# Production of high Omega-3 contained microalgae from food waste: Focus on algal diversity by Oxford Nanopore MinION sequencing and economic analysis

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# INTRODUCTION

## Aim and Hypothesis of the Project

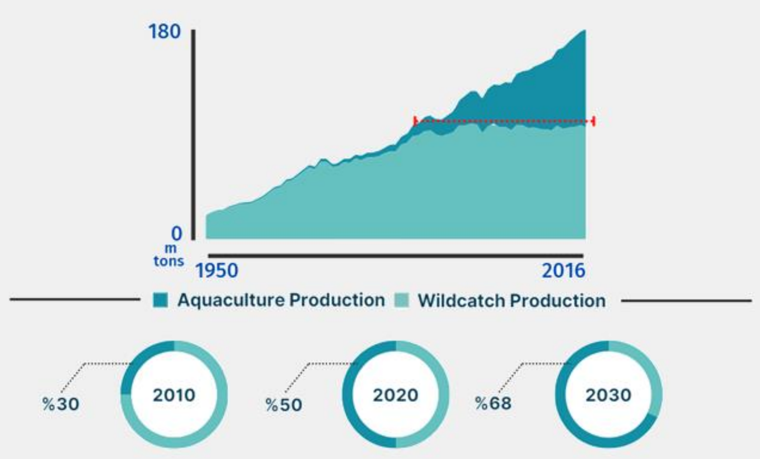
In response to growing environmental concerns, population increases, and the demand for essential nutrients like Omega-3 fatty acids, there's a critical need for accessible, sustainable sources. Microalgae, as primary producers, offer a promising solution, yet industrial production remains costly.

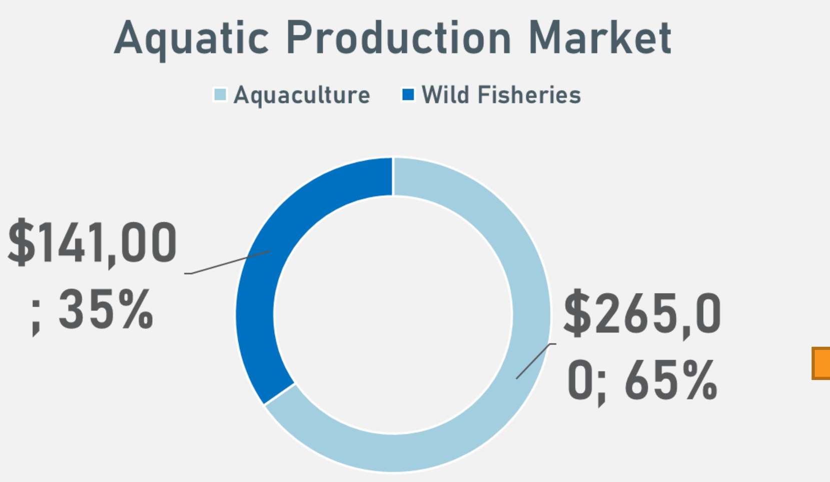
This project seeks to bridge these gaps by employing advanced sequencing technology to explore diverse microalgae strains capable of Omega-3 production from food waste. Through optimization and economic analysis, it aims to establish a scalable and economically viable production system. Ultimately, this initiative aims to contribute to environmental sustainability and public health by providing a renewable source of essential nutrients while tackling food waste challenges.

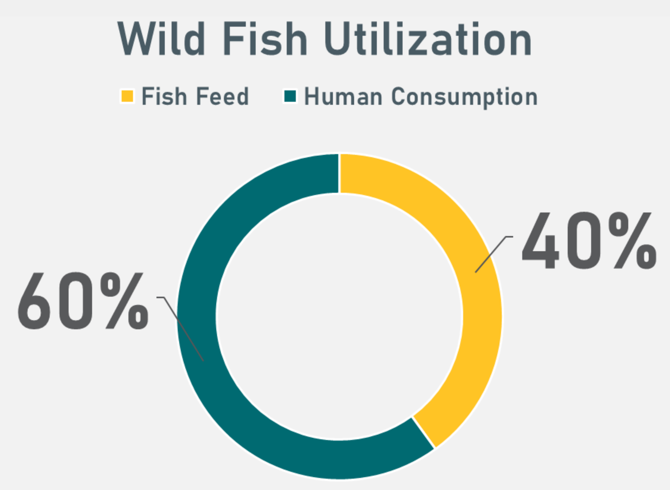
## Aquaculture and Feed Problem

With global population nearing 10 billion by 2050, meeting protein, lipid, and carbohydrate demands requires a 50% food production increase while addressing environmental concerns. The UN's Sustainable Development Goals emphasize urgent need for sustainable food production to combat biodiversity loss and environmental degradation.

