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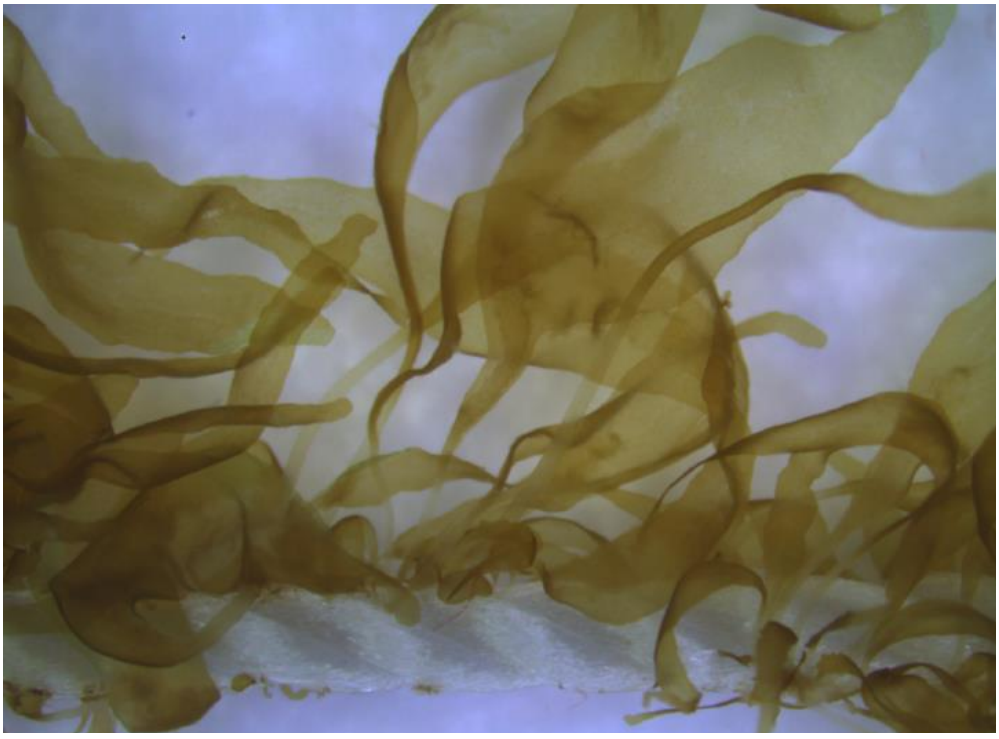
Report

D5.1 Industrial production line for seedlings

MACROSEA WP5

Author(s)
Morten Omholt Alver
[Co-Authors]



SINTEF Ocean AS

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SINTEF Ocean AS

Address:
Postboks 4762 Torgarden
NO-7465 Trondheim
NORWAY

Switchboard: +47 46415000

Enterprise /VAT No:
NO 937 357 370 MVA

Report

D5.1 Industrial production line for seedlings

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AUTHOR(S)
Morten Omholt Alver
[Co-Authors]

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ABSTRACT
The production process for seedlings of macroalgae is analysed with regard to challenges and bottlenecks associated with industrial scale production. Based on issues identified for each sub-process, an action plan with rough estimates of time/cost requirements and proposed prioritization is given as a roadmap to achieve industrial scale seedling production.

PREPARED BY	CHECKED BY	APPROVED BY
Morten Omholt Alver	Torfinn Solvang	Aleksander Handå

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APPENDICES

1 Introduction

This report presents the results of a work assessing today's methods and technology for production and deployment of seedlings of *Saccharina latissima* and *Alaria esculenta*. The production process is evaluated with regard to the potential for large scale industrial production, to identify the limitations and bottlenecks for upscaling. The challenges that are identified are classified with regard to complexity and used to form an action plan.

2 Methods

The information gathering in this work was primarily done in two stages. First, a search for scientific literature within technology for seaweed cultivation was done along with a questionnaire on cultivation technology sent out to all industry partners of MACROSEA. The information gathered in the first step was compiled into the report D5.4 State of the art (Alver et al. 2018). Second, a workshop was held at SINTEF Ocean with participation of SINTEF scientists and two representatives from Seaweed Energy Solutions. Hortimare was invited but did not participate. During the workshop, a process map was drawn up of the steps involved in seedling production from gathering of wild sporophytes to deployment of seeded substrates. The various steps of the process were discussed with regard to production protocols, bottlenecks and challenges for upscaling of the production. The output from the workshop was in the form of a memo summarizing the discussions in the meeting.

