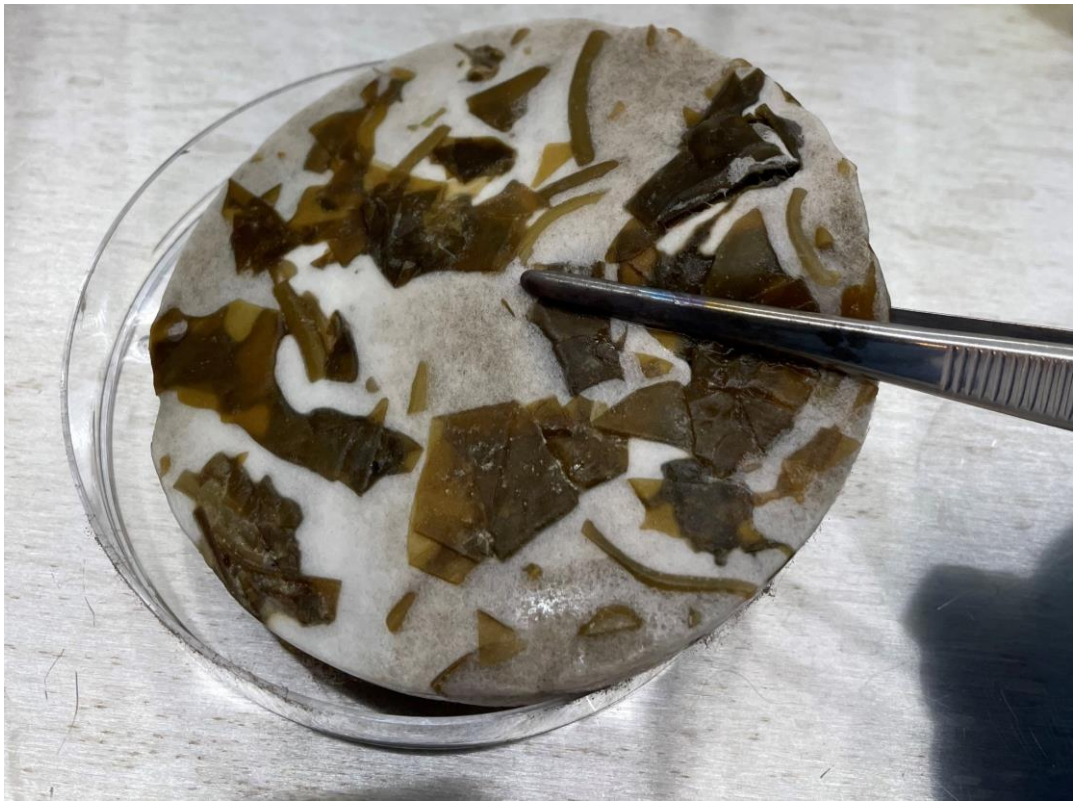
DIVISION BIOECONOMY AND HEALTH
PROCESS TECHNOLOGY, SAFETY AND HYGIENE



Solid-state fermentation of side-streams from Saccharina latissima

Martina Bergentall, Unn Tjörnstrand, Karin Bjerre, Gunnar Cervin, Göran Nylund, Jenny Veide Vilg

RISE Report: **2023:152**

ISBN: **978-91-89896-52-9**

This project has been funded by Blue Food – Centre for future seafood, with contributions from Formas – a Swedish Research Council for Sustainable Development (grant number 2020-02834) and Region Västra Götaland (grant number RUN 2020-00352).

Solid-state fermentation of side-streams from Saccharina latissima

Martina Bergentall, Unn Tjörnstrand, Karin Bjerre, Gunnar Cervin, Göran Nylund, Jenny Veide Vilg

This work was performed by RISE Research Institutes of Sweden AB together with Nordic Seafarm.

Abstract

The cultivation of brown macroalgae, such as *Laminaria* ssp. and *Saccharina latissima*, has increased extensively during the last decades; according to a report by Food and Agriculture Organisation of the United Nations[1]. Already now the cultivation is generating a large amount of side-streams that mostly are used as fertilizers. To better use the side-streams, this project aimed at investigating a potential product made through solid-state fermentation of brown algae side-streams. Different enzymatic pre-treatments were used and combined with either fermenting with *Rhizpous* spores or *Asperigillus* spores. The brown algae side-streams without pre-treatment resulted in the most appetizing product prototype.

