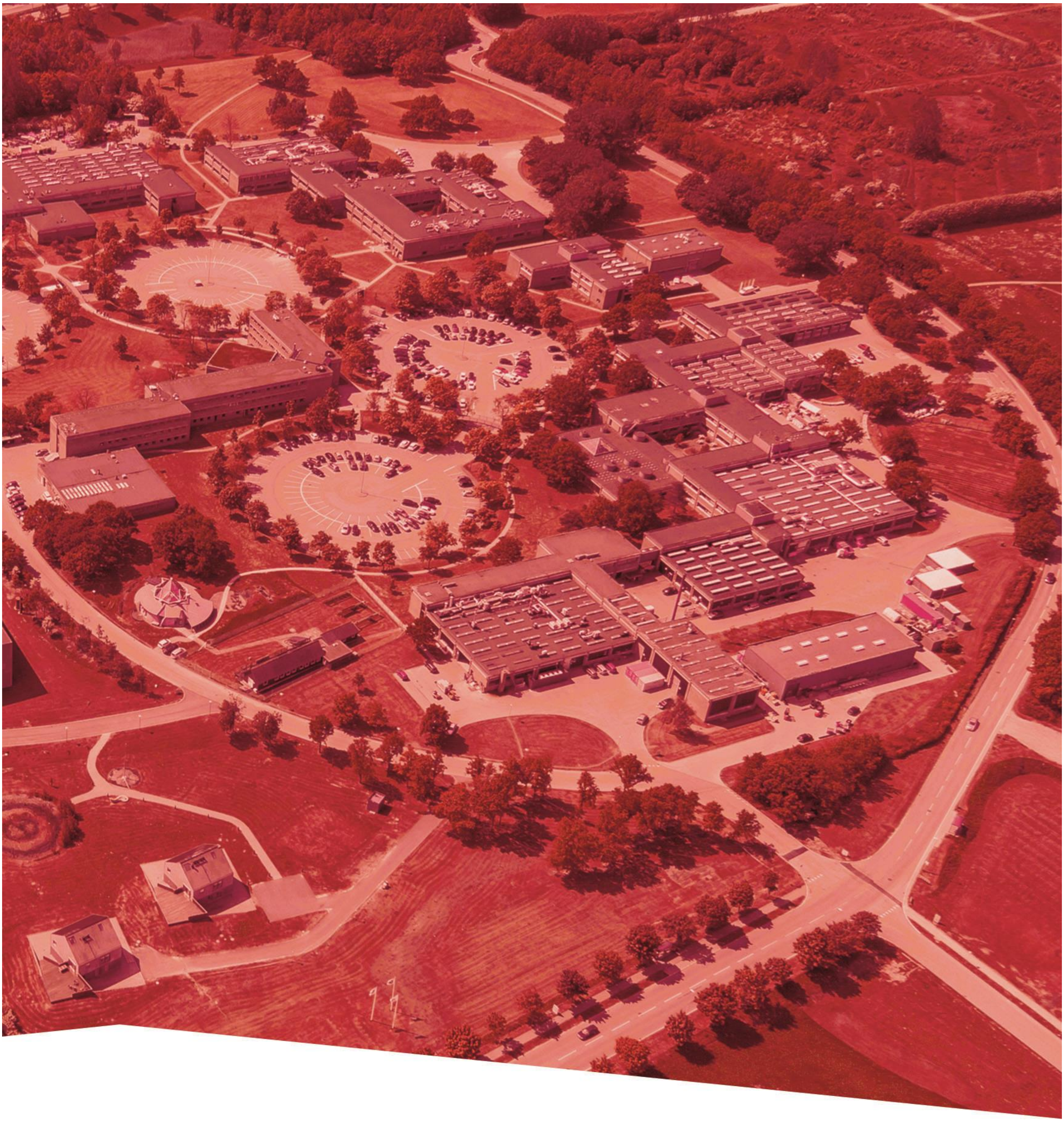

Potential of fermentation

Requirements for facilities

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Potential of fermentation

Requirements for facilities

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1. Contents

2. Introduction

Fermentation technology has experienced significant growth in recent years and is a rapidly expanding global market. Denmark presents a high market potential for fermentation technology due to its strong presence in the food and biotechnology industries. Biosolutions, which involves implementing biotechnology in the food, agricultural, or maritime industries, is a core competence of Danish industry. This report focuses on a market analysis and implementation of fermentation equipment in Denmark, including examples of the use of fermentation or refined products such as enzymes, proteins, biostimulants, and more.

Fermentation encompasses a wide range of biological processes performed by microorganisms. Traditionally, the term refers to processes where microorganisms consume organic molecules in anaerobic conditions. However, in the broader industrial sense used here, it encompasses all processes where replication of microorganisms or production through microorganisms occurs.