Based on the gathered information, a roadmap towards an industrial production line for seedlings has been outlined, including a revised process map and a list of technological requirements that need to be fulfilled.

3 Process map of seedling production

The seedling production process, representing today's state of the art, is summarized in Figure 1. A brief description of each step is provided in Table 1.

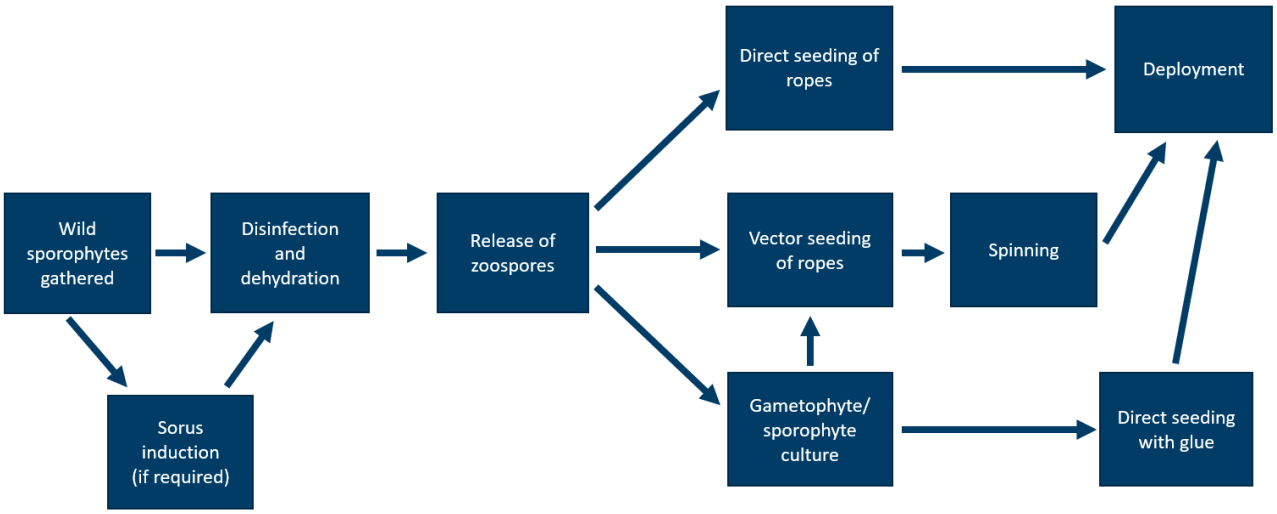

Figure 1: Process map of seedling production, representing today's state of the art.

Table 1: Description of process steps

Process step	Description
Gathering of wild sporophytes	Gathering of wild sporophytes is required to supply the seedling production. The gathering is done manually, utilizing divers or free diving at locations known to contain the appropriate species of macroalgae.

Sorus induction	Removing the growth zone of sporophytes and keeping them under short day conditions in order to stimulate maturation (sorus) and make sporophytes ready to release zoospores.
Disinfection and dehydration	Disinfection of sori to remove contaminating organisms such as cyanobacteria, diatoms and broyozoan. Dehydration to induce osmotic shock to trigger release of zoospores.
Release of zoospores	Dehydrated sori covered with seawater to facilitate release of zoospores. Filtration of water after release to remove remains of sori and tissue debris.
Gametophyte/sporophyte culture	Zoospores can be used to establish gametophyte cultures in red light or sporophyte cultures in white light for the purpose of increasing biomass and increasing the time window for the seeding step.
Direct seeding of ropes	Ropes are sprayed with spore solution or placed in water and exposed to zoospores or gametophytes, which settle on the ropes and grow into seedlings ready for deployment at sea after a period of incubation.
Vector seeding of ropes	Similar to direct seeding of ropes, but done with thin twine that is wrapped around a carrier rope before deployment.
Spinning	The process of wrapping seeded twine around carrier rope before deployment.
Direct seeding with glue	Adding seedlings (gametophytes or small sporophytes) directly to substrate at deployment time, without incubation, using a glue that enables the seedlings to attach to the rope immediately.
Deployment	The process of putting the seeded substrate out in a sea farm for biomass production.

For each process step, the production protocols used by producers outline the methods and inputs to be used; e.g. for *Saccharina latissima* production protocols are described by Forbord et al. (2012) and Forbord et al. (2018).

