SaltGae
algae to treat saline wastewater

Demonstration project to prove the techno-economic feasibility of using algae to treat saline wastewater from the food industry

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WP1 Halotolerant Cultures
Deliverable D1.1 Growth protocol of algae strain in saline wastewater

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Title	Growth protocol of algae strain in saline wastewaters
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Description	This report describes the growth protocols with the best performing conditions obtained for *Dunaliellasalina*, *Spirulina*and *Chlorella* growth on tannery, fishpond and cheesewhey wastewaters.
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Glossary

The glossary of terms used in this deliverable can be found in the public document “SaltGae_Glossary.pdf” available at:http://saltgae.eu/downloads-public/

Abbreviations and Acronyms

Abbreviation / Acronym	Description
N	Nitrogen
P	Phosphorous
COD	Chemical oxygen demand
PBR	Photobioreactor
[ww]	Wastewater concentration

Table 1: Abbreviations and Acronyms

1 INTRODUCTION

SaltGae project (www.saltagae.eu) aims at the development and demonstration of a techno-economically viable solution for the treatment of saline wastewater from food industry. The solution proposed by the SaltGae project consists of an innovative technology based on the use of halotolerant algae/bacteria consortia for large-scale wastewater treatment. Microalgae from Dunaniella and Spirulina genera are known to have very good adaptability to saline environment. Moreover, Chlorella and Tetraselmis genera display very good adaptation to salt stress in wastewaters and can reach high biomass productivity. According to this evidence Spirulina, Chlorella, Dunaliella and Tetraselmis genera were chosen to be tested in agro-industrial waste streams. Bacteria were not selected, since the tested wastewaters were source of bacteria adapted to high salinity condition.

The first task of WP1 (Halotolerant Cultures), led by FPTP, was to set-up lab-scale microalgae/bacteria systems in different saline wastewaters. Three saline wastewaters were chosen in the project framework: tannery, fishpond and cheese whey/dairy wastewaters, since the final goal of the project is to respond to the request of a wide range of industrial sectors, such as leather, food processing and land-based aquaculture.

To identify the best performing microbial consortium for saline wastewater treatment, the halotolerant algae *Dunaliella salina (PTP in-house stock)*, together with selected algal species provided by partners ARAVA (*Spirulina* and *Chlorella* sp.), ARCHI (*Tetraselmis suecica)* from in-house stocks were investigated in batch culturing trials. Based on literature information and partners' expertise on microalgae growth conditions, trials were planned as illustrated in Table 2. *Dunaliella salina* was chosen as elite strain for tannery wastewater treatment since it is the most halotolerant species. *Tetraselmis suecica* is a marine green algae thus is not suitable for fishpond wastewater treatment. *Spirulina* and *Chlorella* are strong and versatile species, able to grow on different wastewaters and accumulate high amount of biomass in short period of time.

	Dunaliella salina	***Spirulina***	***Tetraselmis suecica***	***Chlorella sp***
tannery	✓	✓		✓
fish ponds		✓		✓
cheese whey		✓	✓	✓
dairy		✓	✓	

Table 2: Algal species x wastewater combinations

Here we report the growth protocols obtained for *Dunaliella salina*, *Spirulina*, *Tetraselmis suecica* and *Chlorella sp.* Grown in tannery, fishpond and cheese whey/dairy wastewaters and the best performing concentrations of the latter.

2 TANNERY

2.1 Dunaliella salina

2.1.1 Batch trials setup

Several trials with tannery replacing the growth substrate were performed using not sterilized tannery, in order to evaluate the possibility of using this wastewater as it comes, minimising treatments and allowing co-growth with indigenous bacterial strains.

In each trial, sudden death of microalgae occurred within 5-7 days from the start of the trial. This sudden death might depend on the presence of grazing microorganisms identified through microscope observations. Therefore, sterilized tannery was used aiming at reducing the presence of grazing microorganisms. The strategy proved to be successful.