3. Market potential

Denmark positions itself as one of the best countries in Europe for biotech research and development as ranked by Nordic Life Science News (2019). At the same time Denmark also continuously ranks as one of the most R&D intensive countries based on researchers per capita in Europe, and releases the second most number of scientific biotech publications per capita after Switzerland [1, 2]. According to an analysis issued by the Danish Ministry of Industry, Business and Financial affairs (*Erhvervsministeriet*), the biosolution sector contributes annually with 13 billion DKK to the Danish GDP and employs about 7000 man-years. As such it is ranks as 17 out of 117 industry sectors in terms of productivity when comparing the importance to the Danish economy. At the same time the sector is responsible for an estimated 27 billion DKK in export [2].
The biosolution sector is sustained in large by the strong intellectual property contained in Denmark. According to a recent patent study, Danish companies are at the front when issuing patents within biosolutions. This study issued by the embassy of Denmark in US, which focused on mapping patents spanning from 2000 to 2022, showed that innovation was especially active in animal feed, milk and milk powder preparations and that the yearly number of published patents has increased from 51 to 1000 in the same 22-year period, corresponding to a total of 16,054 patents. Per capita this equates to 4-5 times as many as the average of the 10 leading research nations [3].

In total there are more than 130 companies in Denmark that develop and produce biosolutions focused primarily on fermentation-based technologies [4]. However, access for facilities for testing and upscaling of fermentation processes into mid to large scale within Danish and regional (Zealand) borders has been a growing demand and unfortunately a service which may be difficult to access. The biosolution sector is dominated by few big companies, the five biggest companies in the industry responsible for 85% of the man-years and a large majority of the export. The most active Danish companies with research patents are Novozymes, IFF (formerly Danisco), Chr. Hansen, Arla Foods and Gumlink. Half of all biosolution patents with Danish actors have one of these five companies involved [3]. These companies are leaders in the production of enzymes and other ingredients for the food and beverage industry, and they have been at the forefront of developing new technologies for improving the efficiency and cost-effectiveness of fermentation processes.

Current initiatives to support the growth of the fermentation market are aimed at promoting the development and commercialization of fermentation technology. These initiatives provide funding, technical support, and access to research facilities to companies in the sector. Region Zealand is currently investing in universities and privately owned companies to facilitate access to fermentation equipment and processing technology related to developing, testing and scaling up biotechnological processes. This effort, Biosolutions Zealand, is predicted to ensure skilled labour, create jobs, economic growth and help entrepreneurs and companies get closer to market-ready products [5]. In actuality this may be achieved by e.g. reduction of energy consumptions and better utilizing residues of existing process, which will help reduce the climate impact compared to existing processes [6]. However, the extent to which this impact will have depends in large part by the market demand, resting mainly on the need for equipment[a]. Such demand must be met with considerations of areas of application, equipment scaling, flexibility of system and overall implementation.

3.1. Knowledge exchange with collaborators, stakeholders and potential customers

The Biosolution Zealand consortium consists of a number Danish universities and companies, hereunder Technical University of Denmark (DTU), Roskilde University (RUC), Danish Technological Institute (DTI), Ferm Hub Zealand, 21st Bio, in which equipment supporting fermentation will be implemented benefiting industrial stakeholders ranging from start-ups, SMV's and large enterprises with tasks concerning test and scaling of fermentation process. The aim of Biosolution Zealand is for Denmark to be a global pioneer by supporting the sustainable biotechnologies of the future and support conversion to more energy friendly, bio-based and climate-neutral production worldwide. It furthermore aims to create the optimal framework to develop, test and upscale biotechnological products and solutions, which supports the green transition in Denmark.
In order to benefit from existing knowledge in the field and create more in-depth dialogue, knowledge was exchanged between a total of 40 stakeholders and collaborators in the food, biomass and fermentation industry (including TetraPak, BioInnovation Institute, Lallemand among others) as well as potential customers to DTI's facilities in order to map the current need for equipment. In terms of size, collaborators who specified volumes requested covering of fermentation equipment ranging from a size of 50-2000 L, within the f food and ingredient production, in particular protein production.
Physical visits were also carried out at Danish and European fermentation facilities (Chr. Hansen, Biobase Europe Pilot Plant, NIZO food research and Dutch manufacturers of fermentation equipment).

[a] Based on DTI-customer communications

There was a strong support for the idea of building of expansion of test facilities in the pilot- and demonstration scale within Denmark and having the facilities accessible for research and industrial partners.