4 Industrialization and upscaling of process steps

In this chapter, each process step is treated in turn. A brief description is given of each step, and the main bottlenecks and challenges associated with each step are outlined. Challenges and bottlenecks are categorized into the following problem domains: *regulations*, *knowledge*, *quality control*, and *optimization*. Where possible, the severity of each issue is categorized into low, medium and high, and colour coded as green, yellow and red, respectively.

4.1 Gathering of wild sporophytes

So far, the cultivation of seaweed is dependent on wild gathered sporophytes to initiate seedling production. If and when breeding programmes come up and running, this can replace wild gathering. However, this also requires changes to regulations on local vs. non-local varieties.

Gathering of wild sporophytes - challenges and bottlenecks		
Problem domain	**Description**	**Severity**
Regulations	Seaweed producers need to cultivate local varieties. However, the rules are unclear on what is defined as local. Our present knowledge on the biology and how the spores disperse along the coast is not sufficient to define simple rules for this.	High
Knowledge	Local knowledge is required to gather sporophytes.	Low

Quality control	Individual differences in the amount of spores, mainly dependent on maturity level of the sori. Difficult to identify good ones from bad ones.	Low
Quality control	Biofouling of filamentous brown algae (e.g. Ectocarpus) and other species may be found on sori of wild seaweeds.	Medium
Optimization	Gathering sporophytes takes a lot of time, must be done several times during the year and takes up lab space.	Medium

4.2 Sorus induction

Induction of sorus formation is required if sporophytes are not mature and ready to release spores. This is done by cutting off the growth zone (and possibly the top), and holding sporophytes in tanks with winter conditions (10 °C, low light, 8:16 hours L:D photoperiod) for 6-12 weeks (Forbord et al. 2012)

Sorus induction - challenges and bottlenecks		
Problem domain	**Description**	**Severity**
Optimization	Unpredictable time to complete induction may cause challenges for planning in industrial scale production. Sporophytes need to be monitored to detect when they are fertile.	Medium
Knowledge	Variability in amount of spores.	Medium
Quality control	Too long induction will result in release of spores in induction tanks. Some sporophytes can get mature sorus before others and we need a large amount of sporophytes to ensure sufficient mature sori at the same time.	Low
Quality control	Good tools for assessing maturity are lacking.	Medium
Optimization	Stocking density and degree of aeration in the tanks needs standardisation.	Low
Optimization	Requires space over many weeks.	Low

4.3 Disinfection and dehydration

The sorus is disinfected to remove elements like cyanobacteria, diatoms and bryozoan. However, Ectocarpus cannot be removed in this step. After disinfection the sorus is dehydrated to mimic natural conditions that trigger release of zoospores. Dehydration is done by pre-drying (e.g. with paper towels) and keeping sorus at 4 °C over night, which should trigger release of zoospores when they are placed in water due to osmotic shock. Dehydration and release steps can be repeated to obtain more spores.

Disinfection and dehydration - challenges and bottlenecks		
Problem domain	**Description**	**Severity**
Optimization	The amount of disinfectant and the ratio of water to sori are not standardized (the amount of chlorine to sori is the key variable). Sori are probably the most important source of contamination.	Medium
Optimization	The degree of dehydration depends on the duration (24 h is a typical duration). Current protocols may not be optimal.	Low
Optimization	A disinfection protocol for *Alaria esculenta* is missing.	Medium

4.4 Release of zoospores

After dehydration, sori are placed in autoclaved water at 10 °C (enough water to cover the sori). Mature sori will release spores within 30 minutes but can use up to 90 minutes to release enough spores to get a dense spore solution. The water is filtered to separate the remains of sori and tissue debris from the spores.

Release of zoospores - challenges and bottlenecks		
Problem domain	**Description**	**Severity**
Quality control	The number of spores released is highly variable and needs to be measured. Manual counting under microscope is time consuming, optical density (OD) measurement is quick and can be used as alternative.	Medium
Optimization	Standardise OD measurement as density measurement.	Low
Optimization	Repeated use of sorus for spore release should be standardised.	Low

4.5 Gametophyte/sporophyte cultures

Zoospores can be used directly for seeding of ropes but can alternatively be used to establish gametophyte or sporophyte cultures.