There was a difference in protein/total amino acid content between the samples, where the enzymatically treated algae side-streams had a higher protein content. This can be due to the enzymes that contribute with a protein content. What could be seen was that the amino acid composition was different for the raw material and the algae side-streams inoculated with *Rhizopous* spores.

To be able to use this as a product, the taste of the product needs to be evaluated as well as the microbial and toxicological risks with the product. It would also be valuable to know more about the nutritional profile as well as the bioavailability of nutrients in the product.

Key words: Saccharina latissima, brown algae, solid-state fermentation

RISE Research Institutes of Sweden AB

RISE Report: **2023:152**
ISBN: **978-91-89896-52-9**

[1] *https://www.competecaribbean.org/wp-content/uploads/2021/05/Global-status-of-seaweed-production-trade-and-utilization-Junning-Cai-FAO.pdf*

Content

Abstract **1**
Content **2**
1 Background and aim **3**
1.1 Project setup 3
2 Methods **4**
2.1 Pre-treatment of algae side-streams 5
2.2. Fermentation of brown algae side-streams 5
3 Results **6**
4 Conclusions and future aspects **8**
I. Appendix A – raw data amino acid composition **9**

1 Background and aim

The cultivation of brown macroalgae, such as *Laminaria* ssp. and *Saccharina latissima*, has increased extensively during the last decades; according to a report by Food and Agriculture Organisation of the United Nations[2], the cultivation of brown seaweeds exceeded 16 M tons worldwide as per 2019. At the Swedish West coast, the most cultivated brown alga is *S. latissima* – Sugar kelp – and it is mainly produced for food purposes. The *S. latissima* body consists of a blade, a stipe, and a holdfast. The holdfast and stipe are stiff and tough and not useful for food in its raw state; instead, they become a side-stream in the production.

Fermentation of the stipes and holdfasts could be a way of improving their food properties, but the traditionally used and food-safe microorganisms employed for fermentation of soybeans, cereals, and other plant-based substrates are adapted to grow on plant carbohydrates such as starch and cellulose. This could be an obstacle when attempting to use algae as a substrate, since they are mainly constituted of other carbohydrates, such as alginate, laminarin, fucoidan and mannitol. For fungi to grow on algal biomass there should be a need for enzymes that can degrade the algal carbohydrates to release sugar units to be taken up by the fungal cells, but we did not find any reports on known food-safe fungi specialized on algae. Therefore, we wanted to test different pre-treatments, with the aim to make the algal carbohydrates accessible for established and safe fungi, traditionally used for fermentation of plant material, so that they would be able to ferment the algal biomass.

Our project aimed at assessing the feasibility of using side-streams (stipes and holdfasts) from *S. latissima* as a substrate for solid-state fermentation and to make initial total protein analyses of the product. The goal was to present a proof-of-concept – a model product – for future studies of e.g. amino acid composition, nutritional value, bioavailability, sensorics and environmental impact. The long-term impact target was to enable sustainable and profitable valorization of a presently unused side-stream.

1.1 Project setup

To begin with a literature study was performed to find suitable pre-treatments and fungi to use for the solid-state fermentation of algae. Further we aimed at mapping *Saccharina latissima's* main constituents and what had been done in the solid-state fermentation area of algae.

A number of different pre-treatments could be found in the literature and the most regularly used pre-treatments for macroalgae is drying, blanching, freezing as well as fermentation[3][4]. The different pre-treatments alter the properties of algae, but the main goal is to increase the shelf-life of algae. In our project we used frozen *S. latissima* that was blanched after thawing, which is a method often used by Nordic Seafarm. Blanching decreases the iodine content and gives it a greener color.

[2] *https://www.competecaribbean.org/wp-content/uploads/2021/05/Global-status-of-seaweed-production-trade-and-utilization-Junning-Cai-FAO.pdf*

[3] https://www.who.int/publications/i/item/9789240058538

[4] Bruhn, A., Brynning, G., Johansen, A. *et al.* Fermentation of sugar kelp (*Saccharina latissima*)—effects on sensory properties, and content of minerals and metals. *J Appl Phycol* **31**, 3175–3187 (2019). https://doi.org/10.1007/s10811-019-01827-4

When looking at the main constituents of brown algae, alginate, cellulose, laminarin, fucoidan and mannitol are reported as the main carbohydrates in the literature[5]. These polysaccharides can be enzymatically degraded to sugars and other monomers that are more available to microorganisms during fermentation. Therefore, we aimed at breaking down these polysaccharides, either by using enzymes or microorganisms that potentially could produce appropriate enzymes to be able to grow on the side-streams.