A few key takeaways from the visits range from strategic to design considerations:

- Pilot plant with concepts similar to what is envisioned at the expansion of DTI's biorefinery down-processing facility exist and are able to carry out the type of tasks requested by potential customers with good financial turnover and continuous capacity for expansion and growth.
- Fermentation equipment for scaling up processes in Europe is in heavy demand. Test facilities are often over capacity in terms of handling R&D and customer projects
- Facilities capable of running multiple projects need strict implementation of rules regarding customer IP
- Work should be as automated as possible. In many cases it was seen that workers worked around the clock, however with automation and remote monitoring, it is possible to keep fermentations running without the need for at-location personnel in off-hours.
- Fermentation tanks need a heavy equipment infrastructure around it to support the operation, ranging from water purification systems, to steam generators, electrical grid to name a few.
- Implementation of ATEX safety is an issue that needs consideration based on individual customer needs, but is needed for cases where e.g. alcohol content exceeds a certain level (>20%) or use of flammable/explosive gasses (e.g. hydrogen)
- GMO risk classification 1 is almost universally needed as a minimum to serve potential customers
- Certain features that are not standard can be requested at point of manufacturing which will minimize or ease workload during operation

Figure 2. Reviewing buildings, skid systems, and fermentors at external company visits.

3.2. Implementation of upscaling equipment

An internal focus group focusing on fermentation was formed at DTI consisting of 23 colleagues from four different centers: Water & Biotechnology, Food Technology, Plant Technology and Bioresources. In the workgroup a series of tasks was put out to further define the market potential through strategic planning and communication plans, with the goal of conceptualizing an accessible pilot- and demonstration sized fermentation plant. As a starting point the internal workgroup collaborated on a keyword overview (Three 'W's) to define the target group of interest, the contents of interest and an outline of the purpose.

Table 1. Defining the three W's of the fermentation facilities

What?	For whom?	Why?
• New fermentation platform • Fermentation solutions • Bridging the gap • Proof-of-concept • Enable companies to grow • Fermenting the future	• The customers • SME's and large companies • The society • The general industry	• Enable a sustainable future (food, feed, products) • Enable the green transition • To make more solid business cases

The focus group at DTI made a SWOT analysis for identifying external/internal factors affecting the success of establishment of fermentation equipment in Denmark. Among the external factors to be aware of, adding additional value to customers already involved with bioresource technologies, e.g. by adding a fermentation step to the downstream value chain or fermenting things later to be processed in the existing facilities, was seen as an important opportunity and strength, respectively for companies already having such facilities implemented. At universities for instance, these are already supported with a network of downstream processing plants along with professors, scholars and workers that facilitate new fermentation equipment. At locations It was also seen as a powerful incentive in creating partnering or creating a co-sponsorship with external collaborators and technology suppliers.

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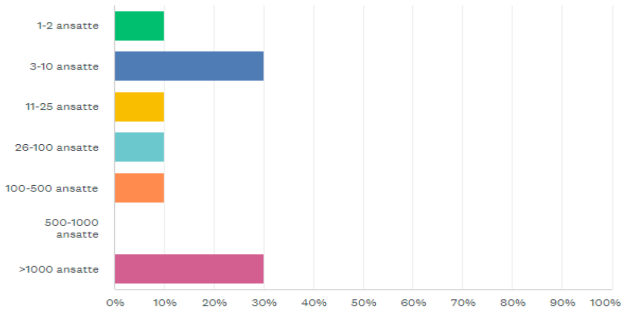
A TOWS analysis was made derived from the SWOT analysis. Based on the results from the SWOT analysis, the changes needed were identified by combinations of the identified based on strengths, weakness, opportunities and strengths and formulating activities to meet the future vision of the concept. The TOWS analysis is thus a useful tool for translating the current strategic situation of DTI and the future goal of Biosolution Zealand. Among the actions identified, a strong external communication and positioning based on ambitious grant applications is needed, along with e.g. employment of more workers, and implementation of digitalization and automation tools in the workflow.

3.3. Online Survey

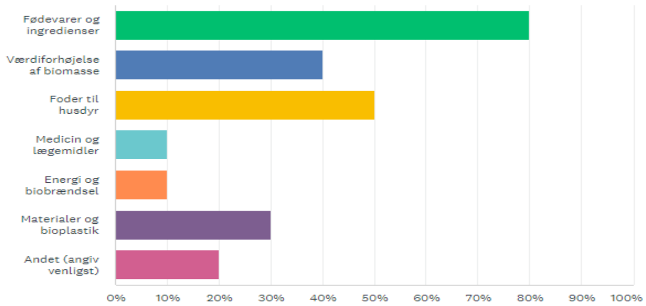
An online survey was formulated as a short 10 question multiple choice page taking approximately 2-4 minutes to complete per user. The survey was shared directly with the internal fermentation focus group, and from there shared partners and internal contacts, as well as publicly on Teknologisk Institute's Future Foods (Fremtidens fødevarer) LinkedIn page [7] which was also shared in the newsletter. The design of the questionnaire was targeted at employees in companies working with fermentation at any level of expertise in fermentation seeking to elucidate on the most important considerations for what type of setup is needed to accommodate the widest span of customers and research projects. The questions and answer distributions (available in Danish and English) were as follows:

1. How large is the company you are working in? (*Hvor stor er den virksomhed du arbejder i?*)



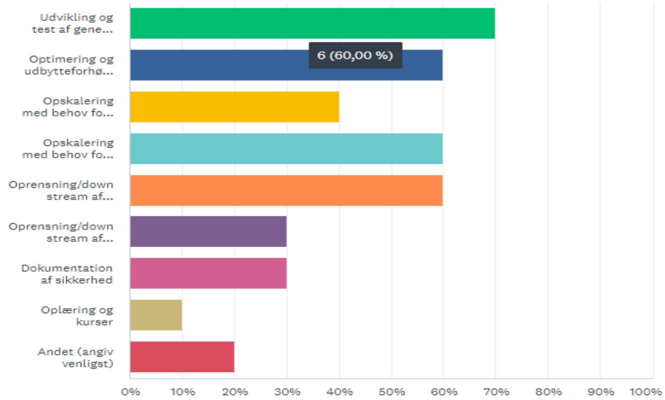
SVARVALG	BESVARELSER	
1-2 ansatte	10,00 %	1
3-10 ansatte	30,00 %	3
11-25 ansatte	10,00 %	1
26-100 ansatte	10,00 %	1
100-500 ansatte	10,00 %	1
500-1000 ansatte	0,00 %	0
>1000 ansatte	30,00 %	3
I ALT		**10**

2. In which categories would your company benefit from utilizing fermentation technology? (*I hvilke områder vil din virksomhed kunne udnytte fermenteringsteknologi?)*



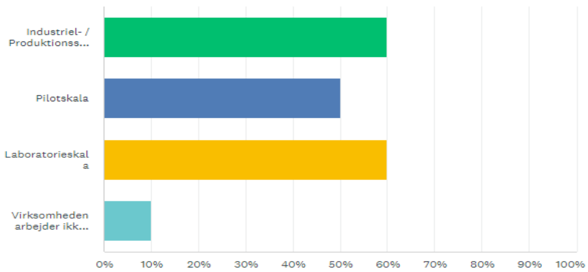
SVARVALG		BESVARELSER	
Fødevarer og ingredienser		80,00 %	8
Værdiforhøjelse af biomasse		40,00 %	4
Foder til husdyr		50,00 %	5
Medicin og lægemidler		10,00 %	1
Energi og biobrændsel		10,00 %	1
Materialer og bioplastik		30,00 %	3
Andet (angiv venligst)	Besvarelser	20,00 %	2
Respondenter i alt: 10			

3. Which of these options best describe your companies' need for external assistance for fermentation? (*Hvilke af disse valgmuligheder beskriver bedst din virksomheds behov til ekstern hjælp med fermentering?*)



SVARVALG		BESVARELSER	
Udvikling og test af generel metode		70,00 %	7
Optimering og udbytteforhøjelse		60,00 %	6
Opskalering med behov for salg af produktet		40,00 %	4
Opskalering med behov for demonstration/dokumentation af konceptet		60,00 %	6
Oprensning/downstream af færdige produkt		60,00 %	6
Oprensning/downstream af sidestrømme		30,00 %	3
Dokumentation af sikkerhed		30,00 %	3
Oplæring og kurser		10,00 %	1
Andet (angiv venligst)	Besvarelser	20,00 %	2
Respondenter i alt: 10			

4. Which scale of fermentation does your company work with internally? (*Hvilken skala af fermentering arbejder jeres virksomhed med internt?)*



SVARVALG	BESVARELSER	
Industriel- / Produktionsskala	60,00 %	6
Pilotskala	50,00 %	5
Laboratorieskala	60,00 %	6
Virksomheden arbejder ikke internt med fermentering	10,00 %	1
Respondenter i alt: 10		

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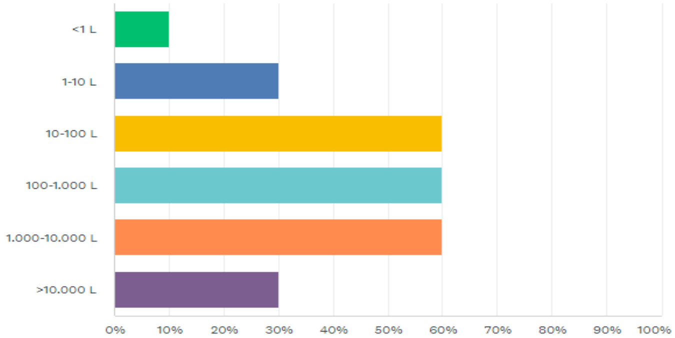
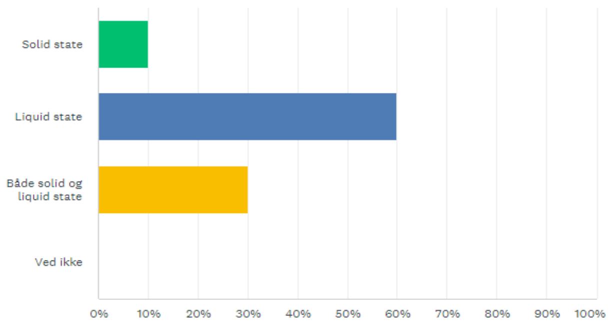
5. Which scale of fermentation is your company seeking assistance for? (*Hvilken skala af fermentering søger din virksomhed Hjælp til?)*