Batch growth conditions are reported here:

- **Light** was provided by cold fluorescent lamp at an irradiance of 210 µE m^{-2} s^{-1} at PBR surface with a light/dark photoperiod: 12h/12h;
- The **pH** was set at 8.2 and it was controlled by using pure CO_2 injection with "on-demand" modality;
- The **temperature** was constant and set at 25°C;
- The microalgae **starter *inoculum*** was introduced into PBRs in order to reach a concentration of 0.3 g L^{-1};
- The **duration of batch trials** was 10 days;
- The **best performing wastewater concentration** was identified in sterilised tannery replacing the growth substrate at 10 %(v/v).

It is important to highlight that microalgae growth conditions, in particular concerning inorganic N supply, can be improved depending on the main focus of the depuration, i.e. to consume COD or Nitrogen. Fine-tuning of nitrogen supply is needed according to carbon content and light seasonal variation.

2.2 Spirulina

2.2.1 Batch trials setup

Trials with tannery replacing the growth substrate were carried out using not sterilized tannery as for *Dunaliella salina*, in order to evaluate the possibility of using this wastewater, minimizing treatments and allowing co-growth with indigenous bacterial strains.

Also with *Spirulina*, sudden death occurred within 5 days from the start of the trials. As with *Dunaliella salina*, sterilized tannery was also used with *Spirulina* in order to decrease the amount of grazing microorganisms present, and as with *Dunaliella* the strategy proved to be successful.

Batch growth conditions are reported here:

- **Light** was provided by cold fluorescent lamp at an irradiance of 210 µE m-2 s-1 at PBR surface with a light/dark photoperiod: 12h/12h;
- The **pH** was set at 9.4 and it was controlled by using pure CO_2 injection with "on-demand" modality;

- The **temperature** was constant and set at 25°C;
- The microalgae **starter inoculum** was placed into PBRs so to reach a concentration of 0.5 g L^{-1};
- The **duration of batch trials** was 19 days;
- The **best performing wastewater concentration** was identified in sterilized tannery replaced to the growth substrate at 10 %(v/v).

2.3 Chlorella

2.3.1 Batches trials setup

Trials with tannery replacing the growth substrate were carried out using not sterilized tannery as for *Dunaliella salina* and *Spirulina*, in order to evaluate the possibility of using this wastewater, minimizing treatments and allowing co-growth with indigenous bacterial strains.

Also with *Chlorella*, sudden death occurred within 5 days from the start of the trials. As with *Dunaliella salina* and *Spirulina* sterilized tannery was also used with *Chlorella* in order to decrease the amount of grazing microorganisms present, and as with *Dunaliella* and *Spirulina* the strategy proved to be successful.

Batch growth conditions are reported here:

- **Light** was provided by cold fluorescent lamp at an irradiance of 210 µE m-2 s-1 at PBR surface with a light/dark photoperiod: 12h/12h;
- The **pH** was set at 8.4 and it was controlled by using pure CO_2 injection with "on-demand" modality;
- The **temperature** was constant and set at 25°C;
- The **starter inoculum** of microalgae was placed into the reactors in order to reach a concentration of 0.6 g L^{-1};
- The **duration of batch trials** was 10 days;
- The **best performing wastewater concentration** was identified in sterilized tannery replaced to the growth substrate at 10 %(v/v).

3 FISHPOND WASTEWATER

Fishpond wastewater was characterized by low content of N, P and COD. Therefore, trials were carried out with fishpond wastewater as it come. Diluted concentrations were not evaluated.

3.1 Spirulina

3.1.1 Batch trials setup

Batch growth conditions are reported here:

- **Light** was provided by cold fluorescent lamp at an irradiance of 210 µE m^{-2} s^{-1} at PBR surface with a light/dark photoperiod: 12h/12h;
- The **pH** was set at 9.4 and it was controlled by using pure CO_2 injection with "on-demand" modality;
- The **temperature** was constant and set at 25°C;
- The microalgae **starter inoculum** was placed into PBRs so to reach a concentration of 0.5 g L^{-1};
- The **duration of batch trials** was 10 days.

