Final report

General information

1. Project details

Project title	CLEO, a carbon neutral fuel for the maritime sector
File no.	64020-1101
Name of the funding scheme	EUDP
Project managing company / institution	A.P. Møller – Mærsk A/S
CVR number (central business register)	22756214
Project partners	University of Copenhagen (IGN), Bio Base Europe Pilot Plant, MAN Energy Solutions, University of Copenhagen (FOOD), Technical University of Denmark (MEK)
Submission date	11 February 2026

2. Summary

Project summary

The purpose of the project

The CLEO project addressed the maritime sector's high CO_2 emissions by developing a sustainable biofuel from lignin and ethanol, researching fuel formulation, enhancing engine compatibility and fuel efficiency. Innovatively, it demonstrated biofuel production at pilot scale, integrating sulfur-free lignin for greener shipping solutions.

Results, conclusions and perspective

Important Results:

- Demonstrated production of CLEO biofuel at a pilot scale, achieving stable blends with up to 60% lignin.
- Enhanced engine compatibility with CLEO fuel, providing guidelines for required marine engine modifications.
- Developed novel methods for improving understanding of fuel properties, e.g. novel lignin size measurement techniques.

Future Use of Results:

- Insights from the project can guide the scaling up of CLEO production and its integration into existing biorefineries.
- The methodologies developed for analyzing and optimizing lignin in biofuels will advance further research and commercial applications. Both in fuels and also for other applications.
- Optimization of processing and solvent composition resulted in full lignin solvation without the need for an evaporation step.

Expected Effects of the Technology:

- CLEO biofuel offers a sustainable alternative to fossil fuels, with the potential to reduce CO_2 emissions significantly in the maritime sector.
- The technology supports the transition to green shipping, aligning with global efforts to decrease maritime emissions and enhance fuel sustainability.
- Long-term, CLEO could foster broader adoption of biofuels in other sectors, contributing to global renewable energy goals.
- However, at present our techno-economical evaluation shows that the technology is not economical viable.

Projektresumé:

Formålet med projektet

CLEO-projektet adresserede den maritime sektors høje CO_2-udledninger ved at udvikle et bæredygtigt biobrændstof fra lignin og ethanol. Projektet forbedrede produktionen, motorkompatibiliteten og demonstrerede produktion af CLEO-biobrændstof i pilotskala.

Resultater, konklusioner og perspektiv

Vigtige Resultater:

- Demonstrerede produktion af CLEO-biobrændstof i pilotskala, hvilket opnåede stabile blandinger med op til 60% lignin.
- Forbedret motor kompatibilitet med CLEO-brændstof, samt retningslinjer for hvilke modifikationer af marine motorer det vil kræve.
- Har gennemført en detaljeret karakterisering af lignin opløst i alkoholer, hvilket er grundstenen i CLEO teknologien.
- Udviklede nye metoder til forståelse af lignin, f.eks. til måling af lignin-molekylestørrelse, hvilket forbedrede forståelsen af brændstofegenskaber.
- Optimering af behandlingsprocessen og opløsningssammensætningen resulterede i fuldstændig opløsning af lignin uden behov for inddampning.

Fremtidig anvendelse af resultaterne:

- Indsigter fra projektet kan vejlede opskaleringen af CLEO-produktionen og dens integration i eksisterende bioraffinaderier – hvis det på sigt bliver økonomisk attraktivt.
- De udviklede metoder til analyse og optimering af lignin i biobrændstoffer vil fremme yderligere forskning og kommercielle anvendelser.

Forventede effekter af teknologien:

- CLEO-biobrændstof tilbyder et bæredygtigt alternativ til fossile brændstoffer, kan potentielt reducere CO_2-udledningen markant i den maritime sektor.
- Teknologien understøtter overgangen til grøn skibsfart, der er i overensstemmelse med globale bestræbelser på at mindske maritim forurening og forbedre brændstofbæredygtighed.
- På længere sigt kunne CLEO fremme bredere anvendelse af biobrændstoffer i andre sektorer og bidrage til globale mål for vedvarende energi.
- Vores teknisk-økonomiske vurdering viser, at teknologien for nuværende ikke er økonomisk rentabel.

