LIFE Project Number

<LIFE13/ENV/ES/445>

FINAL Report

Covering the project activities from 01/07/2014 to 31/12/2016

Reporting Date

<31/03/2017>

LIFE SEACOLORS – Demonstration of new natural dyes from algae as substitution of synthetic dyes actually used by textile industries
1. List of contents:

1. List of contents: .................................................................................................................. 2
2. Executive Summary ........................................................................................................... 3
3. Introduction ....................................................................................................................... 9
4. Administrative part .......................................................................................................... 11
   4.1 Description of the management system ........................................................................ 11
   4.2 Evaluation of the management system ......................................................................... 14
5. Technical part ..................................................................................................................... 15
   5.1 Technical progress, per task ......................................................................................... 15
   5.2 Dissemination actions ................................................................................................. 54
2. Executive Summary

The LIFE+ project SEACOLORS, Demonstration of new natural dyes from algae as substitution of synthetic dyes actually used by textile industries (LIFE13/ENV/ES/445) started the July 1st 2014, and this final report covers the period of execution from July 1st 2014 to December 31th 2016.

The textile industry is named to be the second most polluting industry; being estimated that over 10,000 different dyes and pigments are used industrially and over $7 \times 10^5$ tons of synthetic dyes are annually produced worldwide. Up to 200,000 tons of dyes are lost on effluents every year during the dyeing and finishing operations. Due to these enormous amounts of synthetic pollutants released in the environment SEACOLORS main aim was the demonstration and validation of obtaining natural dyes from a sustainable and renewable source, ALGAE, and their application in the textile industry to replace the synthetic dyes. With this improvement, another goal was to obtain less contaminated wastewater as expected by the higher biodegradability of the natural dyes; thus helping in reducing the water purification process and simultaneously contributing to the application of the politics and community legislation regarding industrial wastewater effluents, the the Normative 2008/105/CE on environment quality in the field of water policy which sets the maximum allowable priority substances and other chemical contaminants, particularly the REACH regulation.

The validation process required by SEACOLORS involved several phases, starting with the validation of the types screening and validation of the most suitable algae strains, from the high variety of microalgae, cyanobacteria and macroalgae species, followed by the optimization of the growth process to achieve the maximum colorant yield per biomass. The pigment extraction process followed an optimization process, which was complementary with the investigation of the variables involved in the textile finishing processes to obtain dyed and printed fabrics with acceptable quality parameters. This is a complex process due to: 1st a huge variety of fibres in the market and the range of colors demanded by the fashion and 2nd by their specific requirements regarding the fastness qualities of a dyed or printed fabric.

Several specific objectives were marked:

- To select algae(micro- and macroalgae) with dye capacity and potential for mass cultivation
- To improve the algal dye content through the optimization of their growing conditions
- To study the extraction conditions to increase the amount of the obtained dye
- To study the dyeing and printing processes and the auxiliaries needed to obtain satisfactory results
- To compare the fastness of the newly obtained natural dyes with the synthetic dyes
- To disseminate and transfer results to stakeholders.

The project started with preparatory action A1, which had as main objective the identification and selection of potential microalgae (including cyanobacteria) and macroalgae species suitable to be used as raw material for the dye industry. The screening of the species was based on literature review, the knowledge of the team involved as well as biomass already
available in the partner’s records. After the first identification of the species, their cultivation was developed to be able to undergo a pigment characterization for a final selection process and a final validation for the textile industry application. At the end of this action, 8 microalgae and 6 macroalgae species were selected to continue the experiments.

Action B1 involved the cultivation of the previously selected algae species, starting from laboratory scale and then updating to a semi-industrial scale, due to the fact that lab pilot observation cannot be extrapolated to outdoor conditions because the physiological behaviour is strain dependent and it may have negative influence due to the uncontrollable external conditions. The final results represented the production of micro and macro algal biomass at pilot scale, with improved yields for prolonged periods in a sustainable way at pilot scale.

During action B2 the cultivated biomass in the previous action was subjected to pigment extraction procedures. These procedures were continuously optimized in order to obtain a general extraction method applicable to several algae strains. By the end of this task three different colorants were successfully obtained: red, blue and yellow; which could be further employed in textile finishing processes.

Action B3 main purpose was the validation and demonstration of algal dyes in the textile dyeing and printing processes. The extracted pigments, in soluble or liquid form, were employed in dyeing and printing processes of cotton and wool fabrics. The processes represented the laboratory simulation of a common industrially exhaustion dyeing process and the most popular pigment-printing process. In order to validate the pigments applicability, the finished fabrics were characterized according to European regulations by means of:
• Determination of CIELab coordinates, to numerically define the color and calculate the color difference for the objective analysis of the different dyeing/printing tests.
• Fastness tests to: laundering, rubbing, light.
• Additional value tests: Protection factor measurement and antimicrobial properties.

Figure 4. Images of finished textiles with algal pigments, A. Dyed cotton (right) and wool (left) fabrics, B. Printed cotton (right) and wool (left) fabrics, C. Printed t-shirts with algal pigments

Action C1 demonstrated a reduction in the environmental impact by using natural dyes as substitutes for the synthetic ones, which represent an important pollution source and, at the same time by reducing the wastewater contamination with their employment. The minimization of the environmental impact occurred in all the phases of the project:

• The impact generated by the obtaining of dyes, was minimized in comparison with the means of production of the other natural sourced colorants or the synthetic ones.
• The impact generated by the employment of these newly obtained dyes has decreased in comparison with their commercial correspondents, in terms of employment of lower process temperatures, higher biodegradability, lower wastewater pollution.
• The impact resulted from the reuse of biomass, was a positive one, making possible the adaptation of the project to the circular economy concept, by further manipulation of the same biomass as a biorefinery in order to obtain different colorants, evaluate antioxidant activity in secondary extracts, giving the biomass another added value.

At the end of action C2, the project demonstrated the ability to employ algae biomass for the obtention of new dyes to be used in the textile industry. In the other hand, wastewater effluents resulting from the process proved higher biodegradability. Furthermore, the used biomass for the extraction of the initial colorants could be used to obtain new colorants and several compounds with added value for other industries. The algal colorants cost was calculated only at laboratory level, and it resulted higher that the currently used dyes. The extrapolation would generate a lower cost due to the relation price-quantity of the auxiliary products used in the algae cultivation, extraction and application processes.

Different surveys were created in order to monitor the knowledge of target audience awareness of the environmental and socio-economic impacts of the project and to measure the acceptability of the newly developed product in the textile industry.

Action D.1 was addressed to the dissemination and communication phase, aiming at reaching the widest possible audience. All partners worked on the development of contents for dissemination purposes, and participated at events of interest at both scientific and commercial level; in action D.2 the webpage of the project was developed in two languages: English and Spanish. It was created in September 2014, but considerably changed in February 2016 in order to improve the contents and to make it more interactive. Also, two Noticeboards were created through action D.3, one at the beginning and the other at the end of the project.
and were placed in the facilities of all the partners. And in action D.4 the Layman’s report was created in three languages: English, Portuguese and Spanish.

Action E.1 promoted the proper functioning of the project, establishing channels for internal and external communication and controlling the time slots for the execution of tasks, this task has been carried out successfully, as the indicators of progress have been achieved on time; in action E.2 AfterLIFE communication plan was done, which ensures the proper exploitation of the results after the end of the project.; Last but not least, Action E.3 resulted in the creation of a database with projects connected with the theme of SEACOLORS project, which were contacted and informed about the projects advances and results. The database contains a total of 60 projects and was used for the final dissemination event. From all contacted projects, 16 answered wishing to join the network and 6 accepted to meet and discuss about the projects.

From the actions and activities performed, several deliverables have been obtained (Attached in the TECHNICAL ANNEX):

**ACTIONS A:**

**D.A.1.1. Microalgae and macroalgae selection for dye extraction.** A complete report based on scientific literature and experience and species records in the partners facilities. It represented the starting point of the project.

**D.A.1.2. Microalgae and macroalgae biomass production at lab scale.** BEA and AlgaPLUS were performing microalgae/cyanobacteria and macroalgae cultivation under laboratory-controlled conditions. Growth conditions and measurements were defined.

**D.A.1.3. Microalgae and macroalgae pigment characterization.** BEA and AlgaPLUS were performing laboratory scale pigments/dyes extraction and characterization (target pigment concentration; purity criteria; stability, etc.) from biomass obtained. Standard methods for pigments extraction and quantification from biomass obtained were used for microalgae/cyanobacteria and macroalgae pigments/dyes characterization.

**ACTIONS B:**

**D.B.1.1. Microalgae/Cyanobacteria production at pilot scale.** The optimum conditions of outdoor algal cultivation were developed, to obtain the highest efficiency in the dye yield. These studies were realized in Spanish Bank of Algae facilities and represented an upscaling of the laboratory cultures.

**D.B.1.2 Report of macroalgae production in an IMTA unit.** A complete report based on the upscaling of the macroalgae species. The increase in the yield efficiency was realized in ALGAPlus facilities and consisted in manipulation of the stocking densities and renewal rates of the seawater inside the cultivation units.

**D.B.2.1. Report of the dye extraction.** This activity characterized the obtained dyes to compare them and determine the changes that occur in the dye, which resulted in a change of process variables or in different batches. Furthermore, sometimes the obtained dye was not suitable for direct application in the textile process so it required prior treatment as conversion to dust or solvent removal. This activity generated three different colors: red, blue and yellow.

**D.B.3.1. Report of the dyeing process to cotton and wool.** This stage validated and demonstrated the application of dyes in the dyeing and printing processes, applied to cotton and wool. It implied various parameter optimization process to reach a generally viable dyeing/printing process for the dyes involved to both tested substrates.
D.B.3.2. Results of the tests done to the dyed fabrics. A full report was developed based on the dyed or printed textiles characterization through which was validated the newly obtained dyes potential to compete with the actual employed colorants. The conformity tests applied to the fabrics revealed good results.

D.B.3.3. Report of the analysis of waste water with natural colours. This report collects all the information about the analysis of waste water after the dyeing process with natural dyes form algae to compare with other dyes.

D.B.3.4. Report on the results of dyeing on an industrial scale. This deliverable collects the validation the dyeing on an industrial scale using the new dyes.

**ACTIONS C:**

D.C.1.1. Environmental impact report. This deliverable collects the monitoring of the impact on the environment, of the project actions, referred to the realization of a quantitative comparative study advantages and saving of the process of manufacture and use of natural dyes comparing with the actual situation. The studies of the environmental impact of a project are suitable to be realized through specific methods of Environmental Impact Assessment (EIA), which can be used for the identification, systematic studies, visualization and impact assessment. Through these methods were identified the direct and indirect impacts.

D.C.1.2. Socio-Economic impact report. This deliverable collects the results of the social and economic impact that the production and the use of algae extracts has on the society and the actual economy.

**ACTIONS D:**

D.D.1.1 Dissemination and Communication Plan. This deliverable includes the tips and way to do the dissemination during the project as well as collects the main dissemination activities developed (fair, congresses and events assistance, number of people informed about the project, impact of the dissemination activities). Moreover, summarizes briefly the dissemination channels used, the dissemination material and the final event results.

D.D.1.2 and D.D.13 Newsletter and Dissemination Material developed during the whole project.

D.D.1.4. Publication, press releases, mailings. The press realises, published articles, social networks dissemination are described in this report.

D.D.1.5. Divulgation material collects the divulgation material and channels used to disseminate the final event.

D.D.2.1 Website of the project. In this deliverable, it has been collected the main section of the website and it has been explained the main functionalities.

D.D.2.2 Partners information in website. Publication in partner’s website are being collected in this deliverable.

D.D.3.1 Notice Board (beginning of the project). Notice board design of the project with the main results of the project.

D.D.3.2 Notice Board (results of the project). Notice board design of the project with the main results of the project.
D.D.4.1 Layman Report. In this deliverable it has been collected the Layman Report in English, Italian and Spanish.

**ACTIONS E:**

**D.E.1.1 Consortium agreement and meeting calendar.** This deliverable has been divided in two parts: the first one with all the consortium agreements signed by all partners with the coordinating beneficiary (AITEX) and the second part in a meeting calendar establishing the meetings carried out.

**D.E.2.1 After LIFE Communication Plan.** In this deliverable it has been attached the communication plan to be followed once the project finish.

**D.E.3.1. Database with information of the main LIFE projects related to this project.** This represents a complete report of a database creation including the most recent LIFE projects in relation with SEACOLORS thematic. This activity was focused on Life+ calls, but also includes information from other EU support programmes like FP7, CIP, etc.

**D.E.3.2. Report of the Final Event.** This report includes a full description of the Final Event with the presentations of the results of the project and presentation of other projects. In this report are collected the numbers of entities and companies to which the project newsletters have been sent and the meetings with the other LIFE project that have been carried out.

Summarizing the main outputs of the SEACOLORS project, the consortium was able to study the pollution levels of some commonly used synthetic dyes in the textile industry, to identify suitable micro (including cyanobacteria) and macroalgae species with the capacity of being cultivated in conditions that generate the highest colorant yield, the most advantageous extraction method of colorants from algal biomass that can be extrapolated to an industrial level and optimum dyeing and printing processes employing the newly, eco-friendly obtained dyes. In this way, it was achieved a significant reduction of the level of pollution generated by the finishing branch of the textile industry and a reduction of the purifying costs of the wastewater resulted from the textile finishing process. The environmental and economic benefits of the usage of the obtained algal dyes have been studied, elaborating impact analysis from the production levels until the end of the final product life and also comparative studies with the actual practices. Thus, SEACOLORS results are considered as fully successful.
3. Introduction

Description of background, problem and objectives

- **Objectives:**
  
  SEAOLORS main goal has been the validation and demonstration of obtaining of natural dyes from a sustainable and renewable source, namely algal species, and their application in the textile industry in order to replace the synthetic ones, with harmful and pollutant effects on the environment.

  The project targets were achieved through the accomplishment of several objectives divided into four phases: selection of algae with dye characteristics and their cultivation process, efficient pigment extraction, used in textile finishing processes as dyeing and printing.

- **Environmental problem/issue addressed**

  Pollution and resource scarcity represent important issues nowadays, generated by the impact of human demands on the environmental health. One of the most polluting industries is the textile sector, as it employs high quantities of water and chemicals, represented mostly by dyestuffs, from synthetic origin, a non-renewable nor sustainable source, mainly oil. They are pollutant during their manufacturing and use, due to the residues content in the wastewater effluents resulting from the dyeing processes.

  Because of this, the foreseen results that were demonstrated during the project has been to obtain natural dyes from a renewable source to use in textile industry, and consequently:

  - New source of natural dye (more efficient that natural dyes from plants)
  - Selection of algae species with high potential of dying
  - Validated dyeing process with the newly obtained dyes
  - Obtaining of a three colours mixture and from there the development of other colours based on the chromatic circle
  - To replace the pollutant synthetic dyes with natural sustainable ones

- **Outline the hypothesis to be demonstrated / verified by the project:**

  The LIFE SEACOLORS project has allowed demonstrating the possibility to obtain natural dye from algae, and demonstrating the use of these dyes in dyeing and printing processes. Different dyeing and printing conditions have been studied in order to obtain a homogeneous and durable colour on the wool and cotton substrates.

- **Description of the technical / methodological solution:**

  Five general phases will be followed during the project:

  - Study and selection of algae with dye capacity
  - Study of growing conditions of algae to increase dyes substance
  - Validation and demonstration of the application of algae dyes in textile dyeing process.
  - Monitoring of the environmental and economic impact
• Dissemination, networking and management activities

- Results and environmental benefits:
  o Selected different micro and micro as pigment/dyes sources to provide the three basic colours employed in dyeing.
  o Optimization of cultivation conditions to increase the Pigment content.
  o Validated the extracting methods to obtain green (chlorophylls), red (phycoerythrin) and yellow (carotenoids) pigments from micro and macroalgae.
  o Obtaining of a tricomy.
  o Definition of the method to dye wool and cotton substrates (eco-friendly method).
  o Dyeing or printing textiles with a good uniformity and reproducibility.
  o Natural algal dyes present 70% of biodegradability.
  o Natural algal dyes production reduces significantly the CO₂ emissions.
  o New trends of textile market reduce uses of synthetic dyes.

- Expected longer term results:

A changing perception of health and pollution drive European demand for sustainable products. European consumers are becoming more aware of the importance of healthy lifestyles and the environment. The same environmental sensibilities that have swept the foodie world (farm-to-table, organic produce) are making inroads in the fashion universe as the environmental movement continues its rise and new technology produces new natural materials and recycled materials.

The textile industry is a large consumer of volatile chemicals and generator of pollutants. The processes that output the most pollutants within the textile industry include the coating, finishing, dyeing and printing processes.

SEACOLORS project has demonstrated the possibility to obtain different dyes from micro and macroalgae and validated the use of these natural dyes in dyeing and printing processes. However, presented technology could be very interesting in additional industrial sectors were dyes are currently used. For that reason, after the end of the project it will be very interesting to continue working in three areas: 1) Selection other algae to obtain new pigments; 2) Implementation the technology in textile industry; 3) Implementation in other kind of industries. AITEX has a lot of clients and contacts in textile industry, therefore the results of the project and the experience obtained will facilitate the introduction of the technology in each kind of company depending on their kind of product and requirements.

Moreover, this project will also contribute to the accomplishment of the environment Directives in order to develop a eco textiles and environment quality.
4. Administrative part

4.1 Description of the management system

4.1.1. Description and schematic presentation of working method and overview

SEACOLORS was composed by 5 types of actions: A (preparatory), B (implementation) which was the technical action and core of the project. C (monitoring of the impact of the project actions) was mandatory, and D (dissemination) and E (management), transversal actions covering the whole project.

Hereunder you can find a calendar where you can see that the project has been executed mainly according to the approved timetable, except for some small delays in finishing actions A and B, which didn’t affect the final results of the project:
### 4.1.2 Presentation of the coordinating beneficiary and associated partners

The **SEACOLORS** consortium was composed by 4 entities from Spain (3) and Portugal (1):

**AITEX** was the coordinating beneficiary, and is a Spanish non-profit making private association formed by textile and related companies. AITEX has a biotechnology research group with great experience in the application of new natural dyes in textile dyeing process. AITEX has been the coordinating beneficiary participating in several actions: A1, B3, C1, C2, D1, D2, D3, D4, E1, E2 and E4.

**ALGAplus** is a private Portuguese company that produces and trades seaweed and seaweed-derived products in an ecological and social sustainable way through continuous innovation and strict parameters of quality and traceability. Algaplus has been the responsible for the implementation of A1, B1 and B2 and has participated in several actions: C1, C2 and D1.

**The Spanish Bioindustry Association (ASEBIO)** brings together companies, associations, foundations, universities, research and technology centers that carry out activities directly or indirectly related to biotechnology in Spain. ASEBIO has been the responsible for implementation of D1 and has participated in D2, D4, E1, E2 and E3.