Using an inoculum of 125 ml of *Spirulina* involved a slight increase of the content of nitrogen and phosphorus into PBRs (coming right from the *Spirulina* synthetic medium) compared to that originally present in the fishpond wastewater.

3.2 Chlorella

3.2.1 Batch trials setup

Batch growth conditions are reported here:

- **Light** was provided by cold fluorescent lamp at an irradiance of 210 µE m-2 s-1 at PBR surface with a light/dark photoperiod: 12h/12h;
- The **pH** was set at 8.4 and it was controlled by using pure CO_2 injection with "on-demand" modality;
- The **temperature** was constant and set at 25°C;
- The **starter inoculum** of microalgae was placed into the reactors in order to reach a concentration of 0.5 g L^{-1};
- **Batch trials** are ongoing.

4 CHEESE WHEY

Cheese whey was used after deproteinization by heat treatment at 115 °C for 15 min and then by filtration of the flocs formed by using a 0.2 μm Whatman filter.

4.1 Spirulina

4.1.1 Batch trials setup

Cheese whey is characterized by very high content of N, P, COD and salts. For this reason, trials were started with low concentrations at 5% and10%.

Batch growth conditions are reported here:

- **Light** was provided by cold fluorescent lamp at an irradiance of 210 μE m-2 s-1 at PBR surface with a light/dark photoperiod: 12h/12h;
- The **pH** was set at 9.4 and it was controlled by using pure CO_2 injection with "on-demand" modality;
- The **temperature** was constant and set at 25°C;
- The **starter inoculum** of microalgae was placed into the reactors in order to reach a concentration of 0.5 g L^{-1};
- The **duration of batch trials** was 10 days;
- The **best performing wastewater concentration** was identified in deproteinized cheese whey replaced to the growth substrate at 5 %(v/v).

4.2 Tetraselmis suecica

4.2.1 Batches trials setup

Cheese whey is characterized by very high content of N, P, COD and salts. For this reason, trials were started with low concentrations at 5% and 10%.

Batch growth conditions are reported here:

- **Light** was provided by cold fluorescent lamp at an irradiance of 210 μE m-2 s-1 at PBR surface with a light/dark photoperiod: 12h/12h;
- The **pH** was set at 7.4 and it was controlled by using pure CO_2 injection with "on-demand" modality;
- The **temperature** was constant and set at 25°C;
- The **starter inoculum** of microalgae was placed into the reactors in order to reach a concentration of 0.5 g L^{-1};
- **Batch trials** are ongoing.

4.3 Chlorella

4.3.1 Batch trials setup

Cheese whey is characterized by very high content of N, P, COD and salts. For this reason, trials were started with low concentrations at 4%, 8% and 10%.

Batch conditions are here reported:

- **Light** was provided by cold fluorescent lamp at an irradiance of 210 µE m-2 s-1 at PBR surface with a light/dark photoperiod: 12h/12h;
- The **pH** was set at 8.4 and it was controlled by using pure CO_2 injection with "on-demand" modality;
- The **temperature** was constant and set at 25°C;
- The **starter inoculum** of microalgae was placed into the reactors in order to reach a concentration of 0.9 g L^{-1};
- The **duration of batch trials** was 10 days;
- The **best performing wastewater concentration** was identified in deproteinized cheese whey replaced to the growth substrate at 4 %(v/v).

5 DAIRY PROCESSING PLANT WASHING WASTEWATER

The following trials were carried out at the demo plant of partner ARCHI. Dairy wastewater appeared as an opaque whitish liquid with white particles in suspension. The effluent was sieved at 32 microns and 3 ml/L of HCl 1:1 were added to neutralize wastewater at pH 7.6.