3. Project objectives

The primary objective of the CLEO project was to address the significant environmental impact of the maritime sector, which accounts for a considerable proportion of global CO_2 emissions due to its reliance on fossil fuels. The project aimed to develop and demonstrate a sustainable, cost-effective alternative to conventional marine fuels through the innovative use of lignin, a by-product from pulping and 2G bioethanol production, and alcohols (ethanol or methanol). This alternative sought to significantly reduce greenhouse gas emissions while ensuring supply chain security and economic feasibility for the maritime industry.

Specific Goals:

- To produce a stable, high-energy-density biofuel suitable for large marine engines.
- To enhance the compatibility of this biofuel with existing marine engine technologies.
- To validate the production process at a pilot scale, ensuring it could be scaled up effectively.
- To assess and optimize the environmental and economic impacts of the biofuel production.
- To research significant unknowns such as the specific solvation patterns of lignin in alcohols, the effects of lignin variability on solvation and fuel quality, and the interactions between CLEO biofuel and marine engine systems.

Description of the Developed Technology: The CLEO project has pioneered a breakthrough in biofuel technology with the development of Cold-processed Lignin in (M)ethanol Oil (CLEO/CLiMO). This innovative technology is distinct in its use of lignin – a byproduct from pulp production and other biorefinery processes – combined with bio-ethanol or green methanol, presenting a sustainable alternative to heavy marine fuels.

CLEO/CLiMO is designed to integrate seamlessly with existing maritime fuel systems, employing lignin extracted from sustainable sources as a primary ingredient. This approach not only utilizes a waste product from other industries but also enhances the biofuel's environmental credentials.

Key Components and Process Details:

- Lignin Extraction and Preparation: Lignin is sourced from the pulp manufacturing industry (only lignin from soda pulping is relevant) and other biorefineries (e.g. from 2G bioethanol plants) where it is typically treated as a low-value byproduct. For use in CLEO/CLiMO, lignin undergoes a purification process to remove impurities that might affect fuel quality and performance.
- Solvent Selection and Preparation: The purified lignin is then blended with bio-ethanol or green methanol. These solvents are selected based on their ability to dissolve lignin effectively at lower temperatures, which is crucial for maintaining the structural integrity of lignin and ensuring efficient fuel combustion.
- Cold Processing Technique: Unlike traditional biofuel processes that require high heat, the CLEO method utilizes a cold-processing approach. This innovative technique involves dissolving lignin in selected solvents at ambient or mildly elevated temperatures. This process is energy-efficient and helps preserve the chemical structure of lignin, resulting in a higher quality fuel.

- Fuel Formulation: The resulting mixture undergoes meticulous testing to ensure it achieves the desired consistency and chemical properties suitable for marine engines. The formulation process is critical to ensuring that the fuel remains stable and homogeneous, preventing phase separation that could occur with changes in temperature or during storage.

To enhance the solvation of lignin in the alcohol mixture, the CLEO project implemented a range of experimental approaches aimed at refining solvent compositions and process parameters. This involved adjusting solvent ratios to identify optimal conditions for lignin dissolution and experimenting with temperature variations to find the most effective settings without compromising lignin's chemical integrity. The project also tested the impact of pH adjustments and various additives like surfactants and stabilizers, which help maintain lignin suspension and prevent particle re-agglomeration. Mechanical methods such as stirring and ultrasonication were utilized to improve the interaction between lignin and solvent, breaking down lignin aggregates to increase surface area and enhance solubility. An iterative feedback loop from small-scale benchtop experiments to scaled-up trials was crucial for refining each variable based on testing results. Compatibility tests ensured the solvated lignin remained stable under different conditions, and advanced analytical techniques like high-performance liquid chromatography (HPLC) and spectroscopy provided precise data to fine-tune the solvation process. These comprehensive efforts have significantly advanced the methodology for optimizing lignin solvation, crucial for the biofuel's performance and practical maritime applications.