The main purpose of using gametophyte cultures is to increase the amount of material available for deployment, or if the species used is fertile at a period not suitable for deployment at sea (e.g Alaria, that is fertile during the spring). Gametophytes grow vegetatively in red light and can be cut into smaller pieces without apparent harmful effects, e.g. using a blender. Gametophyte cultures can be run continuously with regular harvesting of gametophytes. Cultures are typically started in small units containing a nutrient medium and scaled up to units on the order of one litre or less. Aeration and stable pH is preferred. Nutrient medium is replaced periodically, e.g. every 2 weeks, and the culture is homogenised in a blender for upscaling and before seeding.

Sporophytes cultures are produced and maintained in the same way as gametophyte cultures, but are kept in white light to produce sporophytes and are not blended during change of culture medium or before seeding. This method is used when the direct seeding method with glue is used.

Gametophyte/sporophyte cultures - challenges and bottlenecks		
Problem domain	**Description**	**Severity**
Quality control	Gametophytes are prevented from maturing by using red light only (high temperatures can also control maturation). However, one has to periodically ensure that the gametophytes are still able to mature.	Low
Optimization	In cultures newly established from zoospores, gametophytes tend to attach to the glass of culture units and need to be scraped off.	Low
Knowledge	Growth depends on light and nutrient availability. There is so far limited experience with production and use of sporophyte cultures in industrial scale. Production protocols may not be optimal.	High
Optimization	The vegetatively growing gametophytes are regularly broken up into smaller pieces using a blender. The process of filtering, moving to a new container, blending, and moving back is time consuming.	Medium

Optimization	Maintenance of many small cultures is time consuming and takes up space. Significantly bigger units are needed to achieve large scale production.	High
Optimization	Need to use culture containers more suitable for upscaling and automation (e.g. plate cultures or concepts developed for microalgae production).	Medium
Optimization	The conditions for producing sporophyte cultures directly from spores or from already established gametophyte cultures need to be optimised and standardised (time, light, nutrients, aeration and containers).	Low
Quality control	Need methods to measure the amount of gametophytes in cultures. Due to vegetative growth, we need to measure e.g. biomass per volume unit, not number of gametophytes. The use of OD measurement should be standardised for different species.	High
Quality control	Growth rate is hard to predict week by week. Contamination can do a lot of damage through culture crashes. Better quality control is needed. The disinfection step (4.3) has significant effect on the challenges faced here.	Medium

4.6 Seeding of ropes

In this step, ropes are seeded with spores or gametophytes using spraying or bathing methods, or by pouring the spore/gametophyte solution over the ropes. Once these have attached to the ropes and grown to a suitable size, the ropes may be deployed in the sea. The incubation period in the hatchery is typically 6-7 weeks, but this may be shortened. The appropriate size for deployment depends on what time of year they are to be deployed.

Some producers seed carrier ropes directly, while others seed thinner lines that are spun around carrier ropes before deployment. During incubation, filtered and UV-treated deep water at temperatures of 10-12 °C is used. It is uncertain whether extra nutrients might improve results sufficiently to defend extra costs. Light is important in this step, and a variety of light sources have been tested. There is still a need for research on the effects of light regimes and light colours.

Seeding of ropes - challenges and bottlenecks		
Problem domain	**Description**	**Severity**
Knowledge/ Optimization	Density of seedlings on ropes affect final density and size of plants in a nonlinear fashion. More knowledge about choosing optimal density, and how to achieve optimal density, is needed.	High
Quality control	Contaminations of cyanobacteria, diatoms and filamentous brown algae like Ectocarpus originating from the wild sporophyte used to obtain sori, surviving in units since earlier batches or coming through the air. Less problems are associated with ciliates, flagellates, copepods and rotifers.	Medium
Optimization	Space requirements. This is the process step that currently takes up the most space.	Medium
Optimization	Variability of time to deployment. This time is fairly predictable; the risk of crashes is mostly present during the first two weeks.	Low
Quality control	How well seedlings are attached to the rope; dependent on hydraulic conditions in the seeding units. Spores have natural glue, and attach more strongly than gametophytes and juvenile sporophytes.	Low

Quality control	Monitoring of density and size of seedlings on substrate, to ensure high quality of seeded substrate, and choose best time for deployment.	High
Optimization	Design of seeding systems: horizontal or vertically fastened substrate; cylinder tanks vs. flat tanks. Addition and dispersal of spores in tanks.	High
Optimization	Pre-seeding substrate is a potential upscaling bottleneck – direct seeding with glue may be required beyond a certain scale.	High

4.7 Spinning

When vector seeding with twine, the twine needs to be wrapped around and fastened to the carrier rope before or during deployment at sea.