The enzymatic method used in this project was based on two articles, namely Ravanal et al., 2017[6] and Prajapati et a., 2015[7]. Ravanal et al., aimed at releasing glucose from *S.latissima and Macrocystis pyrifera* to thereafter investigate if *Candida utilis* could grow on the algae. Prajapati et al aimed at solubilizing algal biomass for biofuels.

Several studies regard solid-state fermentation with various substrates, as well as with seaweeds as a substrate. There are also examples of solid-state fermentation that aimed at extracting different bioactive compounds from macroalgae[8][9][10].

2 Methods

Below in Figure 1 an overview of how the stipes and holdfast were pre-treated and fermented. All of *S.latissima* was supplied by Nordic Seafarm in frozen form.

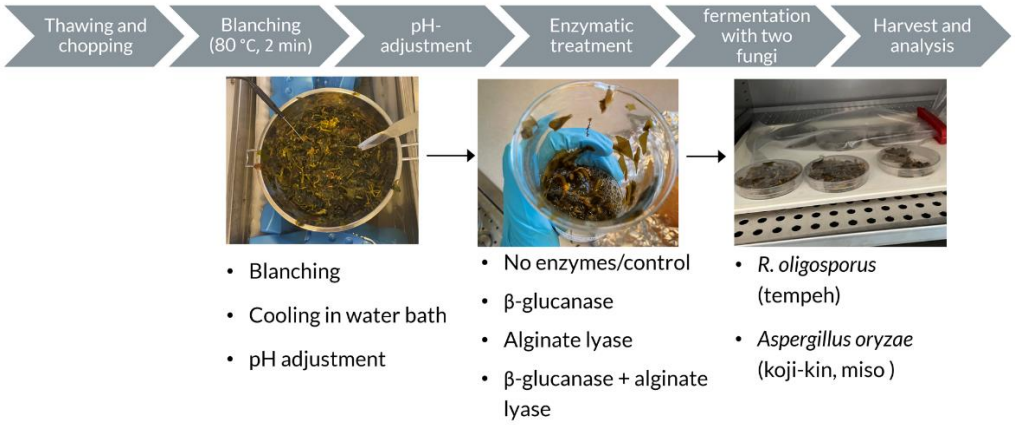


Figure 1. Overview of the different steps in this project.

[5] Li Y, Zheng Y, Zhang Y, Yang Y, Wang P, Imre B, Wong ACY, Hsieh YSY, Wang D. Brown Algae Carbohydrates: Structures, Pharmaceutical Properties, and Research Challenges. *Marine Drugs*. 2021; 19(11):620. https://doi.org/10.3390/md19110620

[6] María Cristina Ravanal, Sandeep Sharma, Javier Gimpel, Felipe E. Reveco-Urzua, Margareth Øverland, Svein Jarle Horn, María Elena Lienqueo, The role of alginate lyases in the enzymatic saccharification of brown macroalgae, Macrocystis pyrifera and Saccharina latissima, Algal Research,
Volume 26, 2017, Pages 287-293, https://doi.org/10.1016/j.algal.2017.08.012.

[7] Sanjeev Kumar Prajapati, Arghya Bhattacharya, Anushree Malik, V.K. Vijay, Pretreatment of algal biomass using fungal crude enzymes, Algal Research, Volume 8, 2015, Pages 8-14, https://doi.org/10.1016/j.algal.2014.12.011.

[8] Ferreira M, Salgado JM, Fernandes H, Peres H, Belo I. Potential of Red, Green and Brown Seaweeds as Substrates for Solid State Fermentation to Increase Their Nutritional Value and to Produce Enzymes. *Foods*. 2022; 11(23):3864. https://doi.org/10.3390/foods11233864

[9] Bonilla Loaiza AM, Rodríguez-Jasso RM, Belmares R, López-Badillo CM, Araújo RG, Aguilar CN, Chávez ML, Aguilar MA, Ruiz HA. Fungal Proteins from *Sargassum* spp. Using Solid-State Fermentation as a Green Bioprocess Strategy. *Molecules*. 2022; 27(12):3887. https://doi.org/10.3390/molecules27123887

[10] A. Pérez-Alva, A.J. MacIntosh, D.K. Baigts-Allende, R. García-Torres, M.M. Ramírez-Rodrigues, Fermentation of algae to enhance their bioactive activity: A review, Algal Research, Volume 64, 2022, 102684, https://doi.org/10.1016/j.algal.2022.102684.