Extensive compatibility testing is conducted to ensure that CLEO/CLiMO can be used as a sustainable fuel in marine engines if the engine is properly modified and optimized for it. These tests assess various performance metrics such as ignition quality, viscosity (important in relation to fuel pumpability, fuel injection, fuel stability, and compatibility with existing systems), mineral content, tribology (the study of friction, wear, and lubrication between interacting surfaces in relative motion), and calorific value to ensure they meet or exceed the standards/expectations required for marine fuels.

The development of CLEO/CLiMO marks a noteworthy advance in biofuel technology by utilizing under-exploited lignin resources to develop a more sustainable fuel alternative. While this technology presents a potential pathway for reducing the maritime industry's dependence on fossil fuels and repurposing a byproduct from other industries, significant challenges remain in terms of economic viability and market acceptance. As efforts continue to refine and scale CLEO/CLiMO, its impact on transforming fuel use within the maritime sector and its contribution to broader climate change mitigation efforts remain cautious yet hopeful aspirations.

4. Project implementation

Project Evolution: The CLEO project was initiated with a clear vision to develop a sustainable marine biofuel by integrating lignin with ethanol. The project progressed through several phases, starting from feasibility studies and laboratory-scale experiments, moving through pilot-scale production, and culminating in engine testing and optimization. Each phase was designed to incrementally validate the technology's practicality and efficiency, ensuring that the biofuel met the stringent requirements of marine applications.

Associated Risks:

- **Technical Risks:** The innovative nature of the CLEO biofuel presented significant technical challenges, particularly in stabilizing high-lignin-content fuel and ensuring compatibility with existing marine engines. The risks were mitigated through rigorous testing and iterative adjustments to the biofuel formulation. However, an additional complexity arises from the inherent variability of lignin, a natural polymer, which poses challenges in developing engines or engine settings that can consistently handle its heterogeneous properties.

- **Scale-up Risks:** Transitioning from laboratory to pilot-scale production involved risks related to process efficiency and cost-effectiveness, especially due to challenges like high water content and phase separation observed in the initial production runs. These risks were addressed by refining the production process to better suit the unique properties of lignin. This included implementing a two-stage evaporation process and making adjustments in solvent compositions and process parameters, such as temperature and pH levels. These iterative improvements were critical to resolving the initial challenges and establishing a more stable and efficient pilot-scale production process.

- **Market and Regulatory Risks:** The acceptance of a new type of marine biofuel by regulators and the market was uncertain. However, this is general challenge with novel fuels in the marine sector, as the standardization practices used for fossil marine fuel (using ISO 8217) can basically not cover any novel fuels. Thus, the main future challenge for further implementation of CLEO, is regarded to be economical rather than regulatory.

Adherence to Milestones: The project implementation largely developed as planned, with key milestones reached successfully (see “Afslutningsskema” for precise list):

- **Feasibility and Lab Testing:** Completed on schedule, confirming the initial viability of using lignin in marine biofuels.

- **Pilot Production:** Although challenging, pilot-scale production was achieved by BBEPP in collaboration with UCph with adjustments to the process to enhance yield and stability.

- **Engine Compatibility Tests:** Conducted as per the timeline, with significant modifications to fuel injection system and engine operation procedure. However, the final long-term test could not be performed before ending the project due to remaining challenges with buildup of CLEO deposits in the laboratory engine.

- **A thorough walk through of all deliverables and milestones,** will be presented under 5. Project results.

- **Insights on Biomass Pretreatment**: The project has elucidated the critical link between biomass pretreatment processes and subsequent lignin reactivity, highlighting how pretreatment methods significantly influence the solvation and performance characteristics of lignin in biofuel applications.

- **Refocusing of WP6:** Due to strategic shifts, not all original milestones for WP6 were achieved as planned. The focus was adjusted to better align with evolving project goals and external market conditions.

Unexpected Problems: Despite careful planning, the project encountered several unforeseen issues:

- **Lignin Supply Variability:** Variations in lignin quality and composition from different suppliers led to inconsistencies in fuel performance, necessitating a more robust preprocessing treatment of lignin. Furthermore, it was demonstrated that the lignin source must meet specific criteria that limits availability: it must be low in ash (particularly abrasive silicates), low in sulfur to comply with regulatory fuel standards (and avoid on-ship scrubbers), and crucially, cost-effective to ensure the economic viability of the final fuel.