Spinning - challenges and bottlenecks		
Problem domain	**Description**	**Severity**
Optimization	Manual spinning is labour intensive and a clear candidate for automation.	High
Quality control	After spinning there are so far no possibilities for quality control before the performance of the seedlings can be evaluated >1 month later.	High
Optimization	It may be necessary to do the spinning on land (not onboard a vessel), which necessitates storage and transportation of ropes before deployment.	Medium

4.8 Direct seeding with glue

Direct seeding of gametophytes and microscopic sporophytes with glue might be a better approach than vector seeding with ropes or other materials as there is no need for incubation of the seeded substrates. The main factors to take into consideration are the availability of effective glue, suitable substrates that can be used with it, and an efficient process for applying the glue and seedlings at deployment.

Some farmers have tested brushing the sporophytes onto the rope in order to force them into the fibers. This strategy might be investigated further as an alternative to glue.

Direct seeding with glue - challenges and bottlenecks		
Problem domain	**Description**	**Severity**
Knowledge	Established products and protocols exist, but are commercial and associated with IP costs	Low

4.9 Deployment

The actual deployment process is not covered by the scope of this report. Special concerns regard the timing, i.e. the time from spinning to deployment of substrate in the sea and the storage conditions.

5 Action plan

Based on the challenges and bottlenecks identified for the seedling production steps, we have defined a number of action items in the form of process changes, research needs and technology gaps. These are listed in Table 2 and Table 3.

Where applicable, tentative estimates are given to indicate the time (and cost) associated with addressing each of the challenges. These are classified as shown in the following tables:

Time horizon	Description
MACROSEA	The challenge is partly addressed within the MACROSEA project
1-2 years	A project period lasting 1-2 years is required to address the challenge
2-5 years	A project period lasting 2-5 years is required to address the challenge
>5 years	A project period lasting longer than 5 years is required to address the challenge

For each action item, a priority level is suggested:

Priority	Description
Low	Recommend low priority
Medium	Recommend medium priority
High	Recommend high priority

Table 2: Action items

#	Process step	Type	Description	How to achieve	Time horizon	Recommended priority
1	Gathering of wild sporophytes	Process change	Today's approach can support a significantly increased production level. However, beyond that limit, the production cycle needs to be closed in order to eliminate the step of gathering wild sporophytes.	Need to supply production through gametophyte/sporophyte cultures. This will open the possibility of breeding to improve performance of farmed seaweed. However, regulations need to be adjusted to allow non-local varieties. This requires research to establish the necessary knowledge on biology, genetics and dispersal of spores to support such a relaxation of regulations.	> 5 years	High
2	Sorus induction	Research need	Optimization of culture conditions and protocols	Research project: Laboratory tests to find optimal conditions and stocking density.	1-2 years	Medium [1]
3	Sorus induction	Research need	Increase predictability of time to complete induction	Research project: Early indicators of maturity state, or protocols that increase predictability.	1-2 years	High [1]
4	Sorus induction	Research need / Technology	Increase predictability of amount of spores, or develop instrumentation or method to predict amount of spores.	Research into causes and indicators of variable spore production.	2-5 years	Medium [1]
5	Sorus induction	Technology	Develop tools for objectively assessing maturity	Develop instrumentation based on machine vision or other technology.	1-2 years	Medium [1]
6	Disinfection and dehydration	Research need	Optimization of protocols with regard to dosage of disinfectant and duration and conditions for dehydration. Disinfection protocol is currently missing for *Alaria*.	Laboratory tests to find optimal protocols, especially for *Alaria*.	1-2 years	Medium

