in co-tutelle). **At the scientific level**: To find and propose a new way of valorization of seaweed biomass (cultured and wild biomass), to propose new natural fertilizers, new biostimulants that improve soil quality, production of food and the crop yield, to find new markets for these ingredients. On the long term, our goal is to find new products and ingredients which will contribute to the reduction of the greenhouse effect and to the increase of soil quality. If our extracts are effective, we may consider large-scale on-site production of the biostimulants of plant growth and evaluate their effectiveness in fields of several hectares. This work can improve and strengthen the seaweed industrial sector in France and Poland and strengthen and maintain collaborative relationships between Université de Bretagne-Sud and Wroclaw University of Science and Technology.

4 - Pour la région Bretagne: adéquation du projet au regard du DIS de rattachement (et/ou du DIS secondaire).

At the economic level, the project aims to **valorize an important biomass** especially that of *Ulva*, *Sargassum*, *Solieria* which remains largely unexploited. The project may create recovery solutions for proliferative biomass, but also the distrust and the growing concern with respect to synthetic chemicals. Valorization of marine biomass for finding molecules or extracts for markets wellness, agricultural industry is part of the regional concerns of our countries. The development of new applications with high added value, especially in the **agricultural industry or health** appears as a real opportunity for socio- economic development of the algae sector.

5 - Si « projet blanc » (hors DIS), préciser les raisons de ce choix :

6 - Si lien avec projet ERC, préciser lequel :

7 - Autres informations utiles (CPER, FEDER, concernant la politique régionale) :

8 - Le cas échéant, précisez le lien du sujet avec les thèmes ISblue

The project contributes to European recognition of **theme 4** about development of marine biotechnology by (1) production of a sustainable liquid biostimulant of plant growth being a rich source of biologically active compound extracted from seaweeds (2) with environmentally friendly extraction process and (3) to improve soil quality and the

crop yield and (4) to use seaweed biomass and post-extraction residues in the bioremediation to remove toxic metals from the environment.

Le cas échéant (si financement ISblue demandé): en regard de la formation par la recherche du futur docteur, perspectives d'insertion professionnelle dans le milieu académique et non académique : Companies specialist in the manufacture/development of natural ingredients in Agriculture field (PRP, Agrival, OLMIX, Algaïa in France ; Seasol, Kelpack, Agrokelp… at International level) ; INRA, Universities

9 - Contexte scientifique et partenarial : éléments généraux

Nathalie Bourgougnon has a recognized know-how in marine biomolecules extracted from seaweeds and biofilm. It possesses a good knowledge on French invasive algae (particularly on physiochemistry of *Solieria* species), extraction and purification of marine substances, screening of biological activity and valuation opportunities (cosmetics and healthcare fields). LBCM is involved in Tremplin Carnot Agrifood transition.

Izabela Michalak (https://scholar.google.fr/citations?user=ONsds1AAAAAJ&hl=fr) from Wroclaw University of Science and Technology (Poland) has good skills in the testing of utilitarian properties of algal extracts in germination test, pot experiments, as well as in field trials. She has also experience in the utilization of the algal biomass as a biosorbent of metal ions. By combining our respective skills, we would like to carry out a laboratory scale preparation of algae extracts from Poland and French biomass (France) and an analysis of biostimulant properties of algal extracts and biosorption properties of the examined biomass (Poland).

In collaboration with **Universitas Negeri Makassar (Indonesia)** and Cereal Crops Research Institute, Allepolea, we have the possibility to study the influence of our extracts on the mineral composition of the soil by measuring greenhouse gas production and to determine the impact of extracts on maize crop growth and soil quality. (PHC Nusantara 2019-2020). They are studying crop enhancement by soil emulsification and the increase of fertility by means of protein or nitrogen (NH_4^+ or NO_3^-). They would also like to analyze the emission of N_2O gases that are released from the nitrification-denitrification process (with Corn (*Zea mays*) as a model). They are using a maize field at pilot-scale (size 10x15 m^2).

IRDL (UBS) Pr. Jean-Louis Lanoisellé : Pulsed electric field assisted extraction of intracellular compounds from macroalgae

10 - Si projet de co-tutelle, internationale, précisez le pays et l'établissement

Wroclaw University of Science and Technology, Faculty of Chemistry, Department of Advanced Material Technologies, Wroclaw, Poland,

11 - Financements Région Bretagne acquis par le porteur au cours des 3 dernières années (titre, montant)

Hugo Pliego Cortes in LBCM (Prestige European program – **SAD** Brittany area financial support March 2018-November 2019): Glycoalg: Extraction, Purification and characterization of novel glycoproteins from different marine seaweeds.

12 - Si projet cofinancé, nom du cofinanceur (**sollicité** et ou acquis)

ETIUDA Doctoral Sholarship (National Science Centre, Poland)

13 - Si cofinancement refusé, autres sources de cofinancement identifiées

Contrat doctoral UBS (1/2), ARED (1/2)

Le – la candidat.e

Profil souhaité du candidat (compétences scientifiques et techniques requises) :

Autonomy, serious, Good knowledge in Phycology, Ecofriendly extraction processes, biochemistry, Plant physiology, English

Projet de thèse en cotutelle internationale

S'agit-il d'un projet de thèse en cotutelle internationale *(oui/non)* **: oui**

Si oui, préciser l'établissement pressenti (*et le pays de rattachement*) : Wroclaw University of Science and Technology,Faculty of Chemistry, Department of Advanced Material Technologies, Wroclaw, Poland

Ce projet de thèse fera-t-il l'objet d'un cofinancement international *(oui/non)* **:** ETIUDA Doctoral Sholarship (National Science Centre, Poland)

(Rémunération du doctorant par l'établissement implanté sur le territoire régional (18 mois sur 36 mois), et l'établissement étranger, qui s'engage également à rémunérer le doctorant dans le cadre de son séjour à l'étranger, soit durant 18 mois -a minima-)

En cas de cofinancement international, préciser -*si vous en avez connaissance***- l'organisation du calendrier des périodes de séjour : 6 mois/an**

Financement du projet de thèse

Part de l'enveloppe financière régionale affectée au projet :

□ Financement Région 100 %

☑ Financement Région 50 % (préconisé)

En cas de financement à 50 %, le cofinancement est-il déjà identifié *(oui/non)* **:** ETIUDA Doctoral Sholarship (National Science Centre, Poland) enn cours

Si oui, préciser la nature du cofinancement (*ANR, partenaire privé, Ademe, etc.*) :

Si le cofinancement n'est pas encore confirmé, date prévue de réponse du cofinanceur : avant l'été

En cas de non-obtention du cofinancement demandé, une autre source de cofinancement est-elle identifiée *(oui/non)* **:** Financement UBS, ISblue