- **Economic Factors:** Fluctuations in the cost of raw materials, particularly sulfur-free lignin, impacted the economic feasibility calculations. The project adapted by exploring alternative lignin sources and adjusting the economic models. Despite these efforts, the challenge of high raw material costs was not fully overcome, continuing to affect the project's economic viability.

- **Technical Delays:** Some engine tests were delayed due to unforeseen technical issues with fuel injection systems, which were not initially designed to handle high-viscosity biofuels which also contain volatile ethanol. This required additional development work to modify the injection system, operation procedure and safety measures.

Conclusion: Overall, the CLEO project managed to navigate the complexities of pioneering a new biofuel technology through proactive risk management and adaptive project planning. While some delays and adjustments were necessary, the project's fundamental goals were achieved, laying a solid foundation for future industrial-scale application and potential market transformation. As of now the main aspect keeping the technology from further development and eventual wider market penetration, is the unforeseen high price of low-sulfur lignin. Unfortunately, there is currently not indication of an immediate price reduction of this feedstock.

5. Project results

Technological Results Obtained:

In the following an overview of the technical results will be presented, on WP level, and with main deliverables and milestones highlighted.

WP1: Process optimization and pilot-scale production using Protobind-1000 lignin

Work Package 1 (WP1) centred on upscaling the CLEO biofuel from lab-scale experiments to pilot-scale production using Protobind-1000 lignin (at BBEPP). The overarching goal was to develop an optimized CLEO solution that could be produced in pilot-scale quantities for engine testing. The main milestone associate to WP1 were: M1.1: Successful upscaling of >4 tons CLEO fuel. While the most important deliverable was: D1.3.1: Pilot Scale CLEO Production.

Work Package 1 embarked on transitioning CLEO biofuel production from laboratory experiments to pilot-scale using Protobind-1000 lignin, starting with an ambitious goal to produce one ton of CLEO in a 4000 L batch (at BioBase Europe Pilot Plant). However, the initial run faced challenges with high water content, prompting a re-evaluation of the process and a scale-down to smaller batch sizes ranging from 200 L to 400 L. This adjustment allowed for more precise control over the biofuel's properties, including refined ethanol concentrations and a redesigned evaporation process informed by collaborative input from UCPH IGN.

Despite the setbacks of the first large-scale batch, subsequent production runs implemented a two-stage evaporation process that effectively reduced water content and stabilized the biofuel mixture. Each iteration brought improvements in handling and quality control, proving critical for achieving a scalable and reproducible pilot production protocol. Alongside these technical successes, the project navigated significant challenges, particularly in managing large volumes of reactive materials in compliance with ATEX safety standards. The lignin-ethanol mixture was prone to clogging and settling, requiring continuous adjustments and enhancements in filter capacity and process automation to ensure uniform quality and reduce labour-intensive interventions.

The learnings from these initial runs have established a robust foundation for the next stages of the project. Further refinement of evaporation and mixing techniques is needed to improve both the efficiency and scalability of the production process. These enhancements are essential for supporting larger-scale production efforts and ensuring the economic viability of the CLEO biofuel as it progresses toward commercialization. However, these lie outside the scope of this project.

WP2. Assessment of applicability on large two-stroke engines, validation and documentation.

Work Package 2 focused on developing a small test engine that could replicate the injection and combustion conditions of large marine engines, enabling testing with small quantities of CLEO fuel. This strategic approach allowed the project to gather crucial operational insights without the prohibitive cost of using a full-scale marine engine test bed for extended periods. The development of the test engine involved adapting features typical of large marine engines such as heated fuel injection systems to lower fuel viscosity, a fuel switching system for engine heat-up and shutdown, and the use of pressurized sealing oil in the injection pump to minimize variations in leak rate when switching between fuels. As a part of this work MSc student, Rasmus Gottenborg Kajbæk, made his thesis titled: *Design and performance test of variable injection timing system for a small experimental compression ignition engine*, 2022.