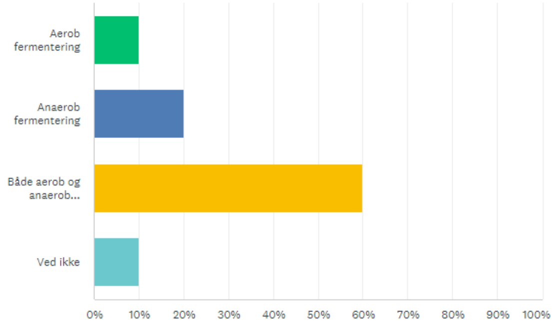
SVARVALG	BESVARELSER	
<1 L	10,00 %	1
1-10 L	30,00 %	3
10-100 L	60,00 %	6
100-1.000 L	60,00 %	6
1.000-10.000 L	60,00 %	6
>10.000 L	30,00 %	3
Respondenter i alt: 10		

6. Is solid state fermentation or liquid state fermentation the main area of interest for your company? (*Har du/I primært brug for Hjælp til solid state fermentering (fast materiale) eller liquid state fermentering (flydende medie)?)*



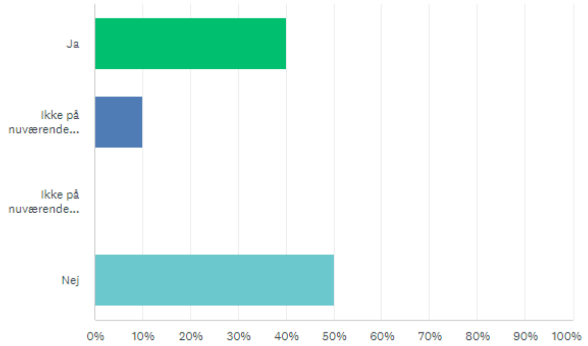
SVARVALG	BESVARELSER	
Solid state	10,00 %	1
Liquid state	60,00 %	6
Både solid og liquid state	30,00 %	3
Ved ikke	0,00 %	0
I ALT		10

7. Is aerobic (with oxygen) or anaerobic (without oxygen) fermentation the main interest for your company? (*Har du primært brug for arbejde med aerob (ilt-rigt) eller anaerob (ilt-fattigt) fermentering?)*



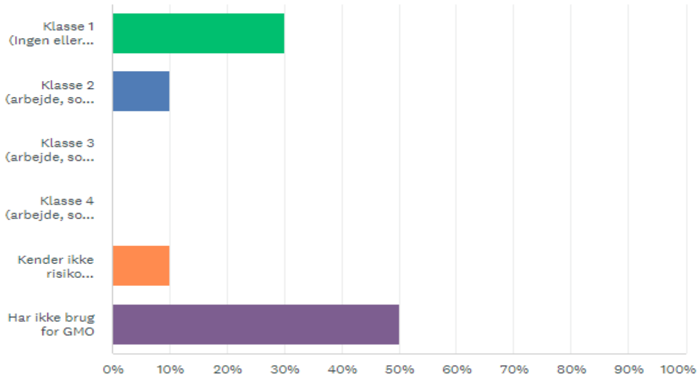
SVARVALG	BESVARELSER	
Aerob fermentering	10,00 %	1
Anaerob fermentering	20,00 %	2
Både aerob og anaerob fermentering	60,00 %	6
Ved ikke	10,00 %	1
I ALT		10

8. Do you have a need for fermentation using genetically modified organisms (GMO)? (*Har du behov for fermentering ved brug af genmodificerede mikroorganismer (GMO)?*)



SVARVALG	BESVARELSER	
Ja	40,00 %	4
Ikke på nuværende tidspunkt, men indenfor 1-2 år	10,00 %	1
Ikke på nuværende tidspunkt, men indenfor 3-5 år	0,00 %	0
Nej	50,00 %	5
I ALT		10

9. If GMO is required, which risk classification? (*Hvis du har brug for fermentering med genmodificerede mikroorganismer, hvilken risikoklassificering falder disse under?)*



SVARVALG	BESVARELSER	
Klasse 1 (Ingen eller kun en ubetydelig risiko)	30,00 %	3
Klasse 2 (arbejde, som indebærer lav risiko)	10,00 %	1
Klasse 3 (arbejde, som indebærer moderat risiko)	0,00 %	0
Klasse 4 (arbejde, som indebærer høj risiko)	0,00 %	0
Kender ikke risiko klassiferingen	10,00 %	1
Har ikke brug for GMO	50,00 %	5
I ALT		**10**