**Universidad de Las Palmas De Gran Canaria (BEA-ULPGC).** The Spanish Bank of Algae (BEA) is a university infrastructure that has got a wide experience on algae (macro- and microalgae) cultivation at laboratory and pilot-scale photobioreactors, tanks and raceways, under the frame of “Marine Agronomy” as an important agro-industrial sector for the development of the Canarian region. BEA-ULPGC has been responsible for the implementation of A1, B1 and B2 and has participated in C1, C2 and D1.

### 4.1.3. Project organization

For the project coordination, a Steering Committee consisting of one representative from each institution was appointed at the beginning of the project. For the project management it has been created a technical and financial team to carry out the necessary tasks within the different actions of the project. This team consists of individuals from each partner as follows: PM, responsible for the executive management of the project (Simona Moldovan, AITEX) and Technical and Financial team (José Antonio Santoja (supported by Eusebio Aranda), AITEX), responsible of the execution of the project, Juan Luis Gómez Pinchetti and Ana Peñuela, BEA; Helena Abreu and Rui Pereira ALGAplus; Marcela Ferrándiz, Elena Mira AITEX; Beatriz Palomo and Pilar Caro Chinchilla, ASEBIO).
Several differences may be observed with respect to the initial organigram included in the project proposal. The differences occurred due to changes in the research groups personnel. The proposed coordinator of SEACOLORS was Miriam Martinez, who due to the overcharge of work was replaced by the coordinator of Life projects in the Biotechnology research group, Luis Miguel Perez, who worked in the project until February 2016, when his laboral activity in AITEX ceased. The new responsible was Simona Moldovan. She had support in project management, coordination and dissemination actions by Rosa López, who replaced the initially proposed Korina Molla, due to her high level of implication in other projects. Marcela Ferrándiz was the Technical coordinator, and she had the support of Elena Mira in all technical tasks. These were the main responsible people but other staff members were involved in the development of the project.

<table>
<thead>
<tr>
<th>Date</th>
<th>Place</th>
<th>Assistants</th>
<th>Event</th>
</tr>
</thead>
</table>
| 08/07/2014 | AITEX facilities, Alcoy, Alicante, Spain | AITEX: Luis Miguel Pérez, Elena Mira y Esther Franco  
ULPGC: Juan Luis Gómez Pinchetti  
ASEBIO: Beatriz Palomo y Pilar Caro  
ALGAPLUS: Helena Abreu | Kick-off meeting |
| 27-28/01/2015 | ALGAplus facilities, Ílhavo, Portugal | AITEX: Luis Miguel Pérez y Elena Mira  
ULPGC: Juan Luis Gómez Pinchetti  
ASEBIO: Pilar Caro  
ALGAPLUS: Helena Abreu  
MONITORING TEAM: Filipa Ferrão | Second project meeting |
| 30/09/2015 | BEA-ULPGC facilities, Las Palmas de Gran Canaria, Spain | AITEX: Luis Miguel Pérez y Elena Mira  
ULPGC: Juan Luis Gómez Pinchetti, Tamara Rodríguez y Carlos Almeida  
ASEBIO: Pilar Caro y Beatriz Palomo Belbel  
ALGAPLUS: Helena Abreu y Andreia Rego | Third project meeting |
| 24-25/02/2016 | ASEBIO facilities, Madrid       | AITEX: Elena Mira, Simona Moldovan y Marcela Ferrándiz  
ULPGC: Juan Luis Gómez Pinchetti y Tamara Rodríguez  
ASEBIO: Pilar Caro y Beatriz Palomo Belbel  
ALGAPLUS: Helena Abreu  
MONITORING TEAM: Filipa Ferrão | Fourth project meeting |
| 14/12/2016 | ASEBIO facilities, Madrid       | AITEX: Simona Moldovan y Marcela Ferrándiz  
ULPGC: Tamara Rodríguez  
ASEBIO: Pilar Caro y Beatriz Palomo Belbel  
ALGAPLUS: Helena Abreu  
MONITORING TEAM: Filipa Ferrão | Fifth project meeting |

Table 1: Project meetings attendants

4.1.4. Changes on the Partnership and Grant Agreements

Since the beginning of the project, 2 reports were sent to the European Commission, which were accepted: the Inception Report (31/03/2015) and the Midterm Report (29/03/2016). Copies of PA, in pdf format, were delivered to the Commission with the Inception Report. Within the Mid-Term Report, was sent the Consortium agreement and meeting Calendar. Also, a Corrigendum to Cooperation Agreement was sent as requirement of the EC after Inception report review. Along with this Final Report is attached an updated Meeting
Calendar, containing the information pertaining to the 2016 meetings in Administrative Annex 1. E.1.1 Consortium Agreement and meeting calendar (updated).

Regarding the Grant Agreement, there were no formal changes to it.

4.1.5. Project coordination

An internal schedule was designed in order to monitor the progress of the executed tasks by the beneficiaries. All beneficiaries were involved in the organization of the 5 planned consortium meetings and Final Event. As in the project schedule, internal meetings were distributed between all partnership members and hosted in each of the beneficiaries’ facilities. Each meeting host was responsible for organisation and travel advice for attendees. Moreover, it is important to mention that phone and videoconferences were held in order to better control the progress of the report.

Regarding the organizational part, there are not significant changes in roles and functions of the beneficiaries (only some beneficiaries have internally included other personnel than foreseen in order to support the original team). This didn’t constitute a problem from any point of view, like deadline modifications of budget fluctuations, due to the fact that all changes were taken by the partner organizations. The changes and reasons for these have been explained and justified in the Comments on the Financial Report section.

4.2 Evaluation of the management system

The management system was established at the beginning of the project and approved by all partners. There were no major incidents, beyond unimportant delays or temporary deviations.

During the implementation of SEACOLORS foreseen activities there was a small delay in the development of action B.3., in the optimization of the dyeing process, which also affected the beginning of action C1. Regardless of this initial delay, both actions were finalized in the correct timetable. The rest of the actions were performed as initially planned. In spite of this fact, the project Work Plan has run successfully and no extensions were needed.

Regarding the process, all partners agreed on tasks division, each one focusing on those with more experience and potential. Thus, Aitex, BEA and ALGAplus focused on preparatory and implementation actions and in the monitoring of the project impact. ASEBIO focused on dissemination activities which are in line with their usual actions.

Communication with the Commission and Monitoring team.

Simona Moldovan (AITEX) as PM participated as intermediary with the EC Project Officers: Rafael Nievergelt, François Delceuillerie and Davide Messina (Technical Desk Officers) and Marija Simic (Financial Desk Officer) and the external monitor, Filipa Ferrão.

Regarding the Monitoring Team, the PM has maintained a close relationship with Filipa Ferrão by mail and phone. In addition to remote contacts, there were presentional meetings in the facilities of ALGAplus on January, 28th 2015 and in ASEBIO´s facilities on February, 25th 2016 and December, 14th 2016.
5. Technical part

5.1. Technical progress, per task

PREPARATORY ACTIONS:

Action A.1: STUDY AND SELECTION OF ALGAE WITH DYE CAPACITY.

(1st September 2014 to March 31st 2015)

The aim of this phase was to identify and select potential micro, macroalgae and cyanobacteria species and/or strains that can be used to obtain dye/pigment for textile industry. Since there has been no delay, the implementation period was completed successfully. This phase was subdivided in the next sub-actions:

A.1.1. STRAINS/SPECIES PROSPECTION AND SELECTION

The criteria to select microalgae and cyanobacteria were: (1) possibilities for being cultivated at pilot scale and consistent biomass production in a sustainable way; (2) pigments/dyes profile and accumulation characteristics (possible nutrient/light effects). On the other hand, the main criterion to select macroalgae was: the possibility to be cultivated under intensive and sustainable aquaculture conditions:

<table>
<thead>
<tr>
<th>Microalgae and cyanobacteria</th>
<th>Macroalgae</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Synechococcus sp.</td>
<td>• Grateloupi turuturu</td>
</tr>
<tr>
<td>• Erythrotrichia cf. carnea</td>
<td>• Gracilaria sp. (gracilis or vermiculophylla</td>
</tr>
<tr>
<td>• Arthrospira platensis</td>
<td>pending DNA confirmation)</td>
</tr>
<tr>
<td>• Leptolyngbya sp.</td>
<td>• Ulva rigida</td>
</tr>
<tr>
<td>• Nostoc cf. commune</td>
<td>• Porphyra dioica</td>
</tr>
<tr>
<td>• Sarcynochrysicy marina</td>
<td>• Porphyra umbilicalis</td>
</tr>
<tr>
<td>• Caesitella pascheri</td>
<td>• Bifurcaria bifurcata.</td>
</tr>
<tr>
<td>• Halochlorella rubescens</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. List of microalgae, cyanobacteria and macroalgae species/strains

Conclusion: Different Microalgae/ cyanobacteria (8 strain) and macroalgae (6 species) were selected as pigment/dyes sources to provide the three basic colours employed in dyeing. The expect result has been successfully achieved (at least 3 microalgae and 3 macroalgae), selecting more species than expected to ensure success.

A.1.2. BIOMASS PRODUCTION

The selected microalgal strains were scaled up (from the test tube to 5 L flasks) and biomass was produced with enriched medium, according previous results and recommendations by BEA. At this laboratory scale, the culture room was controlled in terms of light intensity (<100 μmol photons m⁻² s⁻¹), photoperiod (16:8 L:D), temperature (23 ± 2 °C), aeration and CO₂ pulse addition.
Data for growth characterization were obtained for the different strains in order to compare growth rates (day\(^{-1}\)), duplication rates (days) production yield (g algae dry weight L\(^{-1}\) day\(^{-1}\)) and main pigment/dye yield (g pigment L\(^{-1}\) day\(^{-1}\)). Strains for scale up and further considerations were selected from the obtained results. Logistic model curves for growth characterization were carried out for each strain (depending on the morphological characteristics) through: Optical density, Cell counts, Chlorophyll a, and Dry weight (DW) or ash free dry weight (AFDW).

Specific growth rates were calculated from the exponential growth phase; and filtration or centrifugation, depending of strain morphology, was used for biomass concentration and the obtained paste was frozen at -20 °C until pigment extraction. For further analysis, biomass samples were also freeze-dried.

ALGAplus was able to produce biomass for all the species listed in the Table 2 of Deliverable A.1.1. Microalgae and macroalgae selection for dye extraction (sent with the Midterm Report) except for Chondracanthus teedei and Dilsea carnosa (cultivation was unsuccessful and they are very rare in the wild).

From the species tested in lab culture or small tanks, the most promising results were obtained with *Porphyra dioica* (produced in 127L outdoor tanks), *Scinaia furcelatta, Codium tomentosum* and *Calliblepharis jubata* (tested in 500L tanks). For all the other tested species, either the growth rates were too low or contamination occurred making it impossible to consider these species to be grown in large scale for obtaining pigments for textiles. Nonetheless, as mentioned, samples were available for pigment extraction and some of the

---

**Table 3.** Laboratory controlled conditions for biomass production of microalgae

<table>
<thead>
<tr>
<th>Controller parameter</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture media</td>
<td>(1) BG11; (2) F/2</td>
</tr>
<tr>
<td>Light intensity</td>
<td>&lt; 100 μmol photons m(^{2}) s(^{-1})</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>16:8 L:D</td>
</tr>
<tr>
<td>Temperature</td>
<td>23 ± 2 ºC</td>
</tr>
<tr>
<td>Aeration</td>
<td>Yes</td>
</tr>
<tr>
<td>CO(_2) pulse adition</td>
<td>1 min per hour</td>
</tr>
</tbody>
</table>

---

**Figure 6.** An example of logistic growth curve obtained for cultures of BEA 0007 *Arthrospira platensis* under laboratory-controlled conditions. From these data, growth parameters were calculated.

**Figure 7.** General aspect of the culture chamber for strains growth under laboratory conditions at BEA-ULPGC.
species tested in culture can be wild harvested in the Atlantic shores due to their high abundance: *Bifurcaria bifurcata* and *Stypocaulon scoparia*.

In terms of the species grown normally at ALGAplus (*Ulva rigida*, *Gracilaria sp.*), biomass is readily available and milled samples were sent for testing to AITEX.

**Conclusion:** Different microalgae (Action A1.1) strains (including cyanobacteria) and macroalgae species were obtained, mainly under different cultivation conditions and scales, and confirmed as pigment/dyes producers for the textile industry.

Action A.1.2 was accomplished with several species of micro and macroalgae being produced in a scale suited for pigments extraction.

It has been achieved the biomass production at lab scale of 100% of the tested algae as expected.

**A1.3. PIGMENT CHARACTERIZATION**

Standard methods for pigment extraction and quantification from the obtained biomass were used for microalgae/cyanobacteria pigments/dyes characterization. Extractions were carried out on fresh material under liquid nitrogen using aqueous buffers (phosphate; distilled water) for phycobiliproteins (phycoerythrin and phycocyanin; red and blue dyes) and organic solvents (acetone, methanol) for xanthophylls and carotenoids (orange and yellow dyes).

The quantification was carried out with spectrophotometric analysis through equations already described. Purity criteria of the pigments/dyes obtained were used for a complete characterization of the extracts.

**Pigments extraction and characterization**

Development and characterization of the pigments identified in sub-action A.1.3 were mainly focused on the extraction of phycobiliproteins (phycoerythrin (red) and phycocyanin (blue)) and total carotenoids (carotenes (orange) and xanthophylls (yellow)) pigments/dyes obtained from the assayed microalgae and cyanobacteria strains, according to strains growth rhythm and biomass availability.

Concentrated (by filtration or centrifugation methods) fresh algal material was used for extraction procedures, although freeze-dried material was also used for methodological comparisons. Pigments were extracted after biomass had been broken by freeze (-20 ºC) and defreeze, and/or sonication, depending on the morphological characteristics of the strains assayed (unicellular or filament forming; no cell wall vs strong cell wall). Extracts were obtained in distilled water. In the case of cyanobacteria, after centrifugation for extracts clarification, phycobiliproteins were determined by spectrophotometric methods.

For carotenoids, procedures were developed using organic solvents and concentration by drying (and solvent recovery). Chlorophylls and total carotenoids were extracted using methanol as solvent. After clarification, pigment were quantified (µg ml⁻¹) using corresponding equations.
“Waste” biomass remaining after the extraction/centrifugation process was collected and frozen (-20 °C) for further metabolites extraction and reuse, according to Action C.1; Sub-Action C.1.3 – “Study of reuse of biomass generated during the extraction of novel dyes”. This biomass remaining after extraction from micro- and macroalgae was be evaluated and characterized for other substances of interest (other pigments, polysaccharides, lipids, antioxidants or feed complement), according to the “biorefinery” concept.

**Selected microalgae strains for new developments**

According to the obtained results which were presented in detail in *Deliverable A.1.3. Microalgae and macroalgae pigment characterization* (attached to the Midterm Report), new developments were carried out with these selected microalgae strains:

- For red pigment/dye phycoerythrin: REC 0057 *Synechococcus sp*; BEA 0620 *Erythrotrichia cf. carnea* (an original-new bio-prospected strain obtained at the BEA collection, and its taxonomical assignment recently obtained by molecular characterization)
- For blue pigment/dye phycocyanin: BEA 0007 *Arthrospira platensis*; BEA 0946 *Leptolyngbia sp.*
- For orange/yellow pigments/dyes carotenoids: BEA 0069 *Halochlorella rubescens*; BEA 0313 *Sarcynochrysis marina*

Three other strains (BEA0024 - Nostoc cf. commune, BEA0937- Euglena cantabrica and BEA0858 – Anabaena sp.) were also evaluated in posterior developments by considering possibilities for pigment yield improvements.

On the ALGAplus hand, aqueous extracts from *Gracilaria* (2 conditions) and *Ulva* currently in production at ALGAplus were produced in early December 2014 (image below) following the protocol suggested by BEA. All of the extracts were characterized in collaboration with BEA. The residues from extraction were kept frozen at -20°C for future analysis and possible valorization.
In addition to the foregoing, aqueous extracts of the brown algae *Fucus vesiculosus*, *Bifurcariia bifurcata*, *Stypocaulon scoparia* and the red algae *Osmundea pinnatifida*, *Grateloupia turuturu*, *P.Dioica* were produced in February 2015 according to the protocol used by BEA. These were characterized and residues were kept frozen. The results obtained showed that *Gracilaria vermiculophylla*, *Grateloupia turuturu* and *Porphyra* spp. are the best species for red pigment (phycoerythrin). For the chlorophylls (green pigments) *Ulva rigida* and *Codium tomentosum* were chosen, and for the carotenoids (orange/yellow pigment) the brown alga *Fucus vesiculosus* was chosen.

Conclusion: Pigments/dyes belonging to three main groups: chlorophylls, carotenoids and phycobiliproteins were extracted and characterized from the selected algae species/strains as green, yellow, orange, blue and red colours sources.

A1.3. validated the hypothesis of extracting green (chlorophylls), red (phycoerythrin) and yellow (carotenoids) pigments from micro and macroalgae. And it has been achieved to identify the pigment extract of at least 75% of the algae tested as expected.

On the other hand, one tricomy of macroalgae and one of microalgae/cyanobacteria have the right properties to be used in the textile industry.

**A.1.4. METHODOLOGY EMPLOYED TO DYE WITH THE SELECTED DYSES**

AITEX developed a Bibliographic study on the technologies and methodologies used in the textile sector for dyeing processes. The result of this work is summarized in the Deliverable A.1.4. Ancient dyeing process and auxiliaries employed (attached to the Inception Report).

In this study were identified different auxiliary products necessary in the dyeing process with natural dyes. The main auxiliary products are mordants. Mordants are salts (cooper, aluminium, iron) or tannins used in dyeing to fix the dye to the surface of the fabric.

On the other hand this study has allowed defining different steps to apply these mordants in the dyeing process in Action B.3: Before, during and after dyeing.

Conclusion: This action generated a knowledge base on ancient dyeing process with natural dyes, which helped as starting point in the development of action B3. Dyeing process.

It has been defined the method to dye wool and cotton as expected and, it has been included the printing method for the benefits that it brings.

**Action conclusion:**

As general conclusion for action A.1., the microalgae, cyanobacteria and macroalgae strains suitable for further optimized cultivation and extraction processes were selected, based on different criteria related to growth possibilities, pigment content and characteristics and potential for textile dyeing processes. These extracts were validated in further dyeing processes.
At the end of this action 8 microalgae (including cyanobacteria) strains and 6 macroalgae species were selected. The selected algae, which were cultivated at laboratory scale, were then scaled-up to pilot scale, in both responsible partner’s facilities, BEA and ALGAplus. The final strain selection was also based on preliminary pigment characterization including chlorophylls, phycobiliproteins and carotenoids as target pigment groups. The starting point in the development of a suitable dyeing process involving natural colorants was studied in this action.