Seafood, rich in Omega-3, is vital, with aquaculture now supplying over half of the world's protein and Omega-3, surpassing wild fish consumption. Yet, aquaculture's reliance on fishmeal and oil poses sustainability challenges. Research focuses on alternative feeds to boost production sustainably. Embracing biotechnology can mitigate aquaculture's impact, ensuring a sustainable seafood supply for a growing population.




## Microalgaes as Feed Additive

Microalgal biomass, recognized as fishmeal and fish oil substitutes, offers benefits like growth improvement and nutrient enrichment. Spirulina and Chlorella contain protein levels of 50-70%, but their production costs exceed $5/kg and $10/kg, respectively. Fish oil, more critical for ω-3 PUFAs, faces limitations in alternative oils.

Schizochytrium sp. emerges as a promising source, with up to 40% DHA. Its inclusion improves growth and biomass in fish species, albeit with higher production costs ($39.1-$73.9/kg vs. fish oil's $8/kg). Utilizing food waste in heterotrophic production with fermenter tanks can enhance economic viability.

| Components | Spiriluna Sp. | Chlorella Sp. | Schizochytrium sp |
|---|---|---|---|
| Crude Protein (%) | 55-70 | 37-48 | 12-25 |
| Crude carbohydrates (%) | 12-25 | 18-27.5 | 32 |
| Crude fats (%) | 4-8.2 | 13-21 | 40-72 |
| DHA (g/kg) | <1.0% | <26.0 | 104-204 |
| EPA (g/kg) | <1.0% | <4.0 | <30 |
| Ash Content (%) | 8.7 | 6-7 | 8 |

## Food Waste Usage

Academic studies show that high-sugar food waste can replace artificial glucose in Schizochytrium sp. nutrient mediums, reducing production costs. However, widespread adoption faces challenges in transitioning from lab to industrial scale, where costs would rise significantly. Scaling up production remains a major challenge in microalgae production.

The most promising solution involves conducting a techno-economic analysis for industrial-scale production within a circular economy framework. This includes designing energy and water-efficient processes, utilizing state incentives, and integrating food waste with electricity production. Additionally, DNA/RNA sequencing will analyze high-fat content samples to enhance genetic engineering efforts for improved production outcomes.

| Study Title | Food Waste Source | Key Findings |
|---|---|---|
| Li et al., 2023 | Food waste hydrolase from local restaurants | Increased total fatty acid accumulation in Schizochytrium sp. when produced with seawater isolation. |
| Kurajwska et al., 2021 | Waste glycerol | Waste glycerol identified as the best carbon source for biomass, total fatty acid, and DHA buildup in Schizochytrium sp. |
| Song et al., 2015 | Maize starch hydrolysate | Cultivation with maize starch hydrolysate yielded 85.27 g/L cell biomass and 20.7 g/L DHA. |
| Ren et al ., 2013 | Molasses syrup | Schizochytrium sp. cultivated in high sugar contained molasses syrup resulted in increased biomass and DHA output. |
| Bao et al ., 2021 | Sweet potato waste | Optimizing total sugar content in sweet potato waste to 90 g/L led to increased biomass and DHA output. |
| Surenaga, 2023 | Traditional Japanese food wastes (sake lees and yellow spinach) | EPA and DHA accumulation in Schizochytrium sp. increased with biomass due to high sugar content |

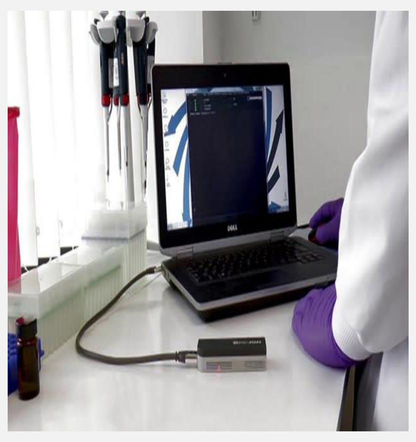
# MATERIAL & METHOD

## First Part of the Project

All the Schizochytrium sp samples were cultivated with different carbon concentrated food wastes. After that, lipid analysis were applied with GC-MS. The most promising ones advanced further through Project.