The 10th question regarded contact details for follow up questions, which are not reported here. From the answers a range of all sizes of companies showed interest, however the main interest of areas were food and ingredients, feed, and adding value to biomass. A minority also answered materials, energy, biopesticides and pharmaceuticals. They worked internally with lab scale to pilot scale and industrial scale with distribution about evenly among the three, however it was not requested by the survey what volume defined each of the three scales, so that remains for interpretation. A majority of answers said they requested need for equipment handling 10-100L, 100-1.000 and 1.000-10.000 L scale, primarily for liquid state fermentation, both aerobic and anaerobic. 60% of answers pointed to no need for GMO, however 40% answers requested the use for GMO (primarily GMO risk class 1, but a single response for class 2) which makes the need implementation of GMO a real consideration.

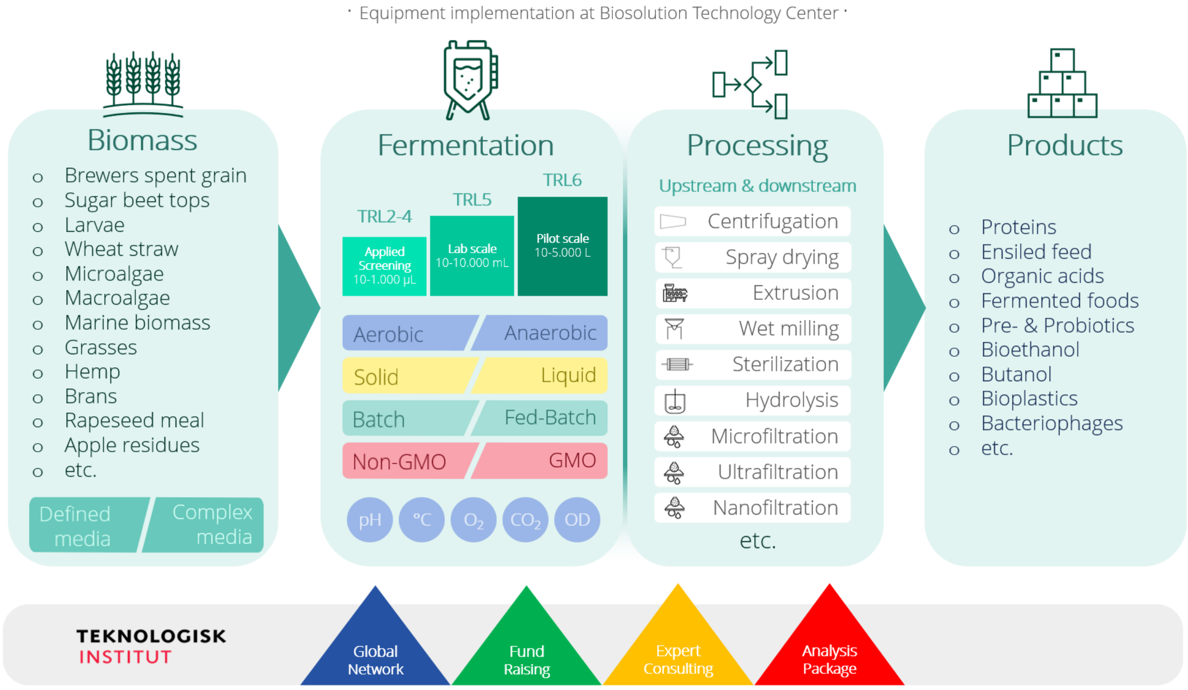
Among the services that needs to be implemented were development and tests of methods and optimization (yield) or upscaling with the purpose of creating a final sellable product, producing documentation. It was confirmed that there was a need of the existing biorefinery plant based on the answers requesting downstream processing of the finished product or sidestreams.

As was evident on the number of correspondents (n=10), the sample size only represents a small fraction of stakeholder relevant for DTI. However, in this case the low quantity of data was later backed up with several more in-depth dialogues between DTI and potential collaborators, stakeholders and potential customers in the following section.

4. Requirements for fermentation facilities

Summarizing the need of the system and considering existing biorefinery plant at DTI, a sketch of the implementation of the equipment was drafted:

Figure 3. Drafting of how the fermentation user requirements will coincide with the existing biorefinery pilotplant at DTI



The equipment must ensure the equipment and implementation of bioreactors for fermentation in a proposed system consisting of four different scaling steps with roughly a factor 10 between each step: 4x1L, 20L, 200L, 2000L working volume. This includes a laboratory scale of four 1L as well, as this will allow to work in parallel with small scale research or optimization tasks, and serve as proof-of-concept before upscaling to pilot scale.