Despite the success in replicating these features, CLEO posed additional challenges due to its unique composition, combining lignin with high viscosity and no volatility, ethanol with low viscosity and high volatility, and water, which adds no heating value but has a high heat of vaporization. Achieving the correct proportion of these components was critical to maintaining CLEO as a stable and liquid fuel. This task proved more challenging than anticipated due to the low flash point temperature of ethanol, which necessitated enhanced safety measures in the laboratory, including automatic leak detection and shutdown systems for the engine.

One significant hurdle was the precipitation of lignin in the injection system during fuel switching, which led to system failures. The project team engaged in a rigorous trial and error process to identify an effective switch-over fuel formulation that would prevent such issues. Moreover, the initial batches of CLEO produced at the pilot plant contained a higher water content than was viable for proper ignition in an engine. Extensive testing was required to adjust the engine's charge pressure and temperature to address misfires and prevent the buildup of unburned lignin deposits, which was a more challenging task than expected.

Throughout these experiments, it was clear that maintaining the physical and combustion properties of CLEO in the fuel system up to injection was pivotal. The project made significant advancements in managing the fuel's characteristics, such as ensuring it remained stable and pumpable when heated to temperatures up to 120°C under pressure and optimizing the engine back pressure to prevent issues like flash boiling of ethanol in the injector tip.

The extensive testing and development culminated in the formulation of a final version of CLEO that displayed adequate ignition quality for engine conditions similar to large two-stroke marine engines without the need for a pilot flame. The project also developed innovative methods to measure the build-up of deposits on the cylinder wall without disassembling the engine, significantly reducing the time needed to optimize the injection system for any given fuel.

Despite not achieving long-term testing due to the challenges with deposit formation on the cylinder walls, the results from WP2 have provided invaluable learnings for future testing of CLEO and other sustainable fuels. The work conducted under this package has laid a robust foundation for potentially redesigning injection and fuel supply systems tailored for new marine fuel formulations, highlighting the project's substantial contributions to the development of sustainable maritime fuel technologies.

The evaluation of CLEO’s technical viability, within large two-stroke engines revealed several critical aspects that are crucial for its successful implementation. While CLEO holds promise as a sustainable alternative to traditional marine fuels, its unique properties pose specific challenges that must be managed to ensure practical engine compatibility and performance.

The primary concern identified was the compatibility of CLEO with existing engine technologies. Extensive engine tests demonstrated that CLEO's higher viscosity and lignin content significantly affect its behavior under standard engine operating conditions. These tests highlighted the necessity for specialized adjustments in fuel handling, particularly the heating mechanisms required to maintain appropriate viscosity levels for efficient atomization and combustion. As a part of this work PhD student Motoki Terauchi published an article titled "*Experimental Study of Lignin Fuels for CI Engines*" (2024), as a valuable contribution to the project.

Further, the stability of CLEO in fuel systems was another focal point. The tests indicated a propensity for lignin components to settle, leading to inconsistencies in fuel composition during longer operational periods. This issue necessitated the development of more robust fuel agitation and circulation systems to maintain uniform fuel characteristics from storage to combustion.

Additionally, the formation of deposits from lignin residues was observed to have an impact on engine components, particularly the injector, cylinder wall and valves. These deposits, if not managed properly, lead to reduced engine performance and failure, thus requiring increased maintenance. Addressing this, the project successfully developed various injection system modifications and operation strategies aimed at minimizing residue accumulation, enhancing the overall reliability and longevity of engine components when operating with CLEO.

In conclusion, while the technical viability of CLEO has been substantiated through rigorous testing, the adaptation of this biofuel for widespread use in maritime applications will require targeted innovations in engine design and fuel management systems. The ongoing development efforts aim to refine these aspects, ensuring that CLEO can meet the operational standards necessary for commercial viability in the maritime industry.

WP3. Fuel formulations.