**Deliverables**

A more detailed description of this Action can be found in:

- **D.A.1.1. Microalgae and macroalgae selection for dye extraction (updated)** delivered within the Midterm Report.
- **D.A.1.2. Microalgae and macroalgae biomass production at lab scale (updated)** delivered within the Midterm Report.
- **D.A.1.3. Microalgae and macroalgae pigment characterization (updated)** delivered within the Midterm Report.
- **D.A.1.4. Ancient dyeing process and auxiliaries employed** delivered within the Inception Report.
IMPLEMENTATION ACTIONS

**Action B.1. STUDY OF GROWING CONDITIONS OF ALGAE TO INCREASE DYSES SUBSTANCE**

*(January 2015 to March 2016)*

The aim of this action was the biomass production at pilot scale and in an IMTA (Integrated Multi-Trophic Aquaculture) cultivation unit, in optimized conditions to obtain high yields of biomass productivity and high content of the metabolite of interest.

Microalgae/cyanobacteria and macroalgae selected as interesting candidates for pigments/dyes preparation per lab scale growth performance, pigment profiles and interest for the dye/textile industry (Action A1), were cultivated intensively at pilot plant scale to evaluate:

- Sustainable growth, biomass and pigments/dyes yield to allow industrial developments.
- The effect of growth conditions on biomass yields and pigments/dyes composition.
- The possibility to stimulate pigment/dyes accumulation and yields by controlling culture parameters as medium composition (type and availability of nutrients), light conditions (quality (e.g. UV) and quantity), stocking density or growth stage for pigment accumulation (exponential vs. stationary).
- Comparative possibilities for microalgae and macroalgae as a sustainable source of pigments/dyes for the textile industry: compositions, yields and production costs.

This action was divided in two different tasks: microalgae/cyanobacteria and macroalgae production. The objectives were achieved successfully; BEA-ULPGC (B.1.1) and ALGAPlus (B.1.2) developed and optimized methods to grow micro and macroalgae with high pigment contents.

**B.1.1. MICROALGAE/CYANOBACTERIA BIOMASS PRODUCTION AT A PILOT SCALE**

From the microalgae/cyanobacteria list described in Action A1, some of the strains presenting interesting performances at laboratory scale were monitored for pilot scale possibilities. Microalgae adaptation to natural conditions was carried under greenhouse natural conditions (light, photoperiod, temperature). Under these conditions pumps supply aeration and air-mixed pulses supply CO₂ regularly. Strains were grown at a pilot scale in raceways tanks (8 m²) and photobioreactors (from 5 to 80 L) to increase biomass production for different dyes production. Fresh biomass and target pigments/dyes were obtained in a regular basis according to the methodology discussed and regularly sent to AITEX for testing (considered as indicator of progress).

One of the main goals of algal biomass production is to find new algal strains/species capable of growing in intensive, open or closed production systems, yielding high biomass productivity and high content of the metabolite of interest. In general, lab pilot observations cannot be extrapolated to outdoor conditions because the physiological behaviour is strain-dependent, and when algae are subjected to natural external conditions such as natural light (including UV-radiation) and/or temperature, their physiology and growth performance may
change at different degrees, influencing in example the accumulation of carotenoids playing a role as photo-protectors in many species of macro- and microalgae, particularly during the stationary phase (growth stage). Other physicochemical parameters that can be easily manipulated might have a strong influence on biomass growth and metabolites content. The role of growth medium composition has been well recognized as significant by influencing growth rate, product yield and biochemical composition of many macro- and microalgae. Among numerous macro- and microelements, some are limiting factors for algal growth, whereas others are involved in many reactions for the biosynthesis of many compounds including photosynthetic pigments. A clear example of this fact is the importance of nitrogen on the biosynthesis and accumulation of phycobiliproteins in red macroalgae and cyanobacteria.

Figure 12. Simplified scheme of the pigment biosynthesis pathways of cyanobacteria and microalgae (according to the review by Mulders et al., 2014, Journal of Phycology 50:229) and relationships with nutrient availability (N-ammonia for phycobiliproteins) and stressful conditions (for carotenoids) as controlling factors.

As an example of this approach, previous developments for biomass production of the cyanobacteria *Spirulina maxima* (blue-green algae), with the objective to evaluate phycobiliproteins (c-phycocyanin) purification for biomedical applications were performed at BEA. *Spirulina* biomass was produced in raceways at pilot scale. By controlling growth conditions related to phycobiliproteins accumulation (effect of high nitrogen concentration on medium composition) at the different phases (exponential vs. stationary), cells were harvested by filtration and centrifugation previously to pigment extraction. Biomass yields at such scale varied between 100 and 500 g FW l⁻¹ per cycle. Raw pigment contents (non-purified) were higher than 9.5 mg g⁻¹ FW for c-phycocyanin, representing percentages higher than 40% of the total biomass protein content.

From the list of microalgae/cyanobacteria strains of Action A.1., different approaches were considered to perform the scale up under natural conditions. BEA0007 *Arthrospira platensis* was continuously produced in 8 m² raceways. Biomass harvest was performed regularly by filtering. Biomass obtained was frozen, extracted or freeze dried for further analysis. Experiments, in 3 raceways available, on growth performance under different nitrogen, as nitrate, concentration conditions were carried out to demonstrate phycobiliproteins (phycocyanin) accumulation, both under laboratory/outdoor greenhouse conditions.

Other microalgae and cyanobacteria strains were grown in a low-cost experimental system that holds polycarbonate- recycled 5-8 L water bottles in a vertical light-oriented panel before being scaled up to 80 L tubular columns. Control of variables (nutrients composition of the medium, effect of natural radiation including UV and growth phase) was considered to study pigments content and accumulation characteristics of the biomass produced. Results obtained were compared to those obtained for *Spirulina maxima*.
Figure 13. Pilot scale cultures under natural conditions have been established. (A) Raceways (3x8 m²) have been used for BEA0007 *Arthrospira platensis* biomass production and pigment accumulation demonstration; (B) Low-cost experimental system that holds polycarbonate- recycled 5-8 L water bottles in a vertical light-oriented panel (cultures in triplicate are BEA0946 *Leptolyngbia* sp. (blue-green) and REC0057 *Synechococcus* sp. (red).

Conclusion: Pigment content could be easily modified and increased for most of the species (8 species) included in this project by means of simple modifications and control of culture conditions.

The results obtained confirm that:

- biosynthesis of the different groups of microalgae/cyanobacteria pigments can be stimulated by controlling algae growth conditions under cultivation.

- increase a 25% the biosynthesis of the microalgae/cyanobacteria pigments modifying different parameters during their growing (Temperature, pH, UV light, nutrients...).

- obtain biomass in a sustainable way at a pilot scale.

All the information is reflected in Technical Annex 1 D.B.1.1. Microalgae/ Cyanobacteria production at pilot scale and is delivered within this final report.

**B.1.2. MACROALGAE PRODUCTION IN AN IMTA CULTIVATION UNIT**

Activities carried out in Sub-actions A.1.1 and A.1.2 provided the results to this deliverable. Macroalgae species selection was based on several criteria: 1) the obligation of having a representative of each phylum (Rhodophyta, Chlorophyta and Ochrophyta), 2) their current existence in the Atlantic coast (native or exotic), 3) the known ability of the species to grow by vegetative propagation and/or with known life cycle = easiness to upscale. This selection produced a list of species described in the table below. The list was divided into 1) species which cultivation potential needed to be tested first in the laboratory and/or the small tanks, 2) species already in large-scale culture in the land-based tanks and 3) native and exotic species that exist in abundance in the Portuguese or the Atlantic coasts. Some of these we decided to also tested them in culture due to their marked seasonality (e.g. Bifurcaria bifurcata). The selected macroalgae species were produced in IMTA conditions at the R&D area of ALGApplus, using tanks of 127L, 230L and 500L. Assays controlling nutrients input and light conditions by manipulating stocking densities and water renewal were performed with the purpose of increasing pigment/dye substances while maintaining high biomass yields of the selected macroalgae species (see table below). General conditions of the cultivation stress trials:

- Time: 3 weeks
- Production factors: Stocking density and Water flow: Nitrates (average 10.4umol/l) Amonia (average 14.5umol/l)

  Selected species: Gracilaria sp.; Ulva rigida; Bifurcaria bifurcata; Grateloupia turuturu; Porphyra dioica (conchocelis).
Figure 14. *Porphyra dioica*. From the left: Conchocelis (vegetative, nursery conditions); Young blades ready to go out; Blades in 127L outdoor tanks.

Figure 15. *Ulva rigida* (20,000L tanks) and *Gracilaria* sp. (7,000L tanks) in land-based cultivation at ALGAplus.

Figure 16. Final result after the assay with *Grateloupia turuturu* with a clear difference in pigment content due to cultivation conditions.

Table 4. Results on the growth performance (mean values) of the different seaweed assayed

<table>
<thead>
<tr>
<th>Species</th>
<th>Target pigment/dye</th>
<th>Growth rate (week⁻¹)</th>
<th>Biomass Yield (g DW m⁻² week⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gracilaria</em> sp.</td>
<td>PE (red)</td>
<td>1,4</td>
<td>2</td>
</tr>
<tr>
<td><em>Ulva rigida</em></td>
<td>Carotenoids/chlorophyll (orange/green)</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td><em>Porphyra dioica</em></td>
<td>PE (red)</td>
<td>9,6</td>
<td>15</td>
</tr>
<tr>
<td><em>Bifurcaria bifurcata</em></td>
<td>Carotenoids (orange)</td>
<td>0,4</td>
<td>7 g DW L⁻¹ week⁻¹</td>
</tr>
<tr>
<td><em>Grateloupia turuturu</em></td>
<td>PE (red)</td>
<td>3</td>
<td>2,3</td>
</tr>
</tbody>
</table>

Conclusion: Production of macroalgae biomass (6 species) with improved pigment yield.

The results obtained confirm that:

- biosynthesis of the different groups of macroalgae pigments can be stimulated by controlling algae growth conditions under cultivation.

- increase a 25% the biosynthesis of the microalgae/cyanobacteria pigments modifying different parameters during their growing (Temperature, pH, UV light, nutrients...).

- obtain biomass in a sustainable way at a pilot scale.

All the information is reflected in *Technical Annex 2. D.B.1.2. Report on macroalgae production in an IMTA system* and is delivered within this Final Report.

Action conclusion:

As general conclusions for Action B.1., pigment contents for both micro- and macroalgae could be easily modified and consistently increased by means of modifications and control of culture conditions. As some of the general results show:

- N availability and concentration in the culture medium and the growth phase at which biomass is harvested (i.e., time allowed to grow) are feasible factors in order to satisfactorily increase pigment content for the strains studied.
• Phycobiliproteins concentration in crude extracts can be significantly increased by adding 2-fold nitrate concentration to culture medium and harvesting biomass at the exponential growth phase.

• Carotenoids concentration in crude extracts can be significantly increased by allowing batch cultures to grow until the stationary phase of growth, meaning that cells are under N starvation and hence they are growing under stressful conditions.

• For ALGAplus this was due to the objective of extracting the pigments immediately after the algae biomass production; this prevented pigment degradation but caused some delays in the cultivation trials.

This action was extended for 3 months and finished at the end of March 2016.

**Deliverables**

- **D.B.1.1. Microalgae/ Cyanobacteria production at pilot scale. (Technical Annex 1)**
Action B2: EXTRACTION OF DYES FROM ALGAE

(January 2015 to December 2016)

The objective of this action was the implementation of conventional extraction techniques and check their feasibility with algae. The implementation involved parameter optimization to obtain the best possible yields in each one of the selected techniques.

According to methodology and results obtained in Action A.1, once BEA-ULPGC and ALGAPlus produced biomass from microalgae, cyanobacteria and macroalgae in a sustainable way (Action B.1), some methods had to be improved to optimize dye extraction (yields and final dye quality). This action was discussed by partners and a short technical meeting was celebrated at BEA-ULPGC in May 25-27, 2015, to establish standard protocols and methodologies to get results and conclusions feasible for algal dyeing techniques developed by AITEX. At the end of the action lab scale extractions and extractions of the outdoor trials were finalized. Different methods of extractions including different solvents (water, ethanol and sodium phosphate buffer) were used per type of algae/pigment of interest.

This action was scheduled to start in the second quarter of 2015, but finally it started before because several micro and macroalgae were produced in B1, and it was necessary to extract the dyes to check their properties and propose their use in dyeing processes.

In these sub actions (B.2.1, B.2.2.), more tests than initially planned were performed because more micro/macroalgae were studied due to their dyeing potential, therefore in this action there are more personal cost that initially planned but this modification did not affect the objectives of the project, and these efforts produced more reproducibility and transferability in the dyes/pigments obtained.

During 2015 this action was implemented in parallel with action B3, because the dyes extracted were tested in B3 in different textile processes and also, it is necessary to mention that this increase in the number of dyes samples in B2 did not generate a greater effort in B3.

It is important to mention that, even though the action B1 finished, it was necessary that BEA and ALGAPlus continued producing algae biomass, which was mandatory for the extraction process, which represents the objective of action B2.

B.2.1. DYES EXTRACTION

To carry out improvements on the pigments/dyes extraction protocols from microalgae and cyanobacteria, some parameters had to be optimized to increase pigment yields and extracts quality. Although it has been considered that obtained dyes should not be refined and purified, it was considered as necessary to increase its purity in terms of colour, because in higher concentrations, less dye is used, reducing cost of transport and storage. So, for the production of red, blue and orange/yellow dyes from microalgae/cyanobacteria strains that fill standards established by AITEX, at BEA-ULPGC some improvements were developed by controlling: Growth phase of the biomass produced (exponential vs. stationary); Disruption methods; Number of extraction cycles; Solvents; Extraction periods and Clarification of the extracts (filtration or centrifugation).

This was particularly important for carotenoids extraction, specifically for each strain depending on the main target pigment (carotene or xantophylls), fresh or dried biomass, the
best solvent or solvent mix, disruption method, optimal extraction time in order to obtain the highest yield and purity of extracts. Techniques for separation and characterization of carotenoids extracts, such as standard Thin Layer Chromatography (TLC), were considered to qualitatively identify the different extracts.

For macroalgae, the pigment extraction was done by ALGAplus in close collaboration (interaction of personnel) with the Chemistry Department of the University of Aveiro due to their better-equipped facilities and knowledge on alternative extraction methods (namely, using ionic liquids as solvents or sequenced extraction processes). As previously described, the extractions were mostly done with fresh and frozen seaweed kept at -20°C (exception with dried seaweed powder described below), that were grinded and mixed with specific solvents. Below, the general description of the methods considered for pigment extraction, quite common to what was done at the other partners:

![Diagram of extraction process](image)

The major improvement in terms of the extraction step (non-toxic solvents and faster) was achieved for obtaining green and yellow pigments from macroalgae, in a single step process (figure below).

![Figure 19. Final result of the extraction of B. bifurcata: separation between carotenoids (yellow) and chlorophylls (green).](image)

Problems encountered: On the ALGAplus side, the major problems involved the application of the different protocols of extraction as well as synchronizing biomass production and extraction to keep with the expected timings. On the AITEX side, the lyophilized pigments
received from ALGAplus and BEA, did not respond in the most efficient way when extracted with the same methods that were applied to the fresh biomass.

**Conclusion: This action major outcomes were:**

1) dye extraction protocols for each micro/macroalgae/cyanobacteria and target pigments as expected.
2) obtaining red, yellow and blue dyes as expected.

### B.2.2. DYES CHARACTERIZATION

This activity was focused on the characterization of pigments obtained from the selected microalgal/cyanobacteria and macroalgae species, represented by phycobiliproteins, carotenoids and chlorophylls. The objective was to compare procedures for stable dyes production in order to control processes variables. Dyes characteristics were obtained through composition, chromatography, colorimetry and stability assays.

Different final products from macroalgae, microalgae and cyanobacteria were produced and delivered, as it can be observed the lyophilized different shades of red, blue and yellow/orange pigments, in the lower figure.

The characterization of the extracts obtained from optimized cultivation protocols were analysed separately using absorption spectrophotometry, a simple and fast method largely used for quantitative analysis of different kinds of compounds. The tables below summarize the concentrations obtained for the different macroalgae species under specific cultivation conditions (details in Technical Annex 4. D.B.2.2. Report of results of dye characterization)

<table>
<thead>
<tr>
<th>Algae species</th>
<th>Pigment</th>
<th>Production Conditions</th>
<th>Concentration (mg pigment/gram of algae)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gracilaria gracilis</em></td>
<td>RED</td>
<td>Low density/High nutrient input</td>
<td>0.333±0.029</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low density/Low nutrient input</td>
<td>0.118±0.026</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High density/High nutrient input</td>
<td>0.468±0.132</td>
</tr>
<tr>
<td></td>
<td>Phycobiliproteins (mainly phycoerythrin)</td>
<td>Low density/Low nutrient input</td>
<td>0.210±0.071</td>
</tr>
<tr>
<td><em>Grateloupia turuturu</em></td>
<td></td>
<td>Low nutrient input</td>
<td>0.022±0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High nutrient input</td>
<td>0.120±0.026</td>
</tr>
<tr>
<td><em>Porphyra dioica (conchocelis)</em></td>
<td></td>
<td>Low nutrient input</td>
<td>0.011±0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High nutrient input</td>
<td>0.034±0.005</td>
</tr>
<tr>
<td><em>Porphyra dioica (blades)</em></td>
<td></td>
<td>Stable nutrient input (commercial conditions at ALGAplus)</td>
<td>0.367±0.000</td>
</tr>
<tr>
<td><em>Bifurcaria bifurcata</em></td>
<td>YELLOW</td>
<td>Low nutrient input</td>
<td>0.002±0.000</td>
</tr>
<tr>
<td></td>
<td>Carotenoids</td>
<td>High nutrient input</td>
<td>0.111±0.045</td>
</tr>
<tr>
<td><em>Ulva rigida</em></td>
<td>GREEN</td>
<td>Low nutrient input</td>
<td>0.304±0.034</td>
</tr>
<tr>
<td></td>
<td>Chlorophylls</td>
<td>High nutrient input</td>
<td>0.383±0.036</td>
</tr>
</tbody>
</table>

Table 5. List of concentrations obtained for the different macroalgae species

Target pigment and main concentration characteristics for microalgae/cyanobacteria strains (C: Cyanobacteria; E: Eukariotic microalgae).
ALGAplus and BEA samples were sent to AITEX, and an UV Spectrophotometric analysis was needed to acquire the qualitative and quantitative assurance of the extraction.

As it was introduced in the subaction A.1.3, after centrifugation, a good separation between the biomass and the supernatant was done, for extracts clarification, phycobiliproteins are determined by spectrophotometric methods according the equations for cyanobacteria by Bryant (1982). In case of not getting good separation with the centrifuge, a vacuum filter system was used, to obtain a ‘clean’ supernatant, with no solid suspensions. The different extracts obtained were characterized by spectrophotometric methods both for concentration and spectra determinations. Examples of main phycobiliproteins and carotenoids spectra are showed in the next figure.