The bioreactors are intended for precision fermentation and fermentation of side streams from biorefining of biomass, and must be able to be used for a wide range of projects such as concept and process development, research, documentation and pilot production. The equipment must be able to handle the reproduction of various microorganisms, including bacteria, yeast and filamentous fungi, microalgae, etc. and must ultimately be used in conditions that also allow it to handle requirements for GMO (class 1), products for food testing and documentation, as well as ATEX.

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Based on the market analysis and survey a User Requirement Specification (URS) was drafted, and in August 2022 sent out to 9 bioreactor distributors in order to provide an offer:

Table 4. User requirement specification

Glass fermentor ~1 L requirements (Laboratory)
- 4 pcs - Working volume 0,7-1,5L if possible - Glass fermentor - pH probe - thermosensor - po probe - Temperature controlled jacket for heating/cooling. - Ports for CO2 , N2, O2, H2 - Airation with mass flow controllers - Minimum 4 pcs pumps (acid, base, antifoam and media) - Sterile sampling - Stirrer - Possibility for pc control/data logging - Network ready - Remote connection for control of proces - Condenser on air exhaust - ATEX - ethanol, methanol, H2 - List over possible inline measurements that can be added to the reactors - Software -

Glass (laboratory) or stainless steel (pilot plant) fermentor ~20 L requirements
- 1 pcs either glass or stainless steel - 1:3 dimension if stainless - Working volume 9-25L if possible - Stainless steel fermentor if possible, otherwise glass - pH probe - Thermosensor - po probe - Temperature controlled jacket for heating/cooling. - Ports for CO2 , N2, O2, H2 - Sterile sampling - Minimum 5 pcs pumps (acid, base, antifoam, media 1 and media 2) - Airation with mass flow controllers - Stirrer - Possibility for pc control/data logging - Network ready

- Remote connection for control of proces
- If stainless steel fermentor - bottom valve for flex hose so the product can be moved after fermentation
- Condenser on air exhaust
- CIP nozzle - if it is a stainless steel fermentor
- SIP - if it is a stainless steel fermentor
- Possibility for fed batch - if it is a stainless steel fermentor
- Can run with pressure op to 3 bar - if it is a stainless steel fermentor
- Light and watchglass - if it is a stainless steel fermentor
- ATEX certified - pure ethanol and methanol in feed, H2 inlet gas
- List over possible inline mesurments that can be added to the reactors
- Top mounted nozzles and vavle for conecting extearnel media tanks
- Minimum 4 ports in bottom of the reactor for probes (pH,PO + 2 undefined)
- If stainless steel fermentor IP65 protection of electronics
- Sotfware

Stainless steel fermentor (Pilot plant) ~200 L requirements

- 1 pcs 1:3 dimension
- Working volume 50-200L if possible
- Stainless steel fermentor
- pH probe
- Thermosensor
- pO probe
- Temperature controlled jacket for heating/cooling.
- Ports for CO2 , N2, O2, H2
- Sterile sampling e.g. (mikroports/keofitt)
- Minimum 5 pcs pumps (acid, base, antifoam, media 1 and media 2)
- Airation with mass flow controllers
- Stirrer
- Possibility for pc control/data logging - Network ready
- Remote connection for control of proces
- Bottom valve for flex hose so the product can be moved after fermentation
- 2 top mounted nozzles and vavle for connecting external media tanks
- Minimum 4 ports in bottom of the reactor for probes (pH, pO + 2 undefined)
- Foam reduction with level sensor
  - Sterile filters for air
  - Light and watchglass
  - Load cells or other control of level
  - Condenser on air exhaust
  - Pressure transsmitter
  - Can run with pressure op to 3 bar

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- CIP nozzle
- SIP
- Possibility for fed batch
- ATEX certified - pure ethanol and methanol in feed, H2 inlet gas
- List over possible inline measurements that can be added to the reactors
- IP65 protection on electronics
- Sotfware

Stainless steel fermentor (Pilot plant) ~2000 L requirements

- 1 pcs 1:3 dimension
- Working volume 1000-2000L if possible
- stainless steel fermentor
- pH probe
- thermosensor
- pO probe
- Temperature controlled jacket for heating/cooling.
- Ports for CO2 , N2, O2, H2
- Sterile sampling e.g. (mikroports/keofitt)
- Minimum 5 pcs pumps (acid, base, antifoam, media 1 and media 2)
- Airation with mass flow controllers
- Stirrer
- Possibility for pc control/data logging - Network ready
- Remote connection for control of proces
- Bottom valve for flex hose so the product can be moved after fermentation
- 2 top mounted nozzles and vavle for connecting extearnel media tanks
- Minimum 4 ports in bottom of the reactor for probes (pH, pO + 2 undefined)
- Foam reduction with level sensor
    - Sterile filters for air
    - Light and watchglass
    - Load cells or other control of level
    - Condenser on air exhaust
    - Pressure transsmitter
    - Can run with pressure op to 3 bar
    - CIP nozzle
    - SIP
    - Possibility for fed batch
    - ATEX certified - pure ethanol and methanol in feed, H2 inlet gas
    - List over possible inline measurements that can be added to the reactors
    - Platform to achieve height
    - IP65 protection

- Software

The dialogue with the distributors aided in dimensioning and description of the system in the URS. In the end, requirements of keeping the system flexible to work with both bacteria, yeast, filamentous fungi and microalgae was proposed, with being careful of having the capacity to expand future addition equipment and probes, as well as ensuring flexibility in the included operating software.