Work Package 3 aimed to increase the robustness of CLEO technology by developing methods to formulate fuels from additional commercially available lignin feedstocks and by evaluating alternative solvent systems. The primary goal was to enhance the solvation and combustibility of lignin particles, thereby improving the overall performance of CLEO biofuel in marine engines. The team at UCPH-IGN focused on assessing various types of commercially available lignin, from different plant sources (such as wood, wheat-straw, and sugarcane-bagasse), and from different technologies (such as organosolv, soda pulping, and hydrothermal pretreatment followed by enzymatic saccharification) for compatibility with the CLEO process. Evaluation parameters included solvation yield in ethanol, mineral and sulphur content, and stability. Lignin samples that demonstrated sufficient solvation yield and stability were forwarded for engine testing and chemical characterization, while those that did not meet the criteria were directed to further processing enhancements. Additionally, the compatibility of other bio-based alcohols, specifically methanol and glycerol, with the CLEO system was examined to determine if they could enhance lignin solubility and engine compatibility.

The Master's thesis by Rebeka Baskaran and Nikki Nafar, titled *"Properties of lignin in ethanol and methanol solutions",* provided valuable insights into the physical properties critical to developing CLEO and CLiMO biofuels. Their work specifically explored the stability, viscosity, and structural properties of these fuels at various concentrations and temperatures, offering foundational data for future formulation improvements. Under the guidance of their supervisors and within the collaborative framework of the CLEO project, their research contributed to enhancing our understanding of lignin's behavior in biofuel applications. The integration of such academic efforts into the project not only propelled the technological advancements but also enriched the academic and practical landscape of sustainable biofuel research.

Significant advancements have been made in understanding the solvation performance of various lignin types within the CLEO process. The study demonstrated that the solubility of lignin varies notably based on its source and extraction method. Notably, alkali and Kraft lignins, which are typically purer, exhibited higher solvation yields compared to biorefinery lignins that contain residual carbohydrates. These carbohydrates have low solubility in alcohols and impede solvation by affecting the lignin's hydroxyl groups, which are crucial for forming stable solutions. The findings underscore the need for tailored processing techniques to enhance solvation yields, especially for lignins that currently show poor solubility. This approach could significantly improve the efficiency and adaptability of the CLEO biofuel, suggesting a move towards more specialized treatments or chemical characterizations for certain lignin types. These adaptations are vital for refining CLEO biofuel formulations to ensure broader application and operational efficiency in marine engines.

Extensive tribological testing provided insights into how CLEO biofuel interacts with engine components, focusing on friction performance and wear characteristics under simulated operational conditions. Innovations included the use of duplex plasma nitriding and diamond-like carbon coatings to enhance the wear resistance of engine components exposed to CLEO biofuel. The work package tackled challenges related to material compatibility and the physical properties of biofuel blends. Adjustments in lignin processing and formulation were critical in overcoming these obstacles, enhancing the biofuel's performance and stability. The introduction of alternative bio-based alcohols also opened new pathways for improving fuel properties. Work Package 3 not only addressed the initial technical objectives but also expanded the potential applications of CLEO biofuel by incorporating a broader range of lignin sources and exploring new solvent systems. This holistic approach aimed to refine the fuel's formulation for better engine performance and environmental sustainability, setting the stage for further development and potential commercialization.

WP4. Advanced characterization.

Work Package 4 (WP4), titled "Advanced Characterization," played a pivotal role in the CLEO project by exploring the intricate physical processes involved in colloidal formation, crucial for understanding and improving the biofuel's storage, bunkering, and performance characteristics. The primary objective of WP4 was to close knowledge gaps by employing advanced characterization techniques to measure rheological properties, identify nano- and micro-scale structures, and characterize lignin sources.

In-depth rheological studies, as detailed in the activities of WP4, provided significant insights into the flow properties of CLEO fuel colloidal systems. These investigations focused on the effects of concentration, temperature variations. The studies confirmed that the unique composition of lignin, ethanol, and water in CLEO significantly influences its viscosity and flow characteristics under different conditions. For example, findings from the *"Kumar et al. 2022"* paper illustrated how temperature and concentration adjustments could modulate the viscoelastic properties of the fuel, which are critical for ensuring compatibility with existing fuel infrastructure and engine systems. (for more info see the paper *“Kumar et al, LEO and LiMO Fuels: Structural and Rheological Characterization of Solvolytically Fractionated Lignin Dispersed in Alcohols”* – do to time issues this paper focussed on the very similar LEO and CLEO fuels, but the findings are applicable to CLEO/CLiMO as well).