2.1 Pre-treatment of algae side-streams

To begin with the algae side-streams (stipes and hold-fast) were thawed at room temperature for around 2 hours and cut into smaller pieces. The side-streams were blanched shortly and cooled down quickly in an ice-bath.

The blanched algae side-streams were enzymatically treated according to the different treatments below:

i) No enzymatic treatment
ii) Only ß-glucanase (Sigma-Aldrich, product no. 49101)
iii) Only alginate lyase (Sigma-Aldrich, product no. A1603)
iv) Combination of ß-glucanase and alginate lyase

2.2. Fermentation of brown algae side-streams

After the literature review, two different fungi were chosen, namely *Rhizopus* spores (tempeh starter from Humlegårdens Ekolager, Sollentuna,SE) and *Asperigillus* spores (koji-kin starter from Humlegården). Samples were prepared in duplicates according to the treatments below:

a) No treatment at all
b) Only inoculated with *Rhizopus* spores
c) Only inoculated with *Aperigillus* spores
d) Beta-glucanase + *Rhizopus* spores
e) Beta-glucanase + *Asperigillus* spores
f) Alginate lyase + *Rhizopus* spores
g) Alginate lyase + *Aperigillus* spores
h) Beta-glucanase + alginate lyase + *Rhizoous* spores
i) Beta-glucanase + alginate lyase + *Asperigillus* spores

2.2.1. Fermentation of S. latissima with Rhizopus spores

The inoculated algae side-streams were put in petri dishes and packed quite densely. The petri dishes were sealed with parafilm to avoid oxygen exposure as much as possible. Thereafter all samples inoculated with *Rhizopus* spores were incubated at 30 °C for maximum one week. The samples were inspected each day, and one of the replicates were harvested and frozen after 3 days.

2.2.2. Fermentation of S. latissima with Asperigillus spores

Half a teaspoon of *Aspergillus* spores was added to 25 grams of algae side-streams. The pH was adjusted to 5.5 using HCl. The plates were not sealed with parafilm and kept in a bag that was filled with air since *Aspergillus* spores prefer to grow with a lot of oxygen.

Samples were incubated at 30 °C for maximum one week. Some samples were harvested earlier, already after 3 days.

2.2.3. Analysis of pre-treated and fermented samples

The samples were sent externally to Eurofins for analysis of total protein content and amino acid composition.

3 Results

The experiments were performed twice, one time in small scale and one time in larger scale to produce enough material to send the samples for external analysis at Eurofins.

During the fermentation, the samples were visually analyzed. Some samples did not result in a solid cake and did not look appetizing. The samples treated with alginate lyase, or a combination of alginate lyase and beta-glucanase were very wet after the enzymatic treatment, probably due to the degradation of alginate. This resulted in a too wet substrate for the fungi to grow on in a good way. The laminarin degradation by beta-glucanase did not have any effect on the growth of the fungi. Many of the enzymatic treatments resulted in growth only on the top of the algae side-streams, probably due to the side-streams being too wet for the fungi to be able to grow down into the matrix.

The treatment that resulted in the most promising product from a visual and tactile point of view was the algae inoculated with *Rhizopus* spores without any enzymatic pre-treatment. This resulted in a solid cake with nice texture. Below is an overview of the visual results from the different treatments can be seen in Figure 2.