In general, carotenoids characterization is difficult and inaccurate because of the interferences with other compounds that can absorb in the same wavelength such as chlorophylls or other carotenoids. Even the determination of the total content of carotenoids is complicated since the extraction procedure with methanol only quantifies the polar compounds soluble in it, whereas the nonpolar compounds are not quantified. HPLC determination and characterization of carotenoids was carried out in collaboration with scientist at the Chemistry Department at ULPGC. However it was extremely complicated due to the complex amount of compounds identified in the samples. Purification processes helped to identify the different carotenoids and the subproducts that can be formed for their possible degradation and transformation with other molecules present in the sample. Furthermore, in AITEX different quantification procedures were employed in order to achieve a complete characterization of the obtained dyes extracts, as:

- Liquid chromatography-mass spectrometry (LC-MS), in order to achieve a selective analysis and characterization of carotenoids and chlorophylls.
The following figure shows the procedure phases employed for the precise concentration determination and extract characterization. Firstly the calibration curve must be realized and then synchronized with the model chromatogram for the pigment at the maximum absorption wavelength, which were finally compared with the resulting chromatogram from LC-MS.

![Calibration curve and Model chromatogram](image)

**Figure 5.** Standard calibration curve (left) and chromatogram (right) for CHLOROPHYLL B

The results obtained are quantified in the following table:

<table>
<thead>
<tr>
<th>Algae strain</th>
<th>Pigment</th>
<th>Concentration (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cystoseira tamarascifolia</em></td>
<td>Chlorophyll B</td>
<td>0.010-0.051</td>
</tr>
<tr>
<td></td>
<td>Chlorophyll A</td>
<td>0.030-0.032</td>
</tr>
<tr>
<td></td>
<td>Beta-carotene</td>
<td>0.062-0.113</td>
</tr>
<tr>
<td></td>
<td>Alpha-carotene</td>
<td>nc*</td>
</tr>
</tbody>
</table>

Table 7. Pigment concentration in the algae strain *Cystoseira tamarascifolia*.

- Thermogravimetric analysis (TGA) for the determination of the thermal behaviour and degradation limits, in term of temperature, for the obtained dyes/pigments.

In order to be able to optimize a general dyeing process, temperature limits for the newly obtained pigments in SEACOLORS were vital, so a TGA analysis was realized. A specific chart resulted from this analysis and revealed the maximum allowed temperature to be applied to the algal pigments.

The results obtained from the TGA analysis reveal a temperature range between 97.7-100°C as highly denaturising for the algal extracts.

- Differential scanning calorimetry (DSC), through which it was determined the heat capacity of the mentioned extracts.

The most accurate limit, where the weight loss of the pigment was also determined was realized with DSC analysis, and the obtained results were presented in the following form:
From this analysis resulted that the denaturation of the pigments starts at approximately 60°C, temperature which could be applied to all the employed algal colorants.

It has to be mentioned that during the whole project in parallel with the decided final 8 strains of microalgae and 5 strains of macroalgae, some other strains were tested, for the case of new potential sources of raw material for industries.

**Conclusion:** At the end of this action three groups of pigment/dyes were characterized and three primary colours (blue, yellow and red) were validated as suitable for further dyeing and printing processes.

From each type of microalgae have been impossible to obtain three colours (blue, yellow and red) as expected, but the different species studied allow obtaining the range of the three colours expected.

These dyes obtained are enough quality to be employed on textile industry.

---

**Action conclusion**

As general conclusions for Action B.2., different methodologies were applied to obtain and evaluate 3 types of pigment extracts from the previously selected strains (8 microalgae and cyanobacteria, and 6 seaweed species) according to physico-chemical characteristics of the biomass and the dyes to be extracted. Extracts were used in further dyeing processes.

The main difference in the extraction of pigments between micro- and macroalgae is that in the case of microalgae, the cells show relatively sensitive walls, which normally does not imply the utilization of very aggressive methods to break them and release the pigments of interest. However, in the case of macroalgae, the cellular wall is stronger, implying the utilization of very aggressive methods.

For microalgae the raw biomass was freezed (-20°C) and thawed (4°C, in the dark) for phycobiliproteins and freeze-dried for carotenoids extracts. Mechanical cell disruption methods were applied in order to break cell walls, allowing extracting pigments into the specific solvent. Supernatant are recovered and concentrated, and finally stored within the fridge (4°C and dark) until used for dyeing and printing tests.

The diverse physico-chemical properties of the different types of pigments, together with the variances in morphology, structural complexity as well as pigment composition for the different microalgae and cyanobacteria strains assayed in this project, makes the search for a standard protocol a difficult task. Slight variations of the standard method were required for some of the strains assayed.

For both micro- and macroalgae, phycobiliproteins are easily extracted using aqueous, cheaper solvents, while carotenoid extractions requires much more complex procedures, including organic solvents.

This action confirmed the possibility of manipulating macroalgae cultivation conditions to induce a higher accumulation of target pigments. As expected, a higher nutrient input clearly has a positive effect in the accumulation of pigments, namely the red phycobiliproteins. This confirms the potential of algae grown in IMTA systems as raw material for pigments extraction.

**Deliverables**
• **D.B.2.2. Report of results of dye characterization (Technical Annex 4)**

**Action B3: VALIDATION AND DEMONSTRATION OF THE APPLICATION OF ALGAE DYES IN TEXTILE DYEING PROCESS.**

*(March 2015 to December 2016)*

The main aim of this action was the evaluation of the viability of the employment of the newly obtained algal colorants in new dyeing and printing processes, through fabric characterization in terms of reproducibility, difference of colour obtained depending on the employment of different batches and every parameter considered relevant.

According to the dyes/pigments obtained during Action B.2, AITEX was studying their application in textile processes: dyeing and printing.

This action started ahead of scheduled due to the need to validate the dyes/pigments obtained. The most important part was performed during 2016, due to the successful identification of the micro/macroalgae and cyanobacteria, study of the dye contents and identification of the methodology for extraction and defined the dyeing/printing processes. All the obtained pigments were tested in all processes, as seen in the following table.

<table>
<thead>
<tr>
<th>Microalgae/cyanobacteria pigments</th>
<th>Color</th>
<th>Macroalgae pigments</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Synechococcus sp.</em></td>
<td>Red</td>
<td><em>Gracilaria sp.</em></td>
<td>Red</td>
</tr>
<tr>
<td><em>Erythrotrichia sp.</em></td>
<td>Red</td>
<td><em>Grateloupia turturu</em></td>
<td>Red</td>
</tr>
<tr>
<td><em>Arthospira platensis</em></td>
<td>Blue</td>
<td><em>Porphyra dioica</em></td>
<td>Red</td>
</tr>
<tr>
<td><em>Leptolyngbya sp.</em></td>
<td>Blue</td>
<td><em>Osmundea sp.</em></td>
<td>Red</td>
</tr>
<tr>
<td><em>Nostoc sp.</em></td>
<td>Red</td>
<td><em>Ulva rigida</em></td>
<td>Green</td>
</tr>
<tr>
<td><em>Halochlorella rubescens</em></td>
<td>Orange</td>
<td><em>Bifurcaria bifurcata</em></td>
<td>Yellow</td>
</tr>
<tr>
<td><em>Sarcinochrysis marina</em></td>
<td>Yellow</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Caespitella pascheri</em></td>
<td>Yellow</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table 8. Pigments used in the dyeing/printing process experiments*

After the process definition, the optimization and validation of the dyeing and printing was realized and completed successfully. The mentioned changes in the action´s timetable didn´t affect the final project´s objectives.

**B.3.1. DYEING PROCESS**

The dyeing tests consisted of a laboratory simulation of the industrial-scale process so the method used, has been by exhaustion, because is the most common industrially used. In these processes, the textile remains in contact with the treatment bath previously prepared throughout the duration of the process and under conditions specified in the corresponding curve of treatment. Time runs and the reagent transfers from the bath to the fibre.

In order to perform a good transfer of the dye to the fabric it was necessary, in some cases, a specific pretreatment of the fabric, the mordanting process, which can be done before, during and after the dyeing process to all types of fabrics. After testing several natural mordants in all the previously mentioned phases, it was concluded that the most suitable application was of the mordant CREAM OF TARTAR in a 6% with respect to the fabric weight.
Mordanting a fabric refers to its treatment with a chemical which will increase the fabric’s adsorption and absorption of the dye molecules. In the dyeing process of this project were tested 3 methods of mordanting, alone or combined between each other.

<table>
<thead>
<tr>
<th>Tested mordants</th>
<th>Applied concentration</th>
<th>Application process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alum</td>
<td>20%</td>
<td>T=85ºC t=45min</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>Cream of tartar</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Tannic acid</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Alum + cream of tartar</td>
<td>20%+6%</td>
<td></td>
</tr>
</tbody>
</table>

Table 9. Fabric pretreatment

In the specific case of wool, it is necessary to perform, before mordanting, the bleaching pretreatment of the fabric, in order to remove natural colouring matters and add-ons during the previous state of manufacturing. The process involved medium temperatures, 55ºC for 60 minutes, and the addition of a small quantity of hydrogen peroxide.

The dyeing process follows the dyeing curve specific for each fibre, with specific times, temperatures and bath ratios. In order to establish of good level of exhaustion of dye to the fibre it was necessary the addition of specific chemicals. The optimization process was realized by modifying, phase by phase, several parameters, from which the main ones were time and temperature, as it can be observed below:

![Figure 8. Optimization of the dyeing process](image)

As the process was optimized it generated a universal dyeing curve for both substrates employed in the finishing processes, which is hereby presented:

![Figure 9. General dyeing curve with natural algal pigments](image)

By applying this curve to all the extract obtained in the SEACOLORS project, on cotton and wool substrates, it was able to validate the application of the natural pigments originating from algae on two natural fabrics. The results are exemplified below.
Apart of the dyeing process, due to the nature of the pigment, the printing technique was also applied. In this case a process optimization was as realized by applying all the obtained pigments via a conventional synthetic paste and also through a natural printing paste.

<table>
<thead>
<tr>
<th>Paste type</th>
<th>Textile material</th>
<th>Treatment</th>
<th>Thickener</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic paste</td>
<td>Cotton</td>
<td>Resina Center STK-100</td>
<td>Clear HC</td>
</tr>
<tr>
<td></td>
<td>Wool</td>
<td>Resina Center Mc-LF</td>
<td></td>
</tr>
<tr>
<td>Natural paste</td>
<td>Resina Center AC60</td>
<td>CMC (CarboxyMethyl Cellulose)</td>
<td></td>
</tr>
</tbody>
</table>

Table 10. Optimization of the printing process employing natural algal pigments

The results of the printing process are presented below.
Conclusion: Action B.3.1 resulted in optimum dyeing and printing processes by employing natural algal pigments. The processes are applicable to cotton and wool substrates and validated for colour uniformity, applicability and replicability.

Textiles dyeing or printing show a good uniformity and reproducibility after to optimize the processes as expected.

B.3.2. CHARACTERIZATION OF FABRICS

This activity aimed to obtain the validation and demonstration of the application of pigment extracts from the 8 microalgae/cyanobacteria and the 6 macroalgae strains, as raw materials in new dyeing and printing processes, by analyzing the CIELab coordinates, the rubbing, washing and light fastness, solar protection factor and antimicrobial properties. All tests were performed on fabrics that were dyed or printed with extractions performed in the project.

In the following figure the obtained results are presented summarized for one extract, *Arthrospira platensis* on cotton substrate. All tests realized follow the same results pattern:

<table>
<thead>
<tr>
<th>IELab coordinates</th>
<th>Fastness to rubbing</th>
<th>Fastness to laundering</th>
</tr>
</thead>
<tbody>
<tr>
<td>L* 88.47</td>
<td>a* -1.00</td>
<td>b* 3.58</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dry staining</th>
<th>Wet staining</th>
<th>Change in color</th>
<th>Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5</td>
<td>3-4</td>
<td>Wool 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acrylic 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polyester 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polyamide 4-5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cotton 4-5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acetate 5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Determination of the ultraviolet factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPF 15 (good protection)</td>
</tr>
<tr>
<td>T (UVA) % 7.91</td>
</tr>
<tr>
<td>T (UVB) % 3.77</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Light fastness</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 (fair)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antimicrobial properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested microorganism</td>
</tr>
<tr>
<td><em>Escherichia coli</em> 46</td>
</tr>
<tr>
<td><em>Candida albicans</em> 0</td>
</tr>
</tbody>
</table>

Table II. Results of characterization test to the dyed/printed fabrics with algal pigments

Conclusion: The tests realized on the dyed/printed fabrics revealed the same quality that the ones dyed with natural dyes employed nowadays in the industry.

The results in term of fastness are worse than synthetics dyes, therefore the textile treated with algae dye aren’t similar or better than synthetics dyes as expected, but the algae pigment show similar results of natural dyes from plants. So, they can apply in sustainable textile, because the in term of fastness is good.

B.3.3. CHARACTERIZATION OF TREATED WATER.

The certification of the quality of the waste water obtained after dyeing with the new natural dyes developed in the project was analyzed using the DOB, COD and pH tests.
The obtained values in the waste water analysis after dyeing with natural dyes was expected to be lower than that of synthetic dyes, before it was treated. But results were approximately similar, which lead to further investigation on the auxiliaries used in the dyeing process, which were influencing the final results.

Information regarding the selected parameters for the characterization of wastewaters coming from dyeing process with synthetic dyes (direct, disperse, reactive…..) was collected.

The aim was to optimize the dyeing process with natural dyes then collect the wastewater, characterize and compare the results with the ones obtained in the previous process. Visual results are presented in the following charts:

![Figure 28. Wastewater analysis and comparison with synthetic dyes (left) and the influence of auxiliaries in the BOD values (right)](image)

**Conclusion:** Wastewater analysis showed the same BOD and COD values as synthetic dyes and when applying a wastewater treatment the values decrease proportionally. The following tests showed that the natural algal wastewater values of BOD were influenced by the auxiliaries used in the process.

The waste water effluents corresponding to synthetic dyes presents biodegradability potential of 7% with respect to effluent corresponding to natural algal dyes which reaches value of 70 %, leading to a significant potential to remove these dyes in treatment wastewater plants. So, the environmental impact of natural algal dyes on water contamination is significantly lower as expected.

**B.3.4. INDUSTRIAL-SCALE DYE TESTS.**

This action was seeking the extrapolation of the research conducted in previous activities once satisfactory results were achieved, and validation of the dyeing process at industrial scale using the new dyes. As the dyeing/printing process reached its optimization with a small delay, it also generated a little delay in the starting of this task, but this did not affect the proposed time table of the process.

During the last quarter of 2015 AITEX launched a survey between 150 textile finishing existing associate contacts, from the Valencian Community, to discover who shows interest in the industrial scale up of the processes developed at laboratory scale. Several companies, approximately 45, responded positively, as being interested in the project’s results. From the 15 companies willing to apply the pigments in their industrial processes, it was selected the most suitable company for the project’s purpose, and small quantities of pigments were delivered, thus achieving experiments at semi-industrial scale.

Due to the limited quantity of pigment, a company with a semi-industrial scale dyeing equipment was selected for the upscaling of the process. The advantage of the equipment was represented by the fabrics loading capacity, comprised between 25 and 1250 kg, so it was able to be tested the lower limit of the capacity.
Dyeing 25 kg of cotton and 25 kg of wool involved the application of 0.5 kg of pigment in each dyeing process. Taking into account the employment of three different pigments, the total semi-industrial tests necessitated 3 kg of pigment in total (1 kg of each pigment).

The extrapolation process to the semi-industrial scale was successful, obtaining cotton and wool fabrics dyed in light colors. The light shades acquired were due to the quantity of colorant that we were able to supply. The higher the amount of colorant employed, generates more intense colors on the textiles. The obtained dyed fabrics at semi-industrial scale were analysed in AITEX facilities, in terms of fastness and the results showed good behaviour to rubbing and laundering fastness:

<table>
<thead>
<tr>
<th>Fabrics</th>
<th>Dry stain</th>
<th>Wet stain</th>
<th>Change in color</th>
<th>Wool</th>
<th>Acrylic</th>
<th>Polyester</th>
<th>Polyamide</th>
<th>Cotton</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>3.4</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4.5</td>
<td>4.5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>4.5</td>
<td>3</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.5</td>
<td>4</td>
<td>5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.5</td>
<td>3.4</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 12. Fabrics dyed at semi-industrial scale

Feedback:

- Different color intensities were observed when dyed two different substrates (cotton and wool)
- The dyeing process must be followed by a finishing process for fastness increase, corresponding to the final application of the textiles.
- The batch dyeing machine was employed at a temperature under 100°C and led to successfully dyed fabrics, due to dye and fabric (wool and cotton) characteristics.
- The resulting dye wastewater effluent showed high degradability of the colorants when subjected to biological wastewater treatment.
- The company that applied the natural dyes in their semi-industrial process was pleased by the obtained results and considers that this process foresees a bright future due to its sustainable character.
Lessons learnt:

- The upscaling of a laboratory dyeing process to semi-industrial or industrial level implies process parameters modifications, as: bath ratio and quantities of auxiliary products. (The bath ratio was reduced 4 times).
- The fastness improvement of the natural colorants must be taken into consideration according to the final application of the product, by that meaning that it does not need excellent fastness levels in all the categories. For example, excellent light fastness is necessary only in the case of textiles with prevised high exposure to intense light.
- Batch dyeing is a suitable method to be applied to pigments obtained from algal sources, on cotton and wool substrates, and is similar to the process applied at laboratory scale.

By reducing the dyeing bath ratio (meaning the water quantity employed in the process), the dilution of the pigment is lower and it generates a slightly more intense coloration of the fabrics.

**Conclusion:** The aim of extrapolating the laboratory dyeing process to semi-industrial scale was realized successfully, fact proved by the fastness tests realized and the visual samples. The fastness tests revealed the results obtained at laboratory level experiments. The industrial scale dyeing process was successfully upscale from laboratory level with the minor changes of the parameter bath ratio due to consume optimization as expected.

**Action conclusion**

Action B3 delivered natural textiles, like cotton and wool, dyed and printed with natural algal dyes, characterized by uniformity of color, fastness to rubbing and laundering defined as good to excellent, light fastness comparable with natural dyes used nowadays in the textile industry.

The additional tests also revealed added value of these finished fabrics, as good antimicrobial properties and ultraviolet protection potency.

In terms of wastewater effluents pollution reduction, it was observed that the natural dyes poses higher biodegradability than the synthetic dyes, but the auxiliaries employed influence the BOD levels.

The validation and replicable character of the dyeing process were proved by semi-industrial dyeing tests, which revealed successful results.