7	Release of zoospores	Research need	Develop standardised protocols for repeated use of sorus for spore release	Experimentally determine the efficiency of repeated use of sorus for spore release	1-2 years	Low
8	Gametophyte/ sporophyte cultures	Technology	Methods to remove gametophytes/sporophytes sticking to container walls.	Development project	1-2 years	Low
9	Gametophyte/ sporophyte cultures	Research need	Optimization of culture conditions and protocols	Laboratory tests to find optimal conditions.	2-5 years	High
10	Gametophyte/ sporophyte cultures	Technology	Method to split large gametophytes into smaller pieces.	Investigate methods such as shaking or other options (ultrasound might be considered, but there are indications that it can be harmful to the cells).	1-2 years	Medium
11	Gametophyte/ sporophyte cultures	Technology	Develop larger culture units adapted for efficient handling, automation and low space requirements	Concept development and testing.	2-5 years	High
12	Gametophyte/ sporophyte cultures	Technology	Develop instrumentation to measure amount of spores/gametophytes/sporophytes per ml	Development project on use of OD measurement.	1-2 years	High
13	Gametophyte/ sporophyte cultures	Technology	Develop method to evaluate culture condition and detect contaminations	Research and development project(s) focusing on machine vision, genetic markers or other approaches. Verification through microscopy or other methods required.	>5 years	High
14	Seeding of ropes	Research need	Research into how final density and size of sporophytes depends on density of seedlings on ropes.	Research project, focusing on both hatchery and sea phases.	2-5 years	Medium
15	Seeding of ropes	Research need	Research into how to control seeding process to achieve the desire density of seedlings on ropes.	Research and development project, testing different methods and evaluating results.	1-2 years	Medium

16	Seeding of ropes	Technology	Develop methods and instrumentation to detect contaminants on substrate	Development focusing on machine vision. Verification through microscopy or other methods required.	1-2 years	Medium
17	Seeding of ropes	Technology	Develop methods and instrumentation to monitor density and size of seedlings.	Development focusing on machine vision.	1-2 years	Medium
18	Seeding of ropes	Technology	Design of seeding systems to achieve efficient use of space, efficient handling and automation of seeding systems, and efficient use of spores.	Concept development and testing. Experimental verification of spore usage vs. seedling density on substrate.	2-5 years	High
19	Seeding of ropes	Research need	When seeding with gametophytes, research into conditions that ensure that seedlings are as well attached to substrate as possible	Research project, setting up simulated ocean conditions and developing methods to monitor performance of seedlings. Testing of the effects of culture conditions on performance.	2-5 years	Medium
20	Seeding of ropes	Process change	Beyond certain scales, the step of pre-seeding substrates may need to be eliminated.	Establishment of knowledge and technology for alternative deployment methods, e.g. direct seeding at deployment time using glue.	2-5 years	Medium
21	Direct seeding with glue	Technology	Availability of products and protocols for direct seeding	Research and development project, testing candidate substances for attaching seedlings to substrate, and testing attachment strength and performance of seedings.	2-5 years	Medium
22	Direct seeding without glue	Research need/ Technology	Mechanically brushing sporophytes onto the rope without using glue might be an alternative method of direct seeding.	Research and development project testing methods and verifying biological performance.	1-2 years	Medium
23	Spinning	Technology	Automated spinning of seeded twine around carrier rope.	Development of equipment for automated spinning.	MACROSEA	High
24	Spinning	Technology	Develop method to assess quality of the deployed material (seedings on substrate spun onto carrier rope).	Development focusing on machine vision.	1-2 years	Medium

1: The suggested priority is given under the assumption that gathering of wild sporophytes remains the main source of sori.

Table 3 Action items listed according to time/cost requirements and suggested priorities.

Priority / Time horizon	MACROSEA	1-2 years	2-5 years	>5 years
High	23: Automated spinning	3: Predictability of induction time 12: Biomass measurement of spores/gametophytes/sporophytes	9: Optimization of sporophyte culture conditions and protocols 11: Gametophyte culture units 18: Design of seeding systems	1: Eliminate wild gathered sporophytes 13: Detection of culture condition and contaminations
Medium		2: Optimization of sorus induction 5: Sorus maturity assessment 6: Optimization of disinfection 10: Method to split large gametophytes 15: Control of rope seeding process 16: Detection of contaminants on substrate 17: Monitoring of seedling density on substrate 22: Direct seeding method without glue 24: Quality assessment of deployed seedlings	4: Predictability of spore count from sorus induction 14: Predictability of size and density of seedlings on rope 19: Conditions to ensure that seedlings are well attached to substrate 20: Eliminate pre-seeding of substrates 21: Protocols and products for direct seeding with glue	
Low		7: Protocols for repeated use of sorus for spore release 8: Removing gametophytes/sporophytes from container walls		

6 Conclusion

This report has summarized an evaluation of the production process for macroalgae seedlings with regard to industrial scale production. For each part of the production process, the challenges and bottlenecks facing producers when increasing production scale have been identified. Based on the identified items, a prioritized action plan has been suggested as a roadmap towards industrial production, including process changes, technological developments and research needs. Most of the required actions go beyond the scope of the MACROSEA project.

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