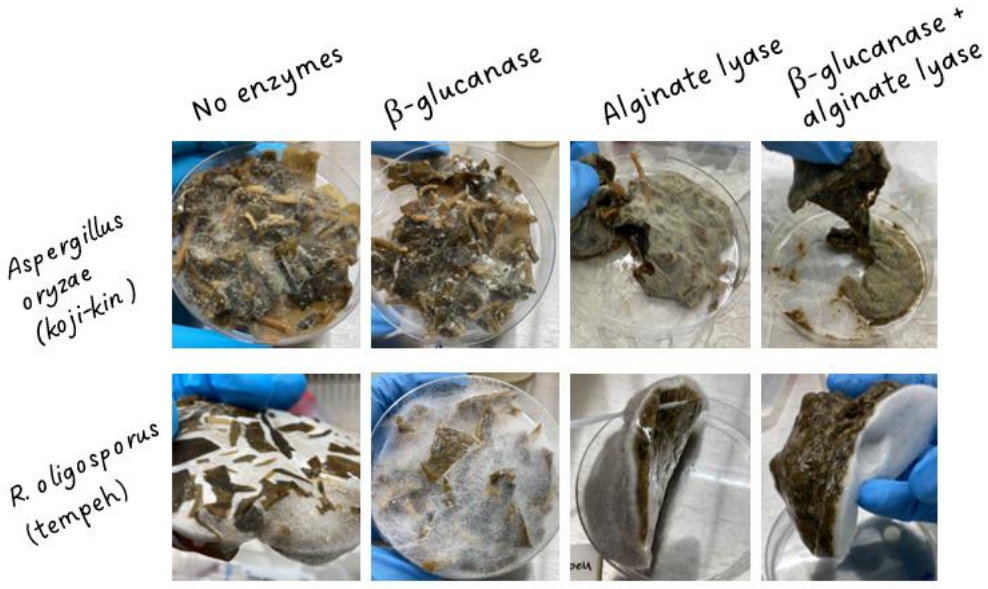


Figure 2. Overview of visual and tactile results for the different treatment.

As mentioned previously, the samples were also sent for external analysis at Eurofins for total protein content of all samples, as well as amino acid profile for some of the most promising samples.

Below in Figure 3 the crude protein content of the samples can be seen. The error bars represent the standard deviation of the mean of the duplicates. No clear pattern can be seen, except for that the treatments including enzymes yielded more protein. This is probably because the enzymes added are composed by proteins that would contribute to the total protein content.

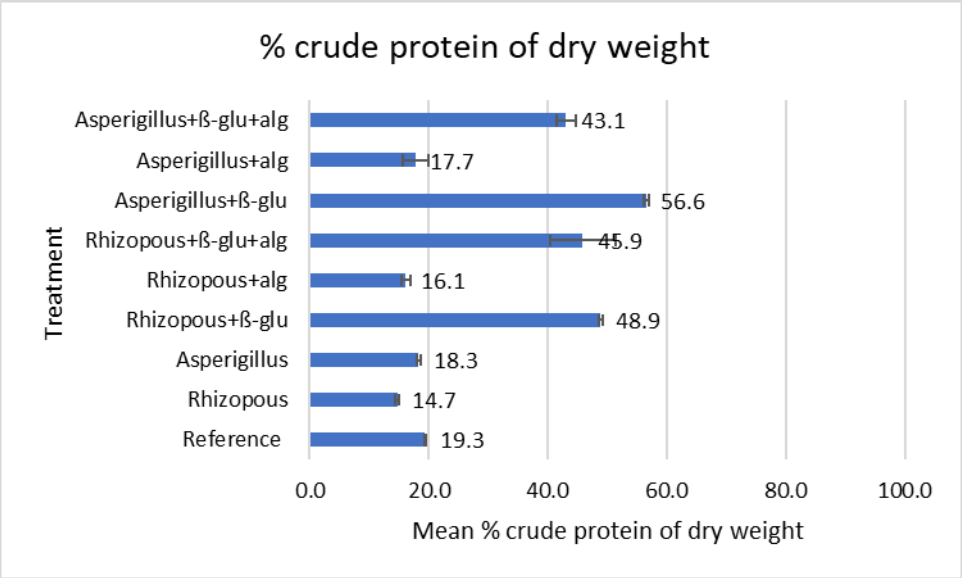


Figure 3. Percentage crude protein per dry weight.

Below in Figure 4, the amino acid profile for blanched *S. latissima* and blanched *S. latissima* inoculated with *Rhizopus* spores can be seen. The amino acid profile for all other samples can be seen in Appendix 1. A different amino acid composition can be seen in the samples inoculated with *Rhizopus* spores can be seen, where histidine is present in the fermented samples, as well as higher levels of phenylalanine, arginine, and isoleucine. Some amino acids were also decreased in the fermented samples.