**Deliverables**

- **D.B.3.2. Results of tests done to fabrics dyed with new dyes (Technical Annex 6)**
- **D.B.3.4. Report of the results on dyeing at an industrial scale (Technical Annex 8)**
**Action C1: MONITORING THE ENVIRONMENTAL IMPACT OF THE PROJECT ACTIONS**

*(January 2015 to December 2016)*

The objective of this action, based on the results obtained from actions B2 and B3, was the evaluation of different approaches and possibilities of monitoring the impact that the projects actions and outputs have on the environment. The following methodologies were used to work on the different sub-actions:

- Analysis, study, comparison, and compilation of the obtained results and data
- Evaluation of the environmental impact of the procedures and products demonstrated
- Evaluation of the efficiency, effectiveness and the feasibility of the materials obtained.

From a technical point of view, BEA-ULPGC worked on one approach of particular interest, when considering the possibility to reduce the environmental impact of wastewaters generated during the dyeing process in the industry. That is, different samples of wastewaters were sent from AITEX to their facilities to assay the possibility to isolate microalgae strains that show the possibility to grow in these waters, facilitating the idea of establishing wastewaters biofiltration treatments, similar to other examples such as agricultural (i.e. purines), urban or aquaculture wastewaters. From the processed samples, two new strains were isolated and characterized according to the procedures established by the collection unit at BEA. On the other hand, specific Environmental Impact Assessment (EIA) was realized using approved instruments of its quantification, as Leopold’s Matrix, which identified the final impact of the project, taking into consideration all direct and indirect impacts.

**C.1.1. STUDY OF ENVIRONMENTAL IMPACT OF OBTAIN DYES FROM ALGAE**

Considering the evaluation of cumulative impacts from the different processes related to algal production and dyes extraction and application, environmental impacts must be reduced to a minimum. For the industrial use of cultivated algae as dyes source for the textile industry, the different procedures were re-evaluated to estimate possible impacts and related solutions.

In the macroalgae cultivation process, were taken into consideration, into the impact measurements, inputs like: the nutrient rich seawater, containing: NH4, NO3 and CO2. Highlighting the fact that there was not necessary the employment of fresh water, no biodiversity loss was generated in harvesting wild strains and no land use (arable nor urban) was needed, due to the fact that the facilities of algae production were situated on an abandoned terrain.

![Figure 29. General procedure of production of macroalgal biomass and phycobiliproteins extracts, inputs and outputs](image-url)
On the other hand, microalgae (including cyanobacteria) production, as mentioned in the macroalgae section, the inputs were similar but in different quantities, taking into account that the strains are considerably smaller than their macroalgae correspondents. In this case, the main inputs are represented by light, temperature and nutrients, as nitrogen.

Furthermore, BEA studies focused on the isolation and identification of algal strains growing under wastewater effluents originating from industrial dyeing process. The results were quantified with the isolation of two algal species (1 chlorophyte and 1 cyanobacteria), potentially applicable in bio filtration treatments or pigments extraction, which were isolated and added to BEA’s algal collection under codes BEA1541B (Halochlorella rubescens) and BEA1542B (Pseudoanabaena sp.).

This approach concerning industrial algae and dyes production for the textile industry, similarly to other bio-industrial developments (i.e. biodiesel production from microalgae biomass), was considered under the Life Cycle Analysis concept, which was evaluated after identification of the major stages, inputs and outputs for each of the actions of the project.

From the arsenal of environmental studies that exist, the most suitable to select was considered the Leopold matrix, through which it was made the connection between environmental factors and anthropic activities of development, and due to the fact that it has a standardized form which ensures the user (evaluator) that any type of impact was omitted. In order to be able to evaluate the impact of the project activities was necessary to involve the partner’s facilities location, for a clear and complete analysis.
Impact factors were evaluated separately for each environmental component relevant for the scope of this study, and scored on a scale from -1 to 5 for impact magnitude, where the negative values represent a negative impact and the positive value give a favourable influence of the project actions towards the environment.

According to the scale of impact magnitude quantification, presented at the beginning of this section, the obtained result, IF=0.24 showed a very low significance impact on the environment, exercised by the obtainment and employment of dyes from algae.

**Conclusion:** The production of algal biomass and the extraction of sustainable dyes is possible, with an environmental impact fairness, taking into account the final purpose of the application, the textile industry, and the second largest pollutant.

The cultivation process of macroalgae and microalgae consume as a medium value of 1 kg CO₂ for 1 m² of algae biomass culture per year, so we can confirm an important reduction of CO₂ from the atmosphere and the cultivation process doesn’t generate this air pollutant. On the contrary, the production of the synthetic dyes generates high quantities of CO₂.

According to the expectations the manufacturing of natural algal dyes reduces the CO₂.

---

C.1.2. STUDY OF ENVIRONMENTAL IMPACT BENEFITS OF EMPLOY NATURAL VERSUS SYNTHETIC DYES

Environmental benefits from biodegradable dyes were anticipated, including less polluted wastewater and reduced water purification demand. Such benefits would help to achieve the goals of the EU legislation covering wastewater (e.g. Directive 2008/105/CE and the REACH regulation).
In order to measure the environmental impact of the project was carried out a quantitative comparative study of advantages and savings of the process of manufacture and use natural dyes comparing with the actual situation. During this task the environmental impact was studied comparing the initial situation with the improved one, in order to confirm the suitability of the demonstrator and the progress achievements. In order to achieve suitable results for this phase, the focus was divided on the pollutant emission when employing dyes, from the point of view of the process parameters (like time and temperature) and the wastewater emissions.

It is estimated that over 10,000 different dyes and pigments are used industrially and over $7 \times 10^5$ tons of synthetic dyes are annually produced worldwide. In the textile industry, up to 200,000 tons of the dyes are lost to effluents every year during the dyeing and finishing operations, due to the inefficiency of the dyeing process.

The production and the employment of algae based colorants was compared with the same processes where were used their synthetic correspondents, and the following differences:

- **Air pollution**: generated by the production of the dyes:

<table>
<thead>
<tr>
<th>Natural dyes</th>
<th>Synthetic dyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algal biomass production does not generate any air pollution due to their environmental friendly growing environment (artificial ponds, seas), and they present a low demand of resources.</td>
<td>Most processes performed in textile mills produce atmospheric emissions. Gaseous emissions have been identified as the second greatest pollution problem for the textile industry.</td>
</tr>
</tbody>
</table>

- **Water pollution**: generated by the substantial amount of water used in its manufacturing process used mainly in dyeing and finishing operations of the plants, and as a conditioning relationship with the previous situation, the amount of water generated and the effluent composition.

<table>
<thead>
<tr>
<th>Natural dyes</th>
<th>Synthetic dyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biodegradable, low stability to light, temperature, water, detergents, chemicals, and other parameters such as bleach and perspiration.</td>
<td>High stability to light, temperature, water, detergents, chemicals, and other parameters such as bleach and perspiration. High thermal and photo stability to resist biodegradation.</td>
</tr>
</tbody>
</table>

In terms of process parameters, it was concluded that the employment of natural, algal based colorants generates less pollution due to the optimum process of employment. By the lower process temperature necessity, it is involved a lower energy consumption, which generates less resource consumption.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Conventional process-synthetic dyes</th>
<th>Conventional process-natural plant dyes</th>
<th>Algae pigments process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>≥85°C</td>
<td>≥85°C</td>
<td>65°C</td>
</tr>
<tr>
<td>Time</td>
<td>60 min</td>
<td>60 min</td>
<td>60 min</td>
</tr>
</tbody>
</table>

Figure 34. Dyeing process parameters comparison, synthetic vs. natural dyes

The wastewater effluents parameters, as analysed in action B.3.3 show values influenced by the employment of process auxiliaries.

The most employed dyes nowadays are the synthetic ones due to several factors:

- Recent legislation has imposed release limits for specific chemicals that are contained in synthetic dyes, fact that help in a reduction of pollution
Due to their economic value and to the high demand of textile products in a short period of time, they seem more suitable to be employed.

Unawareness of consumers regarding the negative effects of the synthetic dyes usage

Taking into consideration the more and more popular concept of durable development, companies should start to adopt in their internal policies, the concept of Corporate Social Responsibility (CSR), which in textile companies can be implemented by switching to the usage of environmentally friendly products, natural dyes, which don’t have negative effects on the environment and create a positive brand name.

Conclusion: Definitely the employment of algal natural dyes presents much more benefits to the environment that their synthetic correspondents, but they reveal slightly lower fastness values and a less diverse colour palette. Adopting a sustainable process in an industry leads to a change in concepts, visibility and acceptance by the market.

The significant results are:

- Reduction of wastewater pollution in dyeing because the natural algal dyes are more biodegradable (70%) than synthetic dyes (7%) as expected.

- Substitution of synthetic dyes in different textile applications like natural or eco fashion, and it represents between 5-10% of the sector.

C.1.3. STUDY OF REUSE OF BIOMASS GENERATED DURING THE EXTRACTION OF NOVEL DYES

Once the procedures for dyes extraction were optimized, biomass “wastes” (biomass remaining after extraction) from micro- and macroalgae were evaluated and characterized for other substances of interest, such as polysaccharides, lipids and fatty acids, substances with antioxidant capacity or potential as feed complement. The basic idea of this sub-action was to apply the concept “biorefinery” to algal/biological material, which significantly improved yields related to algal production and dyes extraction from an ecological/economical point of view.

At BEA-ULPGC, biomass waste from microalgae and cyanobacteria, from which were extracted first water-soluble pigments (phycobiliproteins) or organic solvents-soluble pigments (carotenoids and chlorophylls), were re-extracted in a second process under different polar solvents, which means organic for the first and water for the second. As an interesting result, a high concentration of carotenoids was obtained from BEA0007 Arthrospira platensis. These extracts were also evaluated for antioxidant activity by different methods (DPPH and ABTS), and the obtained results were positive.
At ALGAplus facilities, the same experiments were done. A single step extraction protocol allowed the production of two of the target pigments (green and yellow). Moreover, the waste material from all the macroalgae species presented anti-oxidant activity which can open the door to other high value applications, namely cosmetics.

Another application for the remaining biomass was its usage in the production of nonwovens by WetLaid process, this concept was demonstrated in LIFE SEAMATTER PROJECT (LIFE11ENV/ES/000/600). WetLaid process is suitable due to the characteristics of the machine which can process this type of matter. The applicability of the nonwovens is represented by agroteextiles and green composites.

**Conclusion:** The waste biomass can be reused for new applications by employing the antioxidant activity, creation of nonwovens and application of the biorefinery concept. Therefore, we have obtained at least two different uses for the waste biomass generated after the dyes extraction as expected.

**Action conclusion**

The environmental impact assessment sustains the applicability of the “circular economy concept” to SEACOLORS. As a summary of this concept applied to the project’s activities, it must be mentioned that from a sustainable algae biomass production, followed by efficient colorant extraction, and its application in textile finishing processes, the remaining biomass was subjected to further extraction processes to obtain other pigments/colorant or other compounds of interest to be applied as raw materials in food, feed, agriculture, cosmetics, etc.

**Deliverables**

**Action C2: MONITORING OF THE SOCIO-ECONOMIC IMPACT OF THE PROJECT ACTIONS**

*(January 2016 to December 2016)*

The objective of this action was the measurement, quantification and analysis of the social and economic impact that the production and employ of algae extracts have on the society and the actual economy, and it was achieved by its division in two phases classified in sub-actions: on one hand, surveying three categories’ of possible users and beneficiary of the project results; and on the other, by market studies concerning advantages and inconveniences and perspectives for the employment of the obtained compounds from the project.

During the first stage of the action, a study of the current situation was performed analysing the actual techniques to obtain dyes, the dye process focused on economic aspects (data from previous actions was taken into account). Then a comparative study with the new improved solution was performed taking into account socio-economic factors. This study allowed us to know the economic parameters used in the socio-economic report.

In the second stage, the results and dates of Action B.3 (Validation and demonstration of the application of algae dyes in textile dyeing process) were used to quantify the economic data associated to the use of natural dyes. In addition, this stage also comprised the questionnaires development directed to consumers (buyers), textile industry specialized persons and algae producers, in order to monitor the social impact.

The milestones of this action were the quantification of the socio-economic impacts generated by the cultivation of the selected strains of algae, the extraction of dyes, the waste biomass, and the dyeing process parameters.

**C.2.1. STUDY OF SOCIO-ECONOMIC IMPACT TO OBTAIN DYES FROM ALGAE**

This action was focused in the economic and social improvements achieved during the production process, from growing algae to extraction and modification processes to adapt dyes to textile requirements if finally are needed.

It was made a comparative analysis, based on market studies, between production processes cost and employment cost of synthetic and natural dyes obtained from other sources, and used nowadays in the textile industry. And the main considerations obtained from the market analysis state that the Global Dyestuff Industry faces a series of challenges, regarding the:

**SYNTHETIC DYES:**

- **Environmental considerations:** The industry can prosper if dyes are not hazardous and they are environmentally friendly.
- **Support of the Government and Trade Associations:** Any industry that moves ahead needs the back-end support of the government as well as the trade associations. This helps to promote that industry in other countries, allocation of investments and giving other supports.
- **Problem of over capacity but falling margins:** China and India have high potential as regards production capacity is concerned, due to the shift in the manufacturing
bases from Europe and some other industrialized nations. But there is varying demand across these regions and that is the cause for volatility in the market. Thus affecting prices.

- **Fierce competition**: Due to shift of companies from West to East has resulted in a concentration of all the companies in the Asian region.
- **Research and Development**: Market demands a higher spending for innovation in products like natural dyes.
- **Product quality vis-a-vis competitive prices**: High importance of the quality of products along with competitive prices for retaining market.
- **Classification of products and services**: A decline in the growth for products has prompted the manufacturers to move to specialty products.
- **High cost of energy and interest**: The exorbitant cost of interest can lower the investment in R&D which is at the core of product and service innovations. Also, the high energy cost has also adversely impacted the manufacturing units.
- **Availability of World Class Infrastructure**: As ports and roads are the primary sources of transport, the Governments must emphasis to improve the clearance of the goods at a quicker rate to facilitate trade.

**NATURAL DYES (plant origin):**

- **Commercial structure** to launch products on the technical market. A professional supplier organization must collect raw materials for natural dyes from different agricultural sources and provide a group of dyes available at high standardized quality, deliverable to the dye house within a short period of time, all year long.
- **Technical support**: a rapid introduction of natural dyes into full-scale production processes is of particular importance to react efficiently to the ever-changing fashion demands. The technique of application of the dyes must be described and presented in comparably high technical quality, as done at present by the manufacturers of synthetic dyes. Defined recipes and profound technical support are needed.
- **Full range of colors**: which cannot be provided at this point. To provide a pure black color with natural colorants and the respective environmentally friendly dyeing process needs further investigations. There is a big demand for dark colors; therefore research needs to focus on how to provide natural dark colors in an acceptable and feasible way.

A second part of this study was the realization of a cost comparison, applied to the textile dyeing branch, and the final conclusion is presented in the following table.

<table>
<thead>
<tr>
<th>Dyeing process for 100 kg batch fabric</th>
<th>Synthetic dyes</th>
<th>Natural dyes-plants</th>
<th>Natural dyes -algae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water consumption in LITERS</td>
<td>700-1840</td>
<td>2000-4000</td>
<td>2000-2500</td>
</tr>
<tr>
<td>Energy consumption kWh</td>
<td>569,7-1497,6</td>
<td>269,4-369,4</td>
<td>242,4-332,4</td>
</tr>
<tr>
<td>Dye price (€/kg)</td>
<td>2,67- 35,57</td>
<td>5-70</td>
<td>5-200</td>
</tr>
<tr>
<td>Dye quantity (kg)</td>
<td>16,6</td>
<td>10-30</td>
<td>10-30</td>
</tr>
<tr>
<td>Auxiliary consumption (kg)</td>
<td>8,23-41,91</td>
<td>13,23-46,91</td>
<td>13,23-46,91</td>
</tr>
</tbody>
</table>

Table 13. Production and employment prices of synthetic, natural (plant origin) and natural (algae origin) dyes.
Conclusion: The market studies reveal a tendency of the industry on moving towards sustainable and eco-friendly products, even if it involves higher prices, at the beginning of their introduction on the market. Clothing prices are, in many cases, artificially low, because the society has been trained to buy quantity over quality. The eco/sustainable movement is growing, but is still very new and is a small part of the industry worldwide.

The industrial production of natural algal dyes represents:
- Development of a new niche market with a high impact for the algae producers.
- Inclusion of the volunteer policy of the social corporate responsibility in a company, which generate:
  - working places
  - eco-friendly business
  - popularity due to applied concept

C.2.2. STUDY OF SOCIO-ECONOMIC IMPACT OF EMPLOY NATURAL VS. SYNTHETIC DYES

- The aim of this action was monitoring the socio-economic impact of the new dyes obtained in the project during their use as a raw material in the textile industry. This took into account the cost of temperature and time employed in the dyeing process and the cost employed in purified waste water from natural dyes, versus the cost employed with synthetic dyes. The main outputs of this study are centred on the following parameters: Algae production costs:

<table>
<thead>
<tr>
<th>Production algae cost (fresh weight)</th>
<th>€/kg</th>
<th>Biomass necessary for the obtention of 1 kg of pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroalgae cultivated</td>
<td>2.5</td>
<td>16 kg</td>
</tr>
<tr>
<td>Macroalgae wild-harvested</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Microalgae fresh</td>
<td>30</td>
<td>14 kg</td>
</tr>
</tbody>
</table>

Table 14. Production costs of algal biomass

- The extraction process costs, at laboratory scale:

<table>
<thead>
<tr>
<th>Per extraction</th>
<th>40 gr of Gracilaria sp. (red macroalgae)</th>
<th>60 gr of fresh Bifurcaria bifurcata. (brown macroalgae)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae cost (€)</td>
<td>0.092</td>
<td>0.012</td>
</tr>
<tr>
<td>Water (€)</td>
<td>3.7008</td>
<td>5.04</td>
</tr>
<tr>
<td>Shaker use (€)</td>
<td>0.2 h / 0.0224</td>
<td>0.5 h / 0.056</td>
</tr>
<tr>
<td>Centrifuge use (€)</td>
<td>0.5 h / 0.07</td>
<td>0.5 h / 0.07</td>
</tr>
<tr>
<td>Lead time (h)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Labour costs</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Chemical costs (€)</td>
<td>0.005</td>
<td>4.05</td>
</tr>
<tr>
<td>Total (€)</td>
<td>43.89</td>
<td>49.228</td>
</tr>
</tbody>
</table>

Table 15. Pigment costs, including the extraction procedure costs
It has been impossible to calculate costs at the industrial level, because all the extraction companies contacted have answered that it is difficult to provide this information at these project levels, therefore, all calculations are on a laboratory scale.

Furthermore, the public opinion was quantified in order to measure the perception and the awareness of the society with respect to the results of the project and the acceptance of products developed with the employment of the raw material, algal biomass.