5.1 Spirulina

5.1.1 Batch trials setup

Batch growth conditions are reported here:

- **Light** was provided by cold fluorescent lamp at an irradiance of 300µE m-2 s-1 at PBR surface light 24h/24h;
- The **pH** was set at 9.4 and it was kept by using CO_2/air 2% continuously;
- The **temperature** was constant and set at 20°C;
- The **starter inoculum** of microalgae was 0.6 g L^{-1}; 100 ml of starter inoculum were placed into 500 ml of dairy wastewater (inoculums dilution 1:6), bicarbonate addition to standard Zarrouk medium, Fe addition;
- The **duration of batch trials** was 9 days.
- The **best performing wastewater concentration** was identified in dairy wastewater replaced to the growth substrate at 85% (v/v).

5.2 Tetraselmis suecica

5.2.1 Batch trials setup

Batch growth conditions are reported here:

- **Light** was provided by cold fluorescent lamp at an irradiance of 300µE m-2 s-1 at PBR surface with light 24h/24h;
- The **pH** was set at 8.2 and it was kept by using CO_2/air 2% continuously;
- The **temperature** was constant and set at 20°C;
- The **starter inoculum** of microalgae was 0.65 g L^{-1}; 200 ml of starter inoculum were placed into 400 ml of dairy wastewater (inoculum dilution 1:3) marine salt addition to standard ASW, Fe addition;
- The **duration of batch trials** was 9 days;
- The **best performing wastewater concentration** was identified in dairy wastewater replaced to the growth substrate at 67 % (v/v).

6 CONCLUSIONS

SaltGae project aims at the development and demonstration of a techno-economically viable solution for the treatment of saline wastewater from food industry. Three saline wastewaters were chosen in the project framework: tannery, fishpond and cheese whey/dairy wastewaters.

Some algae were selected for the treatment of these wastewater: Dunaliella salina, Spirulina, Tetraselmis suecica, Chlorella sp. The protocols for their use is summarised next:

	Dunaliella salina	*Spirulina*	*Tetraselmis suecica*	*Chlorella sp*
tannery	Light: 210 µE m^{-2} s^{-1} Ph: 8.2 Temperature: 25°C Starter inoculum: 0.3 g L^{-1} **[ww]: 10 %(v/v)**	Light: 210 µE m^{-2} s^{-1} Ph: 9.4 Temperature: 25°C Starter inoculum: 0.5 g L^{-1} **[ww]: 10 %(v/v)**		Light: 210 µE m^{-2} s^{-1} Ph: 8.4 Temperature: 25°C Starter inoculum:0.6 g L^{-1} **[ww]: 10 %(v/v)**
fish ponds		Light: 210 µE m^{-2} s^{-1} Ph: 9.4 Temperature: 25°C Starter inoculum: 0.5 g L^{-1} **[ww]: NS (normal state)**		Light: 210 µE m^{-2} s^{-1} Ph: 8.4 Temperature: 25°C Starter inoculum:0.5 g L^{-1} **[ww]:NS (normal state)**
cheese whey		Light: 210 µE m^{-2} s^{-1} Ph: 9.4 Temperature: 25°C Starter inoculum:0.5 g L^{-1} **[ww]: 5 %(v/v)**	Light: 210 µE m^{-2} s^{-1} Ph: 7.4 Temperature: 25°C Starter inoculum:0.5 g L^{-1} **[ww]: in progress**	Light: 210 µE m^{-2} s^{-1} Ph: 8.4 Temperature: 25°C Starter inoculum: 0.9 g L^{-1} **[ww]:4%**
dairy		Light: 300µE m^{-2} s^{-1} Ph: 9.4 Temperature: 20°C Starter inoculum: 0.6 g L^{-1} **[ww]: 85% (v/v)**	Light:300µE m^{-2} s^{-1} Ph: 8.2 Temperature: 20°C Starter inoculum: 0.65 g L^{-1} **[ww]: 67%(v/v)**	

Table 3: Summary of algal specific growth conditions and the best performing wastewater concentrations