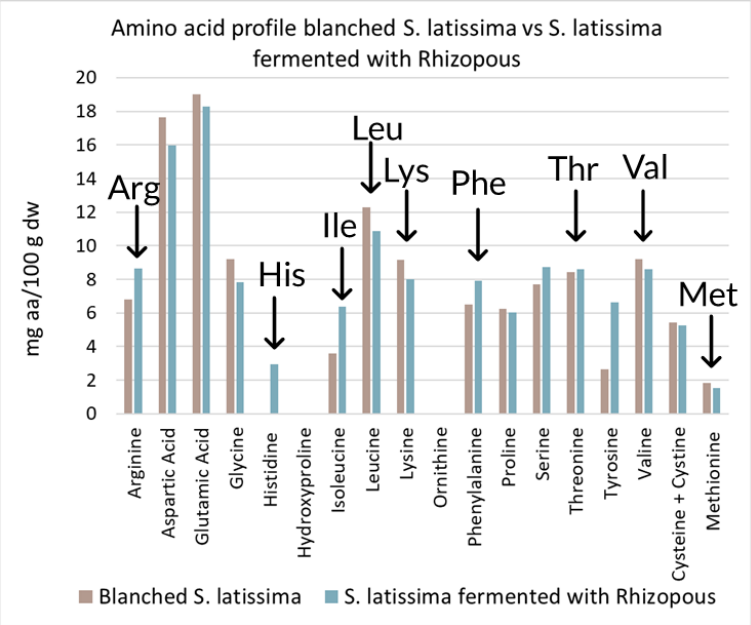


Figure 4. Amino acid profiles of blanched and *Rhizopus*-fermented *S. latissima*.

4 Conclusions and future aspects

The solid cake of *S. latissima* side-streams was formed when no pre-treatment was used and with inoculation with *Rhizopous* spores. This means that the goal to present a proof-of-concept product was fulfilled. Furthermore, the total protein content and amino acid was determined.

There was a difference in protein/total amino acid content between the samples, where the enzymatically treated algae side-streams had a higher protein content. This can be due to the enzymes that contribute with a protein content. What could be seen was that the amino acid composition was different for the raw material and the algae side-streams inoculated with *Rhizopous* spores.

To be able to use this as a product, the taste of the product needs to be evaluated as well as the microbial and toxicological risks with the product. It would also be valuable to know more about the nutritional profile as well as the bioavailability of nutrients in the product.

I. Appendix A – raw data amino acid composition

mg/100g dw	Alanine	Arginine	Aspartic Acid	Glutamic Acid	Glycine	Histidine	Hydroxyproline	Isoleucine	Leucine	Lysine
Blanched *S. latissima*	10.5	6.8	17.6	19.0	9.2	0.0	0.0	3.6	12.3	9.2
S. latissima fermented with Rhizopous	9.4	8.7	16.0	18.3	7.8	2.9	0.0	6.4	10.9	8.0
Rhizopous + alginate lyase	12.0	12.5	20.4	25.3	10.1	4.8	0.0	8.6	15.1	9.7
Asperigillus + ß-glucanase	7.2	6.6	12.8	12.9	6.2	2.5	0.0	4.5	8.1	1.9
Asperigillus + alginate lyase	10.4	6.0	18.8	21.0	8.9	3.0	0.0	6.7	12.2	8.9

mg/100g dw	Phenylalanine	Proline	Serine	Threonine	Tyrosine	Valine	Cysteine + Cystine	Methionine	Total amino acids
Blanched *S. latissima*	6.5	6.3	7.7	8.4	2.7	9.2	5.4	1.8	136.3
S. latissima fermented with Rhizopous	7.9	6.0	8.7	8.6	6.6	8.6	5.2	1.5	141.5
Rhizopous + alginate lyase	10.1	8.8	10.2	10.1	8.1	10.7	5.7	4.2	186.0
Asperigillus + ß-glucanase	5.0	5.2	5.9	6.7	4.3	6.1	4.5	2.8	103.3
Asperigillus + alginate lyase	7.4	5.6	8.8	9.3	5.9	10.0	6.2	4.2	153.4

This project has been funded by Blue Food – Centre for future seafood, with contributions from Formas – a Swedish Research Council for Sustainable Development (grant number 2020-02834) and Region Västra Götaland (grant number RUN 2020-00352).

Through our international collaboration programmes with academia, industry, and the public sector, we ensure the competitiveness of the Swedish business community on an international level and contribute to a sustainable society. Our 2,800 employees support and promote all manner of innovative processes, and our roughly 100 testbeds and demonstration facilities are instrumental in developing the future-proofing of products, technologies, and services. RISE Research Institutes of Sweden is fully owned by the Swedish state.

I internationell samverkan med akademi, näringsliv och offentlig sektor bidrar vi till ett konkurrenskraftigt näringsliv och ett hållbart samhälle. RISE 2 800 medarbetare driver och stöder alla typer av innovationsprocesser. Vi erbjuder ett 100-tal test- och demonstrationsmiljöer för framtidssäkra produkter, tekniker och tjänster. RISE Research Institutes of Sweden ägs av svenska staten.

RISE Research Institutes of Sweden AB
Box 857, 501 15 BORÅS, SWEDEN
Telephone: +46 10-516 50 00
E-mail: info@ri.se, Internet: www.ri.se

Process Technology,
Safety and Hygiene
RISE Report: 2023:152
ISBN: 978-91-89896-52-9