A^3 Project: a new look on algae revalorization

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ABSTRACT

The accumulation of green macroalgae *(Ulva spp.)* responsible for green tides leads to the production of hydrogen sulfide gas (H_2S). Our main goal is to promote the degradation of algae by the sulfate-reducing bacteria (SRBs), and thus accelerate the H_2S produced by those bacteria, before transforming this gas into our final product – sulfuric acid. For this purpose, we aim to develop a bioreactor to enable bacterial growth which will produce sulfatases and other degradation enzymes that will destroy the main component of the cell-wall of algae: ulvan. Once produced, the enzymes will be added to a tank filled with algae taken directly from the beach. Since SRBs are naturally found in green tides, they will also be collected along with the algae. The degradation gas produced in the tank will serve as a base for sulfuric acid production a useful compound for many industries such as the production of detergents, textiles and many others products

Index terms- Green macroalgae, Ulva spp, Green tides, SRBs, sulfuric acid, hydrogen sulfide

I. INTRODUCTION

Green macroalgae (*Ulva spp.*) have been poisoning coast sides for decades. Rising temperatures and eutrophication of coastal waters due to nitrogen fertilizer pollution are mainly responsible for their proliferation. These green seaweed blooms are called “green tides”. This accumulation of algae affects many ecosystems in the world including those in the north-west of France. This phenomenon is getting worse each year and raises many health, economic and environmental concerns.

The significant accumulation of green macroalgae and their degradation causes the formation of anoxia zones, which leads to the production of hydrogen sulfide (H_2S) by the SRBs. Hydrogen sulfide can be an extremely toxic and harmful gas, and its asphyxiation of flora and fauna is just one of the side effects this gas has. Just a few minutes of inhalation might become lethal to humans and animals. In the past few years, this gas has been the cause of several animal and human casualties.

Nantes iGEM team decided to promote green algae, responsible for green tides on the coasts of French Brittany. Our project, therefore, revolves around the development of these algae. Our final goal is to produce sulfuric acid, a compound used in many industries such as the production of detergents, textiles and many other products.

In this article, we will have a look at the methods and pathways for algae degradation by using a specific mix of enzymes.

II. MATERIALS AND METHODS

Enzymes

Our project will be using a total of seven enzymes from the bacterium *Formosa agariphila*. There are three degradation enzymes and three sulfatases.

In order for the sulfatases to work, an additional enzyme will be used – Formylglycine-generating enzyme (FGE). This last enzyme is used to activate the sulfatases.

Enzymes choice

The ulvan degradation cascade described by Reisky *et al.* (2019) shows us that the degradation enzymes P30_PL28, P10_Plnc and P31_GH39 have a significant effect on ulvan. Based on this research, Nantes team decided to use those three enzymes.

Regarding the sulfatases, we based our decision on the same article. The P18_S1_7, P32_S1_8 and P36_S1_25 enzymes show an important activity on ulvan.

Plasmids design

To achieve the goals we set, our project will use two types of plasmids.

- **pET 11 plasmid**

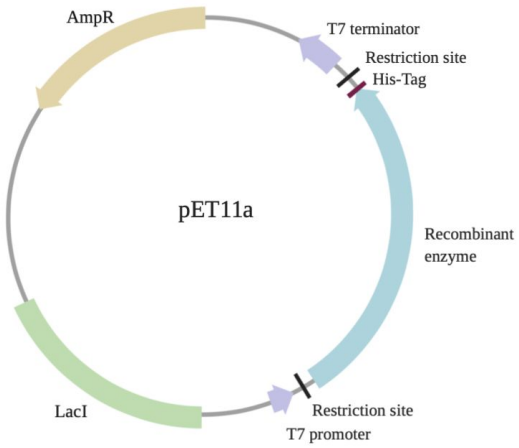

Fig. 1 pET11a plasmid containing each one of the following enzymes : degradation enzymes P30_PL28, P10_Plnc and P31_GH39 and sulfatases P18_S1_7, P32_S1_8 and P36_S1_25.

The pET11a plasmid in Fig. 1 will be used for the expression of the three so-called degradation enzymes and the three sulfatases.