The obtained results reveal a diverse group of people, from the point of view of the location (17 countries) and specialization (textile, algae and consumers). From all the surveyed persons, the majority showed favourability to produce, buy and use products where employed algal compounds.

**Conclusion:** When analysed, the socio economic impact of the employment of natural algal dyes vs. the synthetic ones, it can be concluded that in terms of price, the benefits of algal pigments employment is deficient in nowadays market demands. On the other hand their popularity presents increasing tendency due to the high sustainability and emotional benefits that they could add when incorporated into the practice of a company/industry.

The socio-economic impact difference between the uses of the natural dyes developed vs synthetics is:

- Development of innovative lines of eco-textile.
- Added value of the products developed with the natural dyes (for example: antimicrobial properties, UV protection…)
- They don’t generate allergies.
- Lower pollution (for example: less CO₂ emissions)
- Higher cost at actual production level due to low awareness of the product (low demand generate high price).

**Action conclusion**

The socio-economic impact assessment sustains the applicability of the algal pigments into the textile industry. When focusing on the already mentioned industry, there is a growing movement towards responsible and sustainable disciplines.

In order to make the algal pigment a profitable business it is necessary to increase the awareness at consumers’ level, so the demand for these type of products increases in the same time with market competition, which automatically would make prices competitive.

**Deliverables**

Action D: COMMUNICATION AND DISSEMINATION ACTIONS
(July 2014 to December 2016)
These actions are detailed in chapter 5.2. Dissemination actions

Action E2: PROJECT MANAGEMENT
(July 2014 to December 2016)
This action is detailed in chapter 4. Administrative part

Action E2: AFTER LIFE+ COMMUNICATION PLAN
(December 2016 to December 2016)
This plan, delivered in English, explains the beneficiaries plan to continue applying, disseminating and communicating the results of the project after its end, and how they plan both to continue applying the results themselves and to facilitate / encourage / ensure their wider application by others, thus ensuring the proper exploitation of the results after the end of the project.

A detailed description of the plan is described in the corresponding deliverable.

Deliverables

- D.E.2.1. After LIFE + Communication plan (Dissemination Annex 2)
Action E3: CREATION OF AN INFORMATION EXCHANGE NETWORK WITH OTHER PROJECTS

(October 2014 to December 2016)

The objective of this action was to create a network in order to exchange experiences and results with other LIFE projects, especially LIFE 3 and LIFE + with the same thematic of the present project. The main activities within this action were focused on the identification of related attention-grabbing projects and the realization of a database for the collection of the projects that could be interesting for the development of networking activities, as they were connected to the topics covered or addressed during the project, such as algae applications and cultivation systems, dyes and chemical products, textile wastewater treatments and textile projects.

Several materials were created in order to approach to the project coordinators, presenting the project and the opportunity to collaborate, such as cover letters, noticeboards, brochures, newsletters, or events.

E.3.1. CREATION OF A DATABASE INCLUDING THE MOST RECENT LIFE+ PROJECTS RELATED WITH THE MAIN CONCEPT OF THE PROJECT

A network was created (and was continuously updated during the project life) in order to identify possible LIFE, INTERREG and FP7 projects and partners that might be interested in the SEACOLORS project results. This allowed the partnership to bring new opportunities for the implementation of innovative, sustainable and ecofriendly solutions, and to establish privileged links between diverse projects and partners, creating great opportunities for collaboration and creation of synergies in the future; as well as to contact with different countries and cultures, where it is possible to find and exchange different ways of approaching the same problem.

An initial classification of identified projects was done in order to differentiate several types of projects which could be directly or indirectly related with the SEACOLORS project areas and fields of application, resulting in the identification of 60 different EU projects that can be related or interested in SEACOLORS results (Dissemination Annex 3. E.3.1. Database with some information of main LIFE project), as well, a list with textile, marine, algae and biotech associations, authorities, NGOs, entities, networks, and fairs was created in order to increase the identified target publics promoting dissemination and outreach of the project.

A first contact with those entities was done through cover letters and the newsletters sent. A plan for continuing creating extensive interconnections among them was developed, and several face-to-face meetings and events where consortia met and generated debate about common topics were carried out during project lifetime.

Invitations to the SEACOLORS Final Event were sent to coordinators of the main related projects to assist or participate in it. Finally, a total of 30 people participated in the event, in which we count with the participation of 11 different projects, which shared a similar approach to the one developed within SEACOLORS, due to the use of marine resources, development of processes for diminishing waste generated or applying better treatments to
residues, and the implementation of more sustainable practices within traditional sectors such like the textile industry.

All of this will contribute in the transition towards more sustainable production and consumption patterns in Europe and will lead to the implementation of a circular economy and its long-term benefits.

**Conclusion:** The created network provided an exchange network where partners can share and discuss about their experiences in the development of more sustainable processes within very different sectors, where innovation offers a possibility for improving the efficiency and sustainability of the processes.

Therefore, the creation of this type of platforms, in which different social groups meet together and the public private synergies and collaborations are promoted, will ensure public support for continued development and application of more sustainable and clean technologies and solutions in the industrial processes.

From the technical perspective, the inclusion of this kind of actions within European projects promotes the international cooperation for the progress of scientific knowledge and social development through scientific and technical exchanges, creating the basis for the implementation of bioeconomy schemes in the way forward to reduced greenhouse gas emissions and more efficient, sustainable utilisation of renewable biological resources.

The most important result of these networking actions will be the submission of a new project related to the use of natural dyes from algae in leather industry. This project will be submitted in Call: IVACE from Valencian region, and de partners will be AITEX and INESCO.

In addition several entities have showed interested in the project and the results of the project, such as Nienke Hoogvliet (designer from Holland), Brenner (pharma/food from Israel), GreenSea (France), Tejidos Royo, Rioma, Inescop and DLana, all from Spain.

**E.3.2. Exchange of electronic newsletters in order to inform about the project and topics in relation with it (normative, calls, etc.)**

The objective of the newsletters was to provide regular short updates on the actions of the project to the interested public. The 4 newsletters developed during the project life enclosed all the information about last actions developed within the framework of the project, with a direct link to the SEACOLORS website, and a list and explanation of events assisted as well as future identified events that could be interested for users and followers. Some news from partners, related directly or indirectly with SEACOLORS, were showed, as well as possible interesting calls within the European Commission. This made more interesting the publication itself, increasing the number of users and the network achieved.

The newsletters were sent every 6 months during the project execution to other projects with similar topics of this project (partners involved: ASEBIO).

**Conclusion:** The production of the 4 newsletters (created every six-months) allowed each project partner to write updates on the local implementations of the project, the main results, the events to go, etc. The newsletters are available in English in the following link: [http://www.seacolors.eu/index.php/en/publications-and-events/newsletter](http://www.seacolors.eu/index.php/en/publications-and-events/newsletter)
The Newsletters helped to inform readers and to get people interested on Seacolors, as they reached a wide range of people. They served as a medium to announce most relevant milestones achieved during the project, upcoming events and updates.

Newsletters were sent to stakeholders as a way to know more in detail the evolution and main outcomes that were being developed in the project, giving a more exhaustive description on results and steps carried out that the one showed in general dissemination materials.

The design of the newsletter was improved in a format which was both attractive and easy to handle, differentiating the following sections:
- Project title and direct link to the website
- Approaches (description of selected algae, evolution, main results, etc.)
- Information of consortium meetings / attended events / etc.
- Networking meetings
- News from Partners (200 words)
- Calendar with interesting Events
- Related Legislation/ calls
- Press coverage

The newsletters were sent to 31 contacts at entities related to algae production, to the members of the industrial biotechnology commission, formed by 43 companies, 2 technology centres, 2 scientific parks, 1 foundation and 1 research institute, as well as to 72 members of the project network, making this a total of 152. Thus, the newsletters became an interesting dissemination tool to ensure efficient communication with stakeholders outside the project (scientist, research and industry community, textile and algae enterprises, policy makers and the general public) in order to raise awareness about the ongoing project as well as its results at each level.

E.3.3. Participation in some event of the LIFE network

Beneficiaries participated in networking events of LIFE+ network together with projects working in the same or related subjects to those in SEACOLORS. In these meetings, it was necessary to give clear and direct information about project objectives, its challenges and benefits, highlighting the importance of the results and main impacts to raise awareness effectively among the target audience and to promote eco-friendly and sustainable processes in the industry.

The database with related or interested platforms and entities was defined and updated in order to reach new different kind of profiles, increasing the opportunities of collaboration and networking.

The following table shows the events attended by beneficiaries:

<table>
<thead>
<tr>
<th>Event/Beneficiary</th>
<th>Event purpose</th>
<th>Date</th>
<th>Place</th>
<th>n° assistants</th>
<th>SEACOLORS presentation</th>
<th>Dissemination activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIFE+ INFODAY</td>
<td>Networking event. SEACOLORS presentation</td>
<td>10/07/2014</td>
<td>Escuela de Negocio Lluis Vives, Paterna, Valencia, Spain</td>
<td>57</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>(AITEX)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIFE11 ENV/ES/513</td>
<td>Networking event in the frame of LIFE+ project,</td>
<td>19/11/2014</td>
<td>AIJU –Instituto Tecnológico del Producto Infantil y Ocio (Spain)</td>
<td>30</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>MASTALMOND:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Environmental</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Final report LIFE+  
52
Table 16. Events attended

<table>
<thead>
<tr>
<th>Event/Beneficiary</th>
<th>Event purpose</th>
<th>Date</th>
<th>Place</th>
<th>n° assistants</th>
<th>SEACOLORS presentation</th>
<th>Dissemination activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>improvements in the business: technologies, processes and materials (AITEX)</td>
<td>MASTALMOND</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Networking at CETAQUA (AITEX)</td>
<td>LIFE+ Networking meeting (AQUATIK, aWARE and LIFE WIRE)</td>
<td>27/11/2014</td>
<td>CETAQUA, Barcelona, Spain.</td>
<td>39</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>2nd Industry Expert Workshop, a joint dissemination activity by the MicroB3, MaCuMBa and PharmaSea EU Projects</td>
<td>Networking event in the frame of LIFE+ project, MicroB3, MaCuMBa and PharmaSea EU Projects</td>
<td>30/05/2015</td>
<td>PharmaMar, Colmenar Viejo, Spain</td>
<td>40</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Infoday Regional REDIT Programa Life 2015</td>
<td>Disseminate results and success stories and experience of SEACOLORS</td>
<td>14/07/2015</td>
<td>REDIT (Network of Technological Institutes of Valencia), Paterna, Valencia</td>
<td>30</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>SEACOLORS Final Event</td>
<td>Networking with project identified in database and presentation of project results</td>
<td>29/09/2016</td>
<td>BIOSPAIN 2016, organised by ASEBIO, Bilbao, Spain</td>
<td>30</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Conclusion: All events assisted helped positioning SEACOLORS as a reference in the field, establishing closer relations within project networks contacts, and sharing principal aspects and outcomes as well as expected opportunities. This action increased project awareness and opportunities to establish future collaborations.

Action conclusion

Through the activities carried out, the materials created and the events assisted during project life, SEACOLORS has become a project of reference in the field of more sustainable and ecological textile processes by the use of sustainable practices and resources, the algae.

All these used tools and the information provided has facilitated the consolidation of exchange network and the relationships by increasing the interest of stakeholders and projects. Future activities in the AFTER LIFE communication strategy will take into account this network in order to reinforce the collaboration.

Different companies have shown interest in the project, this interest is being materialized in the development of several proposals for collaboration, such as the already mentioned IVACE call from Valencian region

Deliverables

- Dissemination Annex 3. D.E.3.1. Database with some information of main LIFE projects
5.2. Dissemination actions

5.2.1. Objectives

The principal objectives of the Dissemination and Communication Plan were:

- To optimise and stabilize the flow of information to promote efficient communication, dialogue and exploitation between the institutions participating in the project and possible interested parts or end users.
- To report and communicate the results obtained to public and private entities and European institutions from national and European levels that might be interested in the project to transfer knowledge to other users and policies.
- To ensure that information is shared with appropriate audiences on a timely basis and by the most effective means.

The specific objectives of outreach and external communication were:

- To inform the target audience about the Project Idea: objectives; reason for its creation; composition of the consortium; results; etc.
- To disseminate the progress and results achieved during the project.
- To disseminate and transfer new findings or reference material for interested or related entities to promote future collaborations.
- To engage in effective, transparent and understandable communication with the society in general, focusing on the project ideas and benefits: sustainability, innovation, bioeconomy, viable exploitation, scientific potential, the importance of marine biodiversity and opportunities, etc.
- To create awareness about the development of new processes which are less harmful for the environment and creates environmental, technical and economically feasible solutions to be applied in the textile sector.

5.2.2 Dissemination: overview per activity

Action D.1: DISSEMINATION AND COMUNICATION

(July 2014 to December 2016)

The aim of this action was to achieve a propagation of the projects’ development and results at European level.

D.1.1 Creation of Dissemination Material: Logo, leaflets and posters.

The Promotional material developed consists of leaflets that translated from English to Portuguese and Spanish to be shared at events and official fairs; two noticeboards that are shown at partners’ offices and also in main events and webpage, and newsletters where the major outcomes of the project are showed as well as news, events and next events. These newsletters were sent to stakeholders identified for the creation of an exchange network.

Leaflets included a general overview of the project, main objectives, project context, LIFE and partner logos, goals and members, highlighting the importance of the results and main areas of interest for potential users.
These materials have been used in different events as conferences within the biotech, textile and algae sectors, and the Final Event of SEACOLORS project.

**Conclusion:** The creation and dissemination of the materials containing information on the development and the results of the projects activities was realized successfully.

### D.1.2 Dissemination of the Project in Events by All Partners

2,200 copies of the leaflets were generated and shared with the partners to increase the dissemination of the material at several types of events: 1,800 English; 200 Portuguese; 200 Spanish.

Copies of the leaflets were sent to the partners to increase the dissemination of the material at other types of event related with industrial biotechnology, textile, algae and marine sector.

The project was disseminated in more than 85 major fairs and conferences, with a specialized audience of more than 10,000 people in total, related to topics of algae, biotechnology, textile, chemical and dyes, in which the beneficiaries participated and made the presentations of the project spreading the dissemination material. Some events were not foreseen in the budget but due to the great importance of abovementioned events, the partners attended them in order to spread and disseminate project information.

In the following table are summarized number of events, per year and country during the lifetime of the project:

<table>
<thead>
<tr>
<th>Country</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>8</td>
<td>10</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>Portugal</td>
<td>5</td>
<td>10</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>France</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Italy</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Germany</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>USA</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Colombia</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>UK</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Scotland</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Austria</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Greece</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sweden</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Belgium</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Denmark</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>China</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Ireland</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Norway</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Chile</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Japan</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Czech republic</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>21</td>
<td>41</td>
<td>23</td>
<td>85</td>
</tr>
</tbody>
</table>
85 is the total number of events where SEACOLORS project has been diffused (oral presentations, stands, posters or leaflets), and 35 is the total number of events where SEACOLORS project had a specific dissemination (oral presentation and poster) with specific results and advances.

See deliverable *D.D.1.3. Newsletters and dissemination 2016* for detailed information on the events and SEACOLORS’ participation in 2016, and also in *Dissemination Annex 9* for a table summarizing the events participated during the project’s lifetime.

**Conclusion: SEACOLORS by contributing to scientific conferences and event aimed to present the main objectives and outcomes of the project, to stimulate the interest of the general public in the most important event related to the biotech sector.**

**The project and its results have been disseminated in fairs and conferences related to interesting topics for SEACOLORS. The audience of these conferences were more than 10,000 participants as specialised audience.**

### D.1.3 Organization of a Final Event during BIOSPAIN Fair

The SEACOLORS FINAL EVENT was held successfully in BIOSPOAIN fair, in Bilbao, on 29th of September 2016. It was one of the main dissemination activities as it aimed to announce to the scientific and industrial community the most relevant results obtained in the project, while creating awareness about the opportunities of algae in traditional sectors, such as the textile. As well, it aimed at creating a network in order to exchange experiences and results with other EU granted projects or entities with the same thematic or areas of interest that the present project. The sections in which the event was divided were focused in the following themes, closely related with the scope of the project:

- Applications and opportunities derived from marine resources: algae
- Biotechnology in the textile and leather industry
- New developments in bioeconomy: new added value products and revalorization of wastes
- Societal challenges

A total of 113 entities from the Industrial Biotechnology, Agrofood and Algae field, members of ASEBIO, were invited to the session. As well, 189 FP7, LIFE+ and INTERREG project coordinators (identified in the SEACOLORS network), consumer associations, platforms, authorities, NGOs, Clusters, organizers of related fairs, and other interesting entities working in these fields were contacted and invited to participate in the event. The EC desk officer and the monitoring team officer were also invited to accompany us. Finally, a total of 30 people finally participated in the event.

Sessions in the conference were dedicated to the understanding of marine resources and their possible uses, as well as their opportunities and implementation in traditional sectors. Also, a discussion on the importance of sustainability and the need of extracting value from waste and by-products as indispensable factors to be applied in the value chains of the industry took place. This event provided an opportunity for partnerships to exchange their experience, information and strategies.

Different materials were created for the event and shared with assistants (SEACOLORS notebooks (100 units); SEACOLORS printed cotton bags (50 units); 200 SEACOLORS pen drives; 5 neckerchiefs printed with dyes from algae in a different colour range (pink, green and blue); 60 bags with algae with the SEACOLORS logo for its use in food; 4...
information panels with brief resume about some of the algae used in the project with dyes samples and tissue sample (2 micro and 2 macroalgae); 20 Printed T-shirts with SEACOLORS logo; and 2 roll ups with final event information).

During the event, several surveys were fulfilled by the people attending the event. The objective of these surveys was to understand the acceptability of algae dye by the consumers and/or end users. Surveys are also available at: http://www.seacolors.eu/index.php/en/project/form

In the final event the relationships with the attendees were strengthened, and two of them were very interested in the results of the projects. They asked for samples to test it in other materials like footwear, wood carpets, etc. After the final event, one of the speakers contacted us to try to scale up the project and we are searching different ways to do it.

The ASEBIO stand also sought to draw attention of the assistants to the SEACOLORS Final Event by including a big information panel about the event in one of its walls, and through brochures that were shared not only in the stand, but also during the conferences.

**Conclusion:** The event gave a general overview of some of the topics which were set out within the scope of the project, like algae use and application, cultivation techniques, new approaches in the textile industry, the obtaining, production and application of natural pigments, the environmental and economic impact of these products and processes, waste water treatments, revalorisation of waste and by products, and the importance of social perception.

Through the invitation to other related project to present their experiences, relationships and collaborations were reinforced and networking could take place. This initiative was valued very positively by the audience, as they recommended repeating similar events where partners from different fields could meet and discuss finding solutions for major societal and economic challenges.