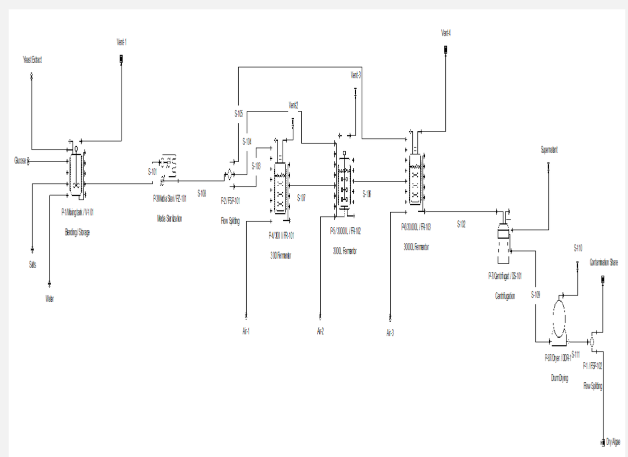
## Oxford Nanophore MinION Sequencing

Samples will undergo DNA/RNA isolation using commercial kits. DNA quality will be assessed using the QubitTM fluorometer, followed by amplification of full-length 16S and 18S rRNA gene regions. Sequencing will be performed using the Oxford Nanopore Technologies' MinION Mk1B device, with taxonomic classification using bioinformatics software.



## Techno-Economic Analysis

TEA were done by assumptions as needed parts other than that, scale-up heterotrophic production choosed and pilot/industrial scale is demonstrated on flow-diagram. All related TEA parameters will calculated to show the potential of the project.

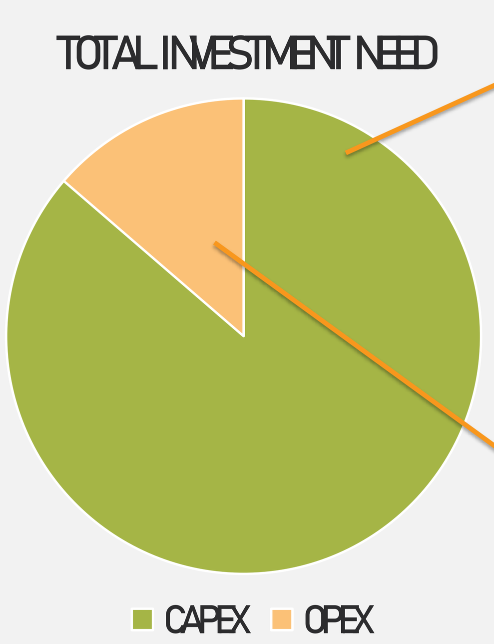


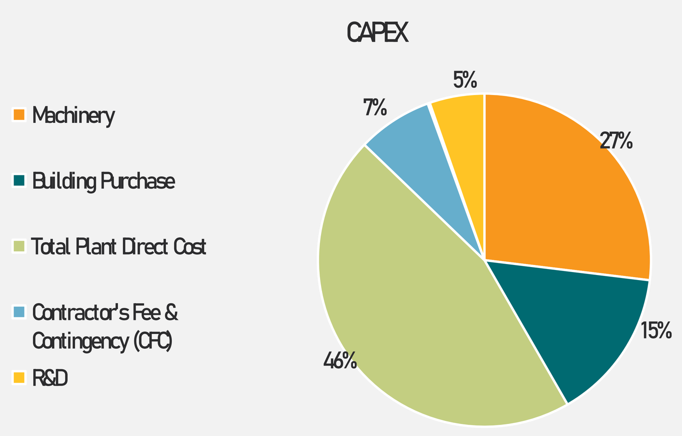
# RESULTS-DISCUSSION

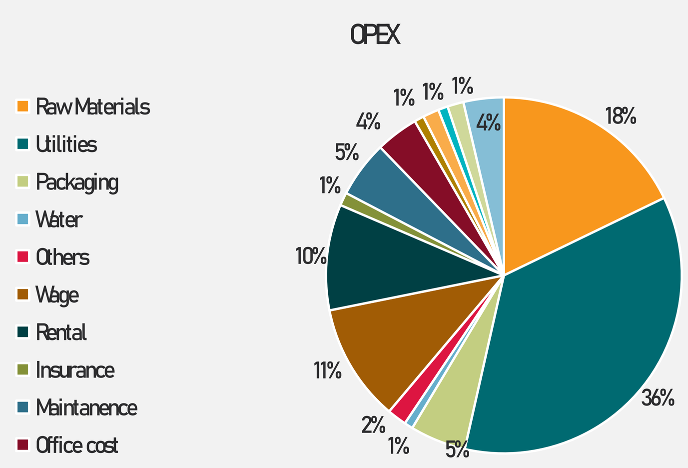
## Results

The techno-economic analysis assumes biomass and oil quantities based on academic literature, as data collection is ongoing. A 5-year financial projection, comprising a 3-year pilot scale and a 2-year industrial scale, was created, with production tonnage determined based on monthly batch numbers in a batch system. All fermenters will work on 80% volume and production tonnage also affected by this parameter too. In terms of CAPEX; all machinery, software and R&D cost were added and as OPEX; rent, wage, labor cost, Office cost, food and marketing cost on industrial scale were calculated with yearly interest rate.