The *"Simonsen et al. 2024"* paper linked the colloidal lignin structures in CLEO with its rheological behaviour, providing detailed information on the arrangement and interactions of lignin particles at various scales, from atomic to several micrometers. Such structural insights are essential for tailoring biofuel formulations to enhance solvation and stability, thereby improving fuel performance in maritime applications. (for more info see the paper *“Simonsen et al, 2024. Characterization and Structural Analysis of Alcohol-Fractionated Lignin Biofuels Processed at Ambient Temperature”*).

Advanced chemical characterization activities involved techniques such as Gel Permeation Chromatography (GPC), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Small-Angle X-ray Scattering (SAXS), Flow-Induced Dispersion Analysis (FIDA), Dynamic Light Scattering (DLS), Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR), and several Nuclear Magnetic Resonance (NMR) spectroscopy based methods such as HSQC, phosphorus NMR and Diffusion-Ordered Spectroscopy (DOSY). These methods were instrumental in defining the molecular architecture of different lignin samples and their chemical behaviour in CLEO formulations. Moreover, microscopy performed at the Center for Advanced Bioimaging (CAB) provided high-resolution images that further aided in understanding the microstructural features of lignin particles within the biofuel.

By providing a deeper understanding of the physical and chemical behaviours of CLEO biofuels, the technological results obtained from WP4 have substantially contributed to refining the specifications for CLEO biofuel formulations.

WP5: Improving lignin-CLEO compatibility.

Work Package 5 (WP5) of the CLEO project focused on elucidating the influence of chemical and structural diversity of lignin feedstocks on the production and stability of CLEO biofuel. This work package aimed to enhance the conversion efficiency of lignin into CLEO and facilitate the integration of this technology into second-generation (2G) biorefineries. Despite some deviations from the initial plans due to various challenges, substantial progress was made in understanding and improving lignin solubility and its subsequent utilization in biofuel production.

Challenges were faced in measuring the zeta-potential of CLEO and 'Pre-CLEO' solutions, which was crucial for understanding their colloidal stability in ethanol. Despite extensive efforts using the Zetasizer Nano ZS and expanding the methodology to include two additional instruments, zeta-potential measurements were not successful. These repeated failures across different instruments led to the conclusion that lignin in CLEO may not behave as colloidal particles as previously assumed. This finding suggests a fundamental reconsideration of how lignin interacts in these solutions, indicating that the charged particle model used to explain colloidal stability might not apply to lignin in CLEO formulations. However, the unsuccessful measurements might also be due to instrument incompatibility with CLEO.

A key achievement in WP5 was the development of innovative biobased solvent mixtures that significantly increased the fractionation yield of lignin. Particularly notable was the use of a novel solvent composition consisting of 40 wt% glycerol and 60 wt% ethanol, which not only enhanced the yield to 89% but also eliminated the need for solvent evaporation, potentially reducing production costs. This breakthrough indicates a promising direction for reducing the economic barriers associated with CLEO production, although the new solvent system's effectiveness in engine tests remains to be evaluated. (for more info see the manuscript *"Simonsen et al, 2024. Improving the production efficiency and sustainability of lignin-alcohol fuel processed at ambient temperature*").

Further advancements were made in modifying the biomass pretreatment steps, a critical phase in CLEO production within a biorefinery context. By integrating additives such as hydrogen peroxide during hydrothermal treatment, researchers were able to produce a lignin with significantly higher reactivity and solubility in alcohol. This modified lignin showed more than double the solvation yield compared to lignin obtained through standard treatments. Such modifications not only enhance the compatibility of lignin with CLEO technology but also expand the potential lignin market for CLEO production by making it feasible to use lignin from existing biorefinery operations like 2G bioethanol plants (for more info see the manuscript *"Simonsen et al, 2024. Enhanced Alcohol Solubility of Lignin-Rich Residues from Wheat Straw Hydrothermally Pretreated with Varied Temperature and Additives"*).