Typical reactor dimensioning of width:height ratio of 1:3 has been defined, as this is a typical industrial scaling that is kept unless working with certain mammalian cell cultures or if space requirements do not allow for such height.

4.1. Concept for implementation

From the strategic planning, an overview of the infrastructure was conceptualized under the name "Biosolution Technological Center", taking into consideration the formation of a steering group backed up by advisory board and technology suppliers. The key concepts explore the use of several different biomasses ranging from mainly blue (marine) and green (agricultural) biomasses, but also keeping the use cases open for utilizing biomass waste streams from horticulture, water treatment and environment. The need for *pharmaceutical grade* and *mammal cell cultivation* was early on in the conceptualization realized to requires the need for strict GMP and QC protocols which would be far too difficult to implement in the same facility, and these areas were therefore omitted from.

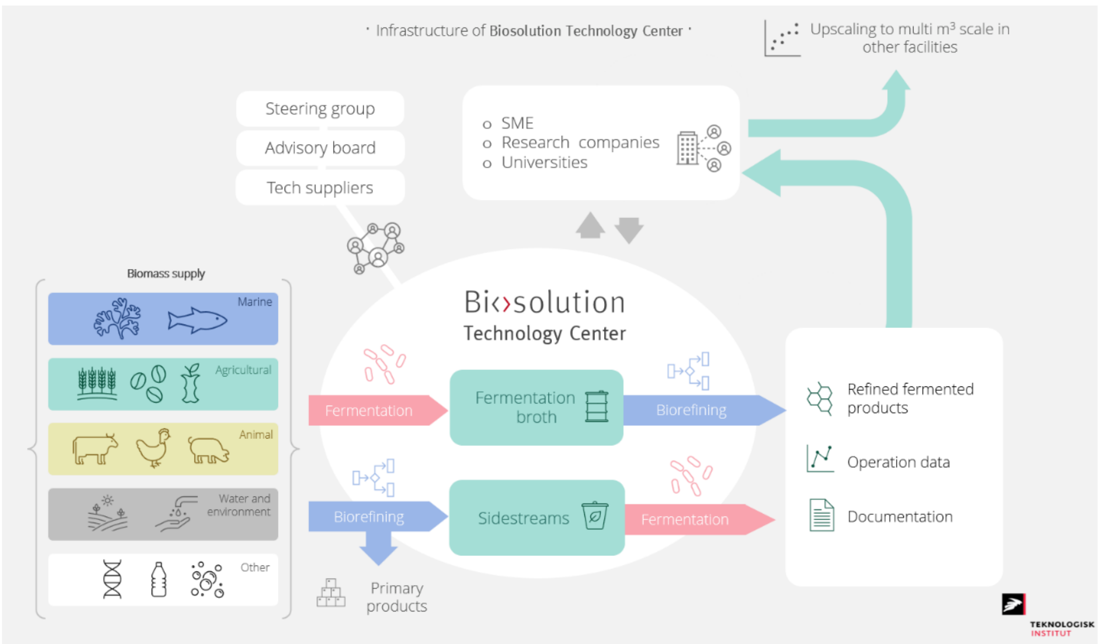

Figure 1. Based on strategic planning an infrastructure of the BTC concept

5. Conclusion

Fermentation technology has seen significant growth in recent years and is a rapidly growing market globally. In Denmark, the market potential for fermentation technology is quite high due to the country's strong presence in the food and biotech industries. The market potential for fermentation technology in Denmark is quite high, and the country has the necessary infrastructure, technology, and government support to drive further growth in the sector. Companies in the food and biotech industries are poised to benefit significantly from this growth, and the fermentation market in Denmark is expected to continue to grow in the coming years.
Efforts to boost the fermentation market's growth currently focus on advancing and commercializing fermentation technology with regards to funding, technical assistance, and access to research facilities to companies operating in the sector. A survey showed an interest heavily favoring food and ingredients, feed, and valorization enhancing of biomass. A majority required equipment for liquid state fermentation using both aerobic and anaerobic methods, while a considerable portion required accessibility for GMO primarily for class 1, with a single response for class 2. . A concept of a fermentation pilot plant at DTI was conceptualized under the name “Biosolution Technology Center”, which based on feedback from potential customers and online surveys, and external input defined a set of requirements for 4 scales of bioreactors and supporting infrastructure.

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