- **pEVOL-1 plasmid**

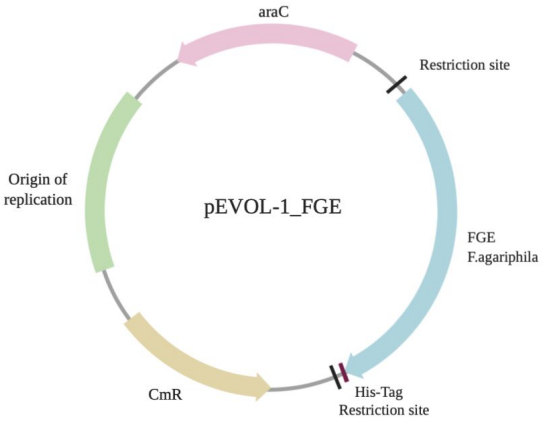

Fig. 2 pEVOL-1 plasmid containing FGE.

The pEVOL-1 *(Fig. 2)* plasmid will be used for the expression of the FGE. FGE and sulfatases will be either co-expressed or put together once produced.

The His-Tag sequence will allow us to purify the enzymes once they are produced, by using an immobilized metal affinity chromatography (IMAC) with nickel resin.

Expression of enzymes

The strain *E.coli* BL21 DE3 was chosen for the expression of our enzymes. This is a popular strain used to express recombinant proteins.

III. BIOREACTOR

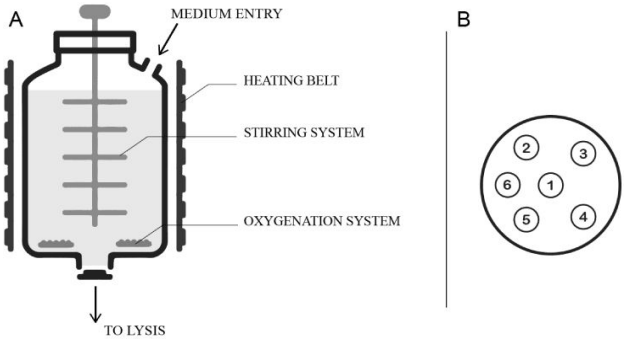

Fig. 3 A Diagram of the bioreactor. **B** Details of the cap: 1. agitation system; 2. sampling catheter; 3. pH probe; 4. temperature probe; 5. overpressure valve; 6. injection catheter.

Our bioreactor will follow the continuous flow method, which means that our BL21 DE3 bacteria will be kept in a constant exponential phase. To do this, an X quantity of medium and bacteria will be taken out and the same X quantity of the medium (sterile) will be added in the bioreactor through the designated inputs and outputs (Fig. 3 A). A stable quantity of degradation enzymes and sulfatases will be produced.

The bioreactor is connected to a reservoir containing ulvan or green algae that need to be degraded. The produced enzymes will be poured into this tank and the degradation will take place in this compartment. Hydrogen sulfide will also be produced in the same tank.

At the end, the hydrogen sulfide (H_2S) that results from the degradation will be collected via a special system.

IV. DISCUSSION

In order to achieve the absolute valorization of green algae and produce sulfuric acid, two groups of enzymes will be produced in an *E. coli* chassis. A group of enzymes composed of three degrading enzymes will allow an accelerated degradation of the ulvan. The second group of enzymes consists of sulfatases which will afterwards promote the release of the sulfates attached to the ulvan. This will therefore enable the production of H_2S by the SRBs. Then, from this released gas and by chemical conversion, sulfuric acid will be obtained in another compartment of the bioreactor.

V. CONCLUSION

The degradation of green algae causes many issues on a global scale. Considering the negative effects it has on human health, on local ecology but also on the touristic field, a solution for valorizing *Ulva spp.* proves useful on many levels. For this purpose, we will put the algae collected in a bioreactor and accelerate their degradation by targeting the ulvan, a sulfated polysaccharide found in the wall of these algae (38% to 54% of the dry weight of the ulvae). Thus, this degradation of the ulvan will allow the release of hydrogen sulfide into the bioreactor.

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