**D.1.4 Publication and Dissemination of the Project Results in Technical Magazines and Press Releases**

There were many appearances related with the project in the printed press. It helped us to disseminate information about the project and its results through different channels among the scientific community, stakeholders and other interested parties. The main purpose of the press releases and articles published was to get attention for the project, its products or another major achievements happening in the project, in order to get the media to report on the results and solutions developed.

Several articles were prepared in order to inform the research community and specific sectors about the SEACOLORS project opportunities. The articles published during the project life can be classified according to their objective, information provided and theme or typology of the magazine. Therefore SEACOLORS publications can be classified in: technical/scientific, specialized sectorial and general dissemination articles. The different magazines, publications and media used for dissemination helped to reach a wider public creating interest for the project topic. A total of 29 publications were launched during project life (press releases, interviews and articles). All these publications led to several appearances in media and TV (more than 50 appearances in media). Detailed information regarding this matter is compiled in the *Dissemination Annex 10. Appearances in media.*
In the following table are detailed the number of publications by type:

<table>
<thead>
<tr>
<th>Type of publication</th>
<th>Nº of publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technical/scientific articles</td>
<td>5</td>
</tr>
<tr>
<td>Specialized sectorial</td>
<td>7</td>
</tr>
<tr>
<td>Press releases</td>
<td>15</td>
</tr>
<tr>
<td>Press articles</td>
<td>44</td>
</tr>
<tr>
<td>TV interviews</td>
<td>3</td>
</tr>
<tr>
<td>Project video</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>75</strong></td>
</tr>
</tbody>
</table>

Conclusion: Through these publications and actions, we get to draw the attention of the research community, the biotech, textile and marine sectors and the consumer and general public to the needs and benefits of the research and demonstration activities carried out during project life, in order to create new materials, textiles and products which provide more sustainable and greener solutions and providing viable and ecofriendly solutions for the textile and dyeing industry.

It is important to highlight that developments in general Actions A and B have been partially used to carry out undergraduate research work by two students from the Faculty of Marine Science at the University of Las Palmas de G.C. (ULPGC). Experiments gave as a result the presentation of the dissertations:

Pablo Botía López. 2015. Effects of culture conditions on the physiology and pigment content in microalgae. Degree in Marine Science – ULPGC


Universidade de Aveiro – 2 Biology students (Letícia Cunha and Helena Trigo, co-supervised by ALGAplus did their thesis reports on SEACOLORS.

Action conclusion

The dissemination activities were an important part of the project actions to announce to the community the most relevant results obtained and to create awareness about the project. To achieve this goal, a variety of activities and materials have been prepared to reinforce the effective dissemination of the project, encouraging science communication.

As a result, a series of materials and different activities have been carried out during project in order to achieve and fulfil these objectives:

- 2,200 copies of the brochures have been shared and showed during events assisted and in partners’ facilities.
- Brochures were created in English, Portuguese and Spanish.
- 2 Noticeboards were created at the beginning and end of the project presenting main objectives, opportunities and results.
- 5 posters have been created explaining major outputs generated during project life.
- 4 newsletters published each 6 months since project begun in order to inform about last news within the project development.
SEACOLORS Merchandising material was developed in order to create an external image on the project. They were shared during SEACOLORS Final Event (SEACOLORS notebooks, printed cotton bags, and pen drives; 5 neckerchiefs printed with dyes from algae in a different colour range (pink, green and blue); bags with algae with SEACOLORS logo for its use in food; information panels with brief resume about some of the algae used in the project with dyes samples and tissue sample (2 micro and 2 macroalgae); 20 printed T-shirts with SEACOLORS logo; and roll ups with final event information Assistance to more than 50 events, where 20 oral presentation took place.

- Organization of a Final Event during BIOSPAIN Fair
- Press releases and 9 articles in specialised and scientific magazines
- Appearances in TV (Telenoticias Canarias and Canarias Hoy)
- Impact on social media (143 followers on Twitter; 198 Facebook followers and 93 members in LinkedIn).

**Deliverables**

- **Dissemination Annex 5. D.D.1.3. Newsletters and dissemination 2016**
- **Dissemination Annex 7. D.D.1.5. Divulgation material**
Action D.2. PROJECT WEBSITE
(September 2014 to December 2016)

The objective of this action was the creation and update of the own full website of the SEACOLORS project which had to include all the information created throughout the life of the project.

The web of the project (www.seacolors.eu) was created on 2014 (in English) with project information and a private area for all the partners and also, with links to the LIFE+ Programme web. Moreover a link of the SEACOLORS webpage has been included in all beneficiaries website. For more information, see D.D.2.2. Information about the project in each partners’ website delivered within the Inception Report.

The initial design of the project website was realized in 2014, and was considered acceptable for the time being, but after the development of the project it was identified that some changes had to be made. The consortium partners agreed, after the Monitoring visit from February 2016, to add new tabs and reorganize the existing ones, maintaining the same design, but achieving the obtention of a more attractive and intuitive page.

The reorganization consisted in:

- Restructuration of the existing information in a simpler and more clear and concise format
- Combination of specific tabs as: Home includes now the Overview tab; the Publications and events comprises the Newsletters, Gallery, Events, Press coverage, Final event and the newly descriptive video of the SEACOLORS project.
- Increased the number of updates of news per month

The impact of the actions is measured by the number of hits on the website. The website has received 21,305 visits during this period. In the following table it can be observed the improvement of the dissemination of the projects action with the increase of the news published on the web. Also it can be observed the diffusion of the project on social networks.
Indicators of progress | September 2015 | February 2016 | December 2016 | Total visits at the end of the project |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of web visits</strong></td>
<td>2,015</td>
<td>10,182</td>
<td>16,686</td>
<td>21,305</td>
</tr>
<tr>
<td><strong>Number of updates and news sent monthly</strong></td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Twitter</td>
<td>61</td>
<td>79</td>
<td>138</td>
<td>143</td>
</tr>
<tr>
<td>Facebook</td>
<td>24</td>
<td>141</td>
<td>197</td>
<td>198</td>
</tr>
<tr>
<td>LinkedIn</td>
<td>7</td>
<td>14</td>
<td>91</td>
<td>93</td>
</tr>
</tbody>
</table>

Table 18. Progress indicators

Through Facebook, the partner ALGAplus achieved a very high dissemination of the project reaching over 1000 people per SEACOLORS-related publication.

Through the web we have received different asks for algae pigments from students in order to facilitate the pigments in doctoral thesis investigations.

In addition, in order to maximize the projects dissemination, the Layman’s reports in all the three languages created (English, Spanish, and Portuguese) were published in the English and Spanish versions of the website. Also, an abbreviated version of the Final Report was uploaded on the website in both existing versions (English and Spanish).

**Conclusion:** The action was developed according to the time plan successfully. The change in the design of the website was considered successful.

**Action conclusion:**

The website of the project was a success and helped the proper dissemination of its information and results, and it also served as means of contact with interested companies and institutes.

**Deliverables:**

- **D.D.2.2. Information about the project in each partners website** delivered within the Inception Report
Action D.3. PROJECT NOTICEBOARDS

(September 2014 to September 2016)

The main objective of this action was making two noticeboards, one at the beginning and one at the end of the project, to help improve the dissemination of the actions and results.

They were created:

- At the beginning of the project: containing general information about the project and the partners. At the end of the project: containing information about project results demonstrated and also the final conclusions (see Dissemination Annex 8. D.D.3.2. Noticeboard (Results of the project).

AITEX and the associated beneficiaries placed the notice boards in their facilities in order to be showed to all the customers, suppliers and associated SMEs.

1st Noticeboard

AITEX

ALGAPLUS

BEA

ASEBIO

2nd Noticeboard

Conclusion: The project’s noticeboards were created on time and presented in the beneficiaries’ facilities and events if possible. The action developed and finished successfully.

Action conclusion

The noticeboards were shown permanently in a very visible area of the partners’ facilities. Noticeboards were shown in events and conferences when possible.

Deliverables

- D.D.3.1. Noticeboard (Beginning of the project) delivered within the Inception Report
- Dissemination Annex 8. D.D.3.2. Noticeboard (Results of the project)
Action D.4. LAYMAN’S REPORT  
(December 2016 to December 2016)  
The aim of this action was to create a final project report, produced on paper and electronically, presenting the objectives, actions and results to the general public.

This report analyses the reasons for the project’s implementation, along with its objectives and main achievements, followed by a description of the actions carried out and its impact, finishing with the most relevant outputs, results and conclusions.

**Conclusion:** The Layman’s report was successfully realized (1,000 copies) and disseminated. It is considered that this action has been completed successfully and with no delay.

**Action conclusion**

The Layman’s report was distributed successfully electronically to a contact list and also presented in the website of the project, and it helped in a better transmission of information concerning the evolution and the achievements of the project. The copies were sent successfully to clients, collaborators, partners for each partner of the project.

**Deliverables**

- **D.D.4.1. Layman’s report - ENG / PT / ES (Dissemination Annex 1)**
The situation of the deliverables (as defined in the Grant Agreement) at the end of SEACOLORS implementation (December 31th 2016) is shown below:

<table>
<thead>
<tr>
<th>Deliverable name</th>
<th>Action</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalgae and macroalgae selection for dye extraction</td>
<td>A.1.1</td>
<td>Completed (delivered with Midterm Report)</td>
</tr>
<tr>
<td>Microalgae and macroalgue biomass production at lab scale</td>
<td>A.1.2</td>
<td>Completed (delivered with Midterm Report)</td>
</tr>
<tr>
<td>Microalgae and macroalgue pigment characterization</td>
<td>A.1.3</td>
<td>Completed (delivered with Midterm Report)</td>
</tr>
<tr>
<td>Ancient dyeing process and auxiliaries employees</td>
<td>A.1.4</td>
<td>Completed (delivered with Inception Report)</td>
</tr>
<tr>
<td>Microalgae/ Cyanobacteria production at pilot scale, Effect of culture controlled conditions of pigment/dye yields and quality</td>
<td>B.1.1</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Report of macroalgae production in a IMTA unit</td>
<td>B.1.2</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Report of the dye extraction</td>
<td>B.2.1</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Report of results of dye characterization</td>
<td>B.2.2</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Protocol to dye cotton with new dyes</td>
<td>B.3.1</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Results of tests done to fabrics dyed with the new dyes</td>
<td>B.3.2</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Report of the analysis of waste water with natural colors</td>
<td>B.3.3</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Reports on the results of dyeing on an industrial scale.</td>
<td>B.3.4</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Enviromental Impact report</td>
<td>C.1.1</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Socio-economic Impact Report</td>
<td>C.2.1</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Dissemination and communication plan</td>
<td>D.1.1</td>
<td>Completed (updated version attached to Midterm Report)</td>
</tr>
<tr>
<td>Newsletters and dissemination material 2014 and 2015</td>
<td>D.1.2</td>
<td>Completed (attached to Midterm Report)</td>
</tr>
<tr>
<td>Newsletters and dissemination material 2016</td>
<td>D.1.3</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Publication, press realeases, mailings</td>
<td>D.1.4</td>
<td>Completed (updated version attached to Final Report)</td>
</tr>
<tr>
<td>Divulagation material</td>
<td>D.1.5</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Website of project</td>
<td>D.2.1</td>
<td>Completed (attached to Inception Report)</td>
</tr>
<tr>
<td>Partner information in website</td>
<td>D.2.2</td>
<td>Completed (attached to Inception Report)</td>
</tr>
<tr>
<td>Notice board (Beginning of the project) showed in beneficiarie’s facilities</td>
<td>D.3.1</td>
<td>Completed (attached to Midterm Report)</td>
</tr>
<tr>
<td>Notice board (Results of the project) showed in beneficiaries’s facilities</td>
<td>D.3.2</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Layman´s report</td>
<td>D.4.1</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Consortium agreement and meeting Calendar</td>
<td>E.1.1</td>
<td>Completed (updated version attached to Final Report)</td>
</tr>
<tr>
<td>Afterlife communication plan</td>
<td>E.2.1</td>
<td>Completed (attached to Final Report)</td>
</tr>
<tr>
<td>Database with some information of main LIFE project</td>
<td>E.3.1</td>
<td>Completed (updated version attached to Final Report)</td>
</tr>
<tr>
<td>Report of the final event</td>
<td>E.3.2</td>
<td>Completed (attached to Final Report)</td>
</tr>
</tbody>
</table>

Table 19. Project deliverables
5.3. Evaluation of Project Implementation

The methodology applied for this LIFE+ Project is considered successful, and the approach of this initiative of interest for the textile (finishing) and biotech sector, and as well for the eco-fashion sector, because it will contribute to solve social trends related to ecological textiles.

No limitation was considered at the beginning of the project, in terms of selection of the algae under evaluation. In fact, SEACOLORS consortium analyzed if different algae can be used to obtain textile dyes considering two different textile finishing methods to apply this dyes: dyeing and printing. Two different types of textile materials were used to apply the dyes: cotton and wool (natural material in both cases).

The project focused on studying the method of obtainment of natural dyes from algae and their textile applications in dyeing and printing processes. It is important to highlight that SEACOLORS partners are highly complementary, the role of every partner was clear since the beginning of the project and no overlapping in demonstration activities occurred.

The first actions (B.2 and B.3) of the project, related to the validation and optimization of the best method of colorant extraction and its application as a dye were extremely important in terms of the optimization process. Several algae and their characteristic colorants produced by BEA and ALGAPLUS were studied in dyeing and printing processes by AITEX. The obtained fabrics were characterized in terms of quality, by measurement of fastness to light, laundering, rubbing order to determinate the best method for dyeing/printing process. The tests revealed successful application of algal pigments to the two tested natural fabrics.

It wasn’t easy to measure the environmental impact by a quantitative study, in this case were compared the dyeing processes using synthetic dyes and natural dyes, but the color (tone and nuance) obtained was different depending on the dye origin (algae strain and cultivation process optimization), and consequently their wastewater had different characteristics.

On the other hand, in order to measure the environmental impact it was agreed to define an LCA as a methodology for the evaluation of the environmental load of a process or activity by means of all inputs and outputs involved during the entire life cycle of the process or activity itself. The results of the analysis revealed that globally the project’s action generated a significantly low impact.

The socio-economic impact was analysed by a quantitative study of advantages and savings of the new dyes used in comparison with synthetic dyes. It resulted that due to the low popularity of the eco-trends at global level, in all industries, the prices were higher than those of synthetic colorants. Due to the increasing awareness of sustainability concepts, a favorable forecast can be confirmed regarding to the popularity of the newly obtained natural dyes.

The following table summarizes the results achieved compared to the objectives:
## Results achieved:

<table>
<thead>
<tr>
<th>Task</th>
<th>Foreseen in the proposal</th>
<th>Achieved</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.1</td>
<td>To study and select of algae with dye capacity.</td>
<td>Selection and assay for growth and pigment/dye from different algae (micro and macroalgae) based on the BEA and ALGAPLUS knowledge. Different Microalgae/ cyanobacteria (8 species) and macroalgae (6 species) were selected. (Deliverable A.1.1) Biomass production for each micro/macroalgae was obtained at lab-scale. (Deliverable A.1.2) A method of dye characterization according to the chemical compound was defined. (Deliverable A.1.3)</td>
<td>In general terms, the results obtained are the expected. The deliverables (D.A.1.1, D.A.1.2, D.A.1.3 and D.A.1.4) were elaborated on time. Indicators: 100% executed</td>
</tr>
<tr>
<td>B.1</td>
<td>To study the algal biomass production and to increase the dye substance contained.</td>
<td>8 microalgae/cyanobacteria have been monitored for pilot scale possibilities. Definition of limiting factors for algae growth. Cultivation of microalgae/ cyanobacteria in a low cost system by BEA. (Deliverable B.1.1) Production of macroalgae by ALGAPLUS. (Deliverable B.1.2)</td>
<td>The information obtained was extremely profitable in order to carry out the experimental activities comprised in Actions B2 and B3. 8 microalgae/cyanobacteria strains were grown at pilot scale and biomass was produced and delivered constantly; 6 macroalgae species were grown at Pilot scale and biomass was produced and delivered permanently; Cultivation techniques for each strain/species were implemented and increase biomass/pigment quality in a reproducible form was confirmed. Indicators: 100% executed</td>
</tr>
<tr>
<td>B.2</td>
<td>The aim of this action was to extraction of dye molecules from different micro and macroalgae.</td>
<td>Definition of extraction protocols from microalgae/ cyanobacteria and macroalgae depending on the dye molecule. Obtaining red, blue and yellow dyes. (Deliverable D.B.2.1) Characterization of dyes molecules. (Deliverable D.B.2.1)</td>
<td>3 colors (red, blue, yellow) were obtained of each type of algae (macroalgae, microalgae and cyanobacteria) in the optimization studies. Dyes molecules studied were: Phycoerythrin, Phycocyanin and Carotenoids. Increased yields were obtained by modifying the conditions of extraction. Reproducibility of colour was validated from different batches of same algae species. The dyes obtained have been sent to AITEX in order to carry out the experimental activities comprised in action B3 and B4. Indicators: 100% executed</td>
</tr>
<tr>
<td>B.3</td>
<td>The main objective of this action was the demonstration and validation of the application of the dyes in dyeing and printing processes.</td>
<td>Definition of the dyeing process, including pre-treatment to increase the process efficiency. Development and characterization of the dyed and printed natural fabrics (cotton and wool). Optimization of the dyeing and natural printing processes. (Deliverable D.B.3.1) In addition, tests which demonstrate added value of the dyed/printed fabrics were developed. (Deliverable D.B.3.2)</td>
<td>Printing process was included in the project as a variant of the dyeing process, because this process allows to obtained different designs. The dyeing and printing processes were optimized, in terms of process parameters, as temperature and time. The experiments showed better results with the employment of the liquid pigments. Optimum dyeing process and protocol was established and validated (65ºC process for 60 minutes). Optimum printing process protocol, involving natural auxiliaries, was developed and</td>
</tr>
<tr>
<td>Task</td>
<td>Foreseen in the proposal</td>
<td>Achieved</td>
<td>Evaluation</td>
</tr>
<tr>
<td>------</td>
<td>--------------------------</td>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td></td>
<td>To certify the quality of the waste water obtained after dyeing with the new natural dyes developed in the project it has been analyzed using the DOB, COD and pH tests. (Deliverable D.B.3.3)</td>
<td>validated; Wastewater analysis, involving parameters of quality were performed. (BOD and COD). The wastewater obtained was compared with the one generated by the synthetic correspondents; Dyeing tests at semi-industrial level were performed and the process was validated.</td>
<td>Indicators: 100% executed</td>
</tr>
<tr>
<td>C.1</td>
<td>To measure the environmental impact by a quantitative study of advantages and savings generated by the natural dyes in comparison with the synthetics dyes.</td>
<td>Study the environmental impact for dye production. Study the environmental impact between dyeing process using natural dyes or synthetics dyes. Life Cycle analysis. Study on the wastewater effluents of natural vs. synthetic dyes. Study on the reuse of the remaining biomass. (Deliverable D.C.1.1)</td>
<td>Assessment of the environmental impact generated by the algae production was realized and resulted in neutral impact, with important benefits. The employment of algal dyes revealed more sustainable than the synthetic dyes, according to the natural vs. synthetic dyeing/printing process impact assessment. Wastewater effluents form natural dyeing and synthetic dyes were compared. The reuse of the remaining biomass after the primary extraction was studied and revealed further active compounds that could be used. Circular economy application was demonstrated. Indicators: 100% executed</td>
</tr>
<tr>
<td>C.2</td>
<td>To measure the socio-economic impact by a quantitative study of advantages and savings of the natural dyes in comparison with synthetics dyes.</td>
<td>Study of the socio-economic impact of obtaining dyes from algae. Study the socio-economic impact between dyeing process using natural dyes vs. synthetics dyes. (Deliverable D.C.2.1)</td>
<td>The socio-economic impact was assessed through the realization of: • Market studies concerning advantages and inconveniences and perspectives for the employment of the new natural dyes obtained. Market studies on production and employment costs were realized on synthetic, natural (plant origin) and natural (algal) dyes. • Surveys applied to: textile industry specialists, algae producers and consumers. (126 surveys applied). Indicators: 100% executed</td>
</tr>
<tr>
<td>D1</td>
<td>To create a Dissemination and Communication Plan, which should outline and structure all the dissemination activities in detail. (Deliverables D.D.1.1, D.D.1.2, D.D.1.3, D.D.1.4 and D.D.1.5.)</td>
<td>All the dissemination actions have been performed on time. (Deliverables D.D.1.1, D.D.1.2, D.D.1.3, D.D.1.4 and D.D.1.5.)</td>
<td>This task has been achieved successfully and its results have been used during dissemination tasks. The dissemination actions have met the main stakeholder’s textile, chemical, algae producers, water purification companies and institutions. Indicators: 100% executed</td>
</tr>
<tr>
<td>D2</td>
<td>Construct a website and keep updating with the results of the project to widen the dissemination and results transfer at the end of the project on all the targeted audience. The web site has been performed and maintained. (D.D.2.1 and D.D.2.2.)</td>
<td>The website was used as dissemination and management tool by the consortium. Indicators: 100% executed</td>
<td></td>
</tr>
<tr>
<td>Task</td>
<td>Foreseen in the proposal</td>
<td>Achieved</td>
<td>Evaluation</td>
</tr>
<tr>
<td>------</td>
<td>--------------------------</td>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td>D3</td>
<td>Design and print two Noticeboards and show them in the partner’s facilities, one at the beginning of the project and the other at the end.</td>
<td>Were designed, printed and posted the two noticeboards expected. (Deliverables D.D.3.1. and D.D.3.2)</td>
<td>Both of the noticeboards were done, the evaluation is positive because it can be seen in the description of the activities all partners show the noticeboard in their facilities. Indicators: 100% executed</td>
</tr>
<tr>
<td>D4</td>
<td>Layman’s Report Producing 1000 copies of the report containing the results achieved by the project</td>
<td>The Layman report was edited with information about main results obtained in the project, in English, Spanish and Portuguese. (Deliverable D.D. 4.1.)</td>
<td>The Layman’s Report has served as a dissemination tool for general public. Indicators: 100% executed</td>
</tr>
<tr>
<td>E1</td>
<td>Relationship and reporting to the EC. (1 Inception, 1 Mid Term and 1 Final Report); Project Financial and Administrative Control; Coordination between partners; Analysis and monitor of the data obtained from the project in each task, ensuring all the specifications required are met; Consortium Agreement Regular meeting Calendar.</td>
<td>Management activities have been carried out successfully, coordination and communication between partners was very fluid.</td>
<td>The schedule proposed in the project has been met; the analysis and monitoring of tasks were carried out by the consortium. Indicators: 100% executed</td>
</tr>
<tr>
<td>E2</td>
<td>To create an After LIFE Communication Plan</td>
<td>The After LIFE Communication Plan was performed at the end of the project.</td>
<td>The report will serve to outline the proper exploitation of the results and also the plan for communicating the project results after the end of the project. Indicators: 100% executed</td>
</tr>
<tr>
<td>E3</td>
<td>Make Life project data base; Create 4 Newsletters; Participate in other Networking events; Organize the project’s Final Event.</td>
<td>The deliverables have been performed on time. Newsletters enclosed all the information about last actions. Networking with other LIFE projects.</td>
<td>A database with information of projects that share interests with seacolors project was created; The events were successful with more attendants than expected and good feedback. Contacts established; The 4 newsletters were sent every 6 months. Contact from companies in the field of textile application contacted to identify the possibility of the application of the new algal pigments in their industry.</td>
</tr>
<tr>
<td>E4</td>
<td>Project Audit</td>
<td>Audit performed.</td>
<td>An external firm performed an audit regarding the financial expenses of all partners.</td>
</tr>
</tbody>
</table>

Table 19. Results achieved
Results that have been immediately visible and which results will only become apparent after a certain time period.

The first result is the selection and production of different algae (microalgae/macroalgae) with dye capacity. During the first part of the project it was achieved the method of algae production and the optimized dye/pigment extraction. The dyes/pigments obtained were characterized in order to meet their dyeing properties.

The second result obtained was the definition of the optimum dyeing process and the approach of the process variables. At the same time in the finishing textile processes was incorporated the optimized printing process, including natural components of the process. These optimized processes can be applied to the textile industry, due to the fact that requires the conventional machinery.

The problem of time arises in the scaling up of the process due to the fact that there are very few companies capable of producing and extracting high quantities of this new, natural pigment. As the eco-fashion gains more popularity, the demand for natural pigments, obtained from a total sustainable source will increase, generating high quantities, at market competitive price of this new raw material for the textile industry.

Effectiveness of the dissemination

All dissemination actions carried out in the project had a high efficiency, providing the main stakeholders knowledge about the project and subsequently the results. As the project results have great impact on several actors of society, we have shared information with big, medium and small companies, researchers and research institutions, universities, professors and students.

SEACOLORS consortium extended its participation in events abroad. Institutions participating in this project have an active attendance to events specialized in the area of algal production and manipulation, biotechnology, where algae products are a topic of interest, as well as the wastewater effluents. Through fairs, congresses and conferences, we contacted with many people interested in the project. The promotional material such as leaflets, posters, newsletters, samples, USBs and Layman’s Report distributed to these contacts with stakeholders have been one of the means to capture the interest. The articles published in specialized magazines and in congresses also have generated interest among stakeholders.

SEACOLORS didn’t have major drawbacks, all channels were open for a very fluid communications, media channels are infinite, and there is a great interest in environmental solutions.

On the other hand, in order to monitor the impact and environmental awareness of the main stakeholder’s regarding the dissemination activities of this project, were carried out dissemination surveys, by AITEX. The results and the surveys format can be consulted in Dissemination Annex 11. Surveys

The dissemination surveys show that reception and knowledge of the project was there since the beginning over the Life + programme, and all the information regarding the project implementation was received openly, stakeholders have accessed to project advances and results through the several channels (website, dissemination material, social networks, face to face meetings, etc.).
5.4. Analysis of long-term benefits

1. **Environmental benefits**

   a) **Direct / quantitative environmental benefits:**
   Several environmental benefits can be considered with regard to SEACOLORS project:
   - Reducing the use of synthetic dyes in dyeing process. This reduction refers to different benefits:
     - Decrease the synthetic dyes production, which are obtained from non-renewable resources like petrol.
     - Decrease the natural, plant origin, dyes production which occupies large arable lands.
     - Decrease of the polluted wastewater effluents generated in the process, because natural dyes are more biodegradable.
   - Lower CO\textsubscript{2} emissions due to production of algae.
   - Inclusion of the textile industry in a circular economy concept, by reutilization of remaining biomass.

   As a concluding remark, by replacing the synthetic dyestuff with a natural, sustainable new one, we generate a reduction in their demand, which leads to a reduction in their production, which implies a reduction in the generation of CO\textsubscript{2}, to which we add less polluted water and less cost of water treatment, leading in this way to environmental benefit.

   The innovation of SEACOLORS project is to use the newly obtained raw material in other fields to solve some of the pollution problems of the chemicals in textile industry. The advantages of dyeing/printing with algal dyes to current techniques of textile finishing is that apart from the more environmentally sustainable compounds and processes employed, the process also allows the reduction of residual water coloration.

   b) **Relevance for environmentally significant issues or policy areas**

   Textile industry is energy, water and chemical-intensive: from 15 -19\% of total costs in the wet textile processes are energy costs. In 2009, 954 million Kg. of textile were produced in Europe, requiring between 40-386 l. of high quality water to produce 1 Kg. of textile. Up to now, due to the new trend of environmentally friendly concept, natural colorants, originating from plants have started to be researched and employed in this most polluting industry, in order to lower the emissions levels. An industrial-scale, technically-viable recovery technology has not been developed, given that it has been always assumed that the dyes would be irretrievably lost. But, due to their cultivation limitations, generated by emissions of CO\textsubscript{2}, employment of massive arable land surfaces, a new solution was demanded. This solution is represented by the algal pigments, which present sustainable cultivation and employment. The main difference with the technology outlined in SEACOLORS is the cultivation process, which does not involve the employment of fresh water, neither arable land, and the pigment obtention does not emit CO\textsubscript{2} or other polluting compounds. On the other hand, after the colorant extraction, is not necessary a further treatment of the pigment, because it can be used directly as obtained. By means of sustainability, it was revealed that it can be realized a secondary processing of the remaining biomass, obtaining a secondary pigment with added antioxidant value, or different active compound which could be applied in food, feed or cosmetics. Like this, by producing one material, several final products can be sustainably obtained.
By means of this project it is intended to solve the problem of reducing the quantity of chemical products used in textile industry by replacing them with sustainable and renewable ones and moreover reducing the pollutants in waste waters.

Therefore, it is foreseen to obtain respectful solutions with the environment in all the phases of its conception and utilization. Furthermore, the project contributes to the application of the politics and community legislation regarding waste waters and more specifically, the normative, 2008/105/CE on environment quality in the field of water policy which sets the maximum allowable priority substances and other chemical contaminants and also in particular the REACH regulation.

2. Long-term benefits and sustainability

a. Long-term / qualitative environmental benefits

Regarding the long-term qualitative environmental benefits associated to SEACOLORS project it must be pointed out that this initiative is demonstrating that algae can be a source of dyes and, the optimization of the production methods can be increase the dyes concentration in algae. This is important if we think in an industrial production for the future.

SEACOLORS project has been focused on obtaining naturals dyes from algae for industrial application. These natural dyes were demonstrated to be used successfully in different textile processes: dyeing and printing. The optimized processes, employing natural, renewable raw materials, involve less energy consumption, due to lower temperature of the dyeing process and treatment of the printing one, and they generate less colored and polluted wastewater effluents. So, it will be understood the positive impact on the European Textile Sector related to SEACOLORS project, in terms of new and sustainable raw material introduction in the process and a generation of a purified wastewater effluent.

As the textile industry is energy, water and chemical-intensive user, it generates a major pollution of the fresh water sources, through the synthetic dyes that remain unfixed after the dyeing process in the waste water effluent. The main aspect in term of wastewater effluent purity is represented by the biodegradable compounds added to the process, and the natural auxiliaries involved. In terms of process parameters, the working temperature also generated less energy consumption, due to lower heating of the dyeing bath needed.

This project intended to solve the problem of reducing the quantity of chemical products used in textile industry by replacing them with new and renewable ones from a vast resource origin, algal biomass. The algal biomass production and pigment extraction processes have been proved to be one of the most sustainable ones from the textile industry.

Another important environmental benefit can be revealed by the CO₂ emissions reduction. As the synthetic dyes are produced using oil, petroleum products, all the recovered dyes will impose a reduction in the production of the synthetic dyes, therefore, a reduction of CO₂ generation. On the other hand, when referring to the natural dyes from plant origin, the necessity of arable land is totally eliminated, and there is no generated the competition for food, which is defined by the need of fresh water (close to the arable land). No fresh water is needed for the cultivation of algal biomass.

When referring to the whole process of cultivation and employment of algal biomass, it can be stated the application of the three R’s (Reduce, Reuse, Recycle) of the environmental
protection policy. Within SEACOLORS project is achieved the reduction of pollutant emissions, the remaining biomass, after colorant extraction, can be reused for further extractions of compounds of interest and the main input in the algal biomass cultivation is represented by recycled aquaculture water as growth medium.

b. Long-term / qualitative economic benefits

Regarding long-term qualitative economic benefits, the following issues are pointed out:

- Cost saving for the production of synthetic dyes from petrol or other non-renewable materials. These raw materials are imported from countries with problems and the cost and availability are not stable.
- Cost saving for the textile industry, because of the usage of a product nationally produced (Spain), and the generated wastewater has lower pollutants. Also lower costs generated by the energy need of the processes.
- New market for algae production companies. Spain and Portugal are surrounded by sea and this kind of company can be very important in this area to produce dyes and other interesting products.

c. Long-term / qualitative social benefits

Regarding long-term qualitative social benefits, the following issues should be pointed out:

- This project will contribute to solve social concerns related to eco-fashion. This is a part of the growing design philosophy and trend of sustainability, the goal of which is to create a system which can be supported indefinitely in terms of human impact on the environment and social responsibility.

- If extrapolating the already mentioned environmental benefits, direct effect in human health can also be the reduction of $\text{CO}_2$ emissions generated by the production of synthetic dyes. And also the fight for food, which involves necessity of arable land and fresh water, are reduced when producing natural algal biomass.

d. Continuation of the project actions by the beneficiary or by other stakeholders.
Regarding the continuation of the project, it is expected to enlarge the network of stakeholders related to the main objective of the project, so results obtained could be implemented in the short time at industrial level. AITEX will promote the use of natural dyes in dyeing and printing process to obtain new eco-textiles. AITEX is continuing this research line focused on natural dyes from algae. We are considering applying for another project funded by the EC where we want to involve SMEs able to bring the products to the market. At this stage we are thinking in contacting one SME that wants to submit a SME Instrument in order to develop and commercialize the industrial algal pigments and their textile applications.

As for ASEBIO, the organisation is awaiting the final resolution of a project where they had planned to hold conferences calling the attention of society and consumers to particular areas of biotechnology, including the idea of dealing with applications and opportunities of marine biotechnology. Another action arising from the project was the collaboration with agents identified in the networking network. In this line, a project invited them to participate in an event that will take place in June 2017, where an Industry Conference with exhibition is back-to-back with an Open Science Meeting on Marine Microbiology.

3. **Replicability, demonstration, transferability, cooperation:**

SEACOLORS’ methodology of dye production from algae could be implemented to solve the environmental problems of other sectors or industries that use synthetic materials, because algae allow extracting different molecules that can be interesting in sectors like cosmetic, pharmaceutical, food/feed, energy, etc. Dissemination activities will be performed in these sectors also after the end of the project.

Microalgae and Macroalgae are an economically important source of biomolecules and metabolites and constitute a direct source of nourishment, medicine and biofuels, among others. Dyeing and printing processes studied in the project are applicable to any textile industry, because the studied are being performed in the same conditions, and may be these pigments will be applicable in leather industry.

- The main limitation is the cost of algal pigments.

It is possible to find companies focused in manufacturing of algae, but most of them are concentrated in the Asian part, due to historical employment. In Europe, resources and capacities already exist and they are considered viable from economical point of view. The main limitation is represented by the industrial level of algae production focused on specific extraction as compounds of interest to be used in food, feed and cosmetics. Due to the fact that this is an emerging field and this new raw material with various applicability in industry is getting researched and discovered from only a short period of time, the market demand, compared to the correspondent synthetic compounds, used nowadays, did not reach a competitive level.

The awareness of the sustainability concept due to resource scarcity and pollution must be supported, in order to increase the knowledge regarding the nowadays problems. By informing the population, the demand for more sustainable industry will increase, and in this manner the companies would have a higher susceptibility of implementing the Corporative Social Responsibility (CSR). This is a chain reaction which finally will have an impact in this new product’s popularity and accessibility. The higher the demand, generates higher competition and production, and influences the lower final cost.
The final conclusion is the increasing awareness campaigns, support of the research process and diffusion of the results of the applicability and viability of projects like SEACOLORS, which already has awaken interest in the area, due to the fact that these pigments can be generally implemented in already existing textile finishing technologies.

In order to impulse the development at industrial level AITEX published a demand of partnership in the European network EEN.

- Other limitation is the industrial level of pigment extraction. Through diffusion and networking actions, SEACOLORS’s project was contacted by companies capable of producing and extracting high quantities of algal pigments. The main solution would be the projects’ results transfer in order to raise awareness of this possible solution to a part of the pollution problems generated by the textile industry. These results must be disseminated through already created industrial facilities of algae production, and with the guidance provided by the After LIFE + Communication plan (D.E.2.1.).

- Sustainable consumer awareness
  This is the most important parameter, because if there is a consumer demand willing to pay a reasonable cost, the industry will offer this product.

4. **Best Practice lessons**

The results obtained within SEACOLORS project are relevant in terms of a new application of algae, obtaining dyes from a natural source (algae) and also in terms of new dyeing/printing process more sustainable. For this reason, SEACOLORS consortium will consider within the after-actions of the project to get in contact with EC working groups dealing with best practices guidelines for environmental issues.

The Best practice lessons were developed during 2016, through comparisons of the dyeing/printing process with natural and synthetic dyes in terms of the pollutants, water and energy consumption, etc. The results revealed a significant positive impact in the employment of natural algal pigments in the same conventional processes developed nowadays in the industry. The application of these newly obtained pigments does not involve extra efforts nor costs, in terms of implementation in the industries facilities.

5. **Innovation and demonstration value**

SEACOLORS methodology can be defined as relatively innovative. It is important to note that algae is a source of interesting compounds, and there are a lot of initiatives to obtain different products from algae in sectors like food, cosmetic, energy but previously to the development of this project, no other initiative was known regarding natural dyes extracted from algae for dyeing textile process. Some other strategies like biofuel, biomass production for food/feed obtaining from algae are the most common known.

The use of the AITEX dyeing/printing pilot plant (semi-industrial capacity) and the use of industrial plant from industrial AITEX partner allow assuring the demonstration value of the project. In addition, the algae production pilot plants employed by BEA and ALGAPLUS are being used nowadays for investigation and semi-industrial production.

6. **Long term indicators of the project success**

Below are listed the long term indicators of the project success:
- Kg of algae produced
- Kg of dyes obtained
- Kg of dyed/printed textiles
- Number of companies that introduce the new pigments in their production systems
- Nº compounds further extracted from the ´waste´ biomass remaining after the primary pigment extraction
- Nº different applications/industries where the secondary obtained compounds are applied