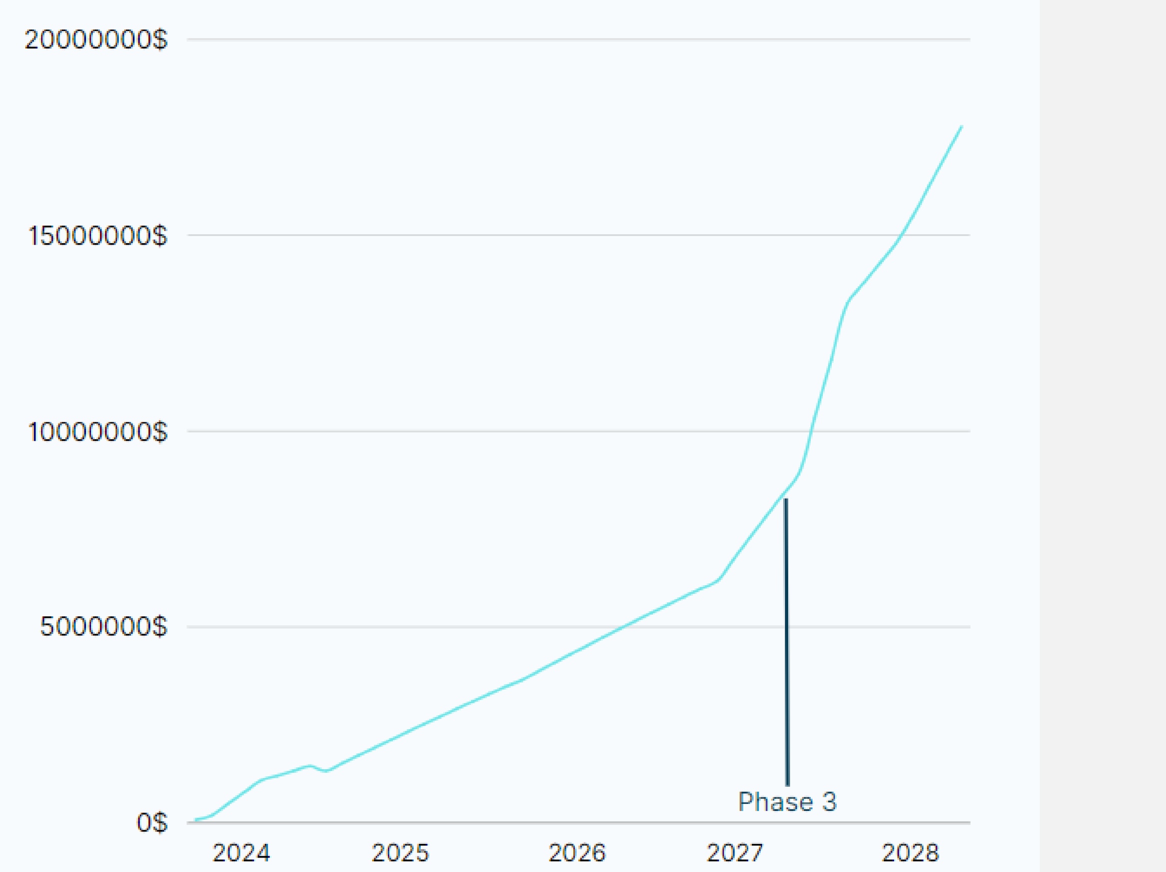
Sales price strategy variations were planned due to undisclosed market prices. Profits, EBITDA, and net cash flow were calculated based on the assumed average price. Profitability after 5 years, depicted graphically, notably rises parabolically with industrial production, mainly due to a tenfold increase in production from the addition of a high-volume fermenter tank. This illustrates the potential of scaled-up production and large fermenter tanks to address global protein and lipid needs effectively. Following financial feasibility assessments and ROI calculations, discussions with potential investors will evaluate both domestic trade and export potential, considering consumption patterns and market potential in various countries to model sales potential effectively.




| | Revenue |
|---|---|
| 2024 | $1.845.520,00 |
| 2025 | $2.227.918,00 |
| 2026 | $2.729.453,70 |
| 2027 | $14.074.476,30 |
| 2028 | $16.665.246,50 |




# FUTURE APPLICATIONS

Establishing a waste supply chain and circular economy aspect is crucial for consistent microalgal production. Optimization of waste streams is necessary to maximize microalgal biomass yield. Genomic approaches could enhance strain tolerance and boost cultivation in adverse conditions, although their full potential remains underutilized.

Additionally, leveraging techniques like MinION for biodiversity and enzyme enhancement potential, could provide insights into microbial communities' dynamics and contribute lipid accumulation efforts within microalgae cultivation systems to be a excellent solution for upcoming nutrition demand.