Additionally, WP5 introduced a high-throughput method for characterizing the hydrodynamic radius and estimating molecular weight of lignin, pivotal for understanding its behavior in biofuel applications. This method, based on Taylor Dispersion Analysis (TDA), offers a rapid, reliable, and solvent-versatile alternative to traditional Size-Exclusion Chromatography (SEC) for analyzing molecular size, which is crucial for optimizing production processes and ensuring consistent biofuel quality (for more info see the *manuscript "Simonsen et al, 2024. Lignin Molecular Weight Estimation by Dispersion Analysis"*).

These technological results obtained in WP5 not only deepen the understanding of lignin's role in biofuel production but also pave the way for more efficient and economically viable CLEO biofuel formulations. These innovations are expected to facilitate the broader adoption of CLEO technology in the maritime sector, contributing to the overall goal of reducing reliance on fossil fuels and promoting environmental sustainability.

Additionally, in Work Package 5, four student projects contributed significantly to the advancement of the CLEO project, each focusing on different aspects of biofuel production using lignin.

1. Xiaoyu Sun, made a “project outside course scope”, where she examined the particle size effects on lignin yield and ethanol solvation, which are crucial for enhancing the practical application of lignin in biofuels and ensuring consistent fuel quality.
2. Xiaoyu continued in her MSc, where she focused on the enzymatic hydrolysis of lignin and its effect on the yield of solvated lignin from wheat straw. Her work provided insights into how enzymatic treatments could be optimized to increase the effectiveness of lignin solvation, which is critical for the successful application of this technology in commercial biofuels.
3. In Jasmin Dilgen’s MSc, she explored the impact of different solvents on lignin solvation, specifically examining how various solvent mixtures could enhance the solubility and stability of lignin from poplar in biofuel formulations. Her findings have helped in identifying optimal solvent conditions that could potentially improve the efficiency of lignin-based biofuels.
4. In an internship, Hugo Montanvert investigated the potential of different ratios of glycerol and ethanol in stabilizing and solubilizing lignin, identifying a specific mixture that maximized lignin yield at 49%. His research contributes to understanding the critical parameters for improving the solvation process in industrial applications.

These student projects have collectively enriched the understanding of lignin behavior in biofuel applications, offering new approaches to tackle the challenges of lignin solvation and stability that are vital for scaling up this promising technology.

In the final period of the project, significant results were obtained based on the collective learning thus far. We managed to produce CLEOs using a new recipe with enhanced shear effects and increased heating (50°C), where we achieved 100 % yield from various lignins (from grass, softwood and hardwood), exceeding 40% lignin concentration with complete stability avoiding the energy-demanding evaporation step, which was previously essential for the CLEO technology. This streamlined production process not only simplifies the methodology but also has the potential to substantially reduce production costs, making the technology more economically viable for broader application. At UCPH-IGN, we hope to continue this development.

WP6: Sustainability Assessment

Throughout 2022 and 2023, WP6 concentrated on assessing the energy balance and the environmental and economic sustainability of the CLEO technology. The techno-economic assessments (TEAs) were refined using advanced modeling tools such as Aspen+. These assessments provided crucial data on CAPEX for 2G ethanol and CLEO production, helping to clarify the economic viability of the technology. Despite the comprehensive data provided by these models, certain aspects like the full economic impacts and lifecycle analyses were not as extensively covered due to re-prioritization within the project.

WP7: Business Development & Exploitation

In parallel, WP7 focused on refining business strategies and exploitation plans, aided significantly by the insights from the TEAs. Workshops involving key stakeholders, including APMM, DTU, and UCPH, were pivotal in identifying the most critical elements affecting the commercial potential of CLEO. These workshops highlighted the importance of considering various factors such as the combination of lignin and alcohol types, process conditions, and detailed cost analyses (including CAPEX, OPEX, and feedstock costs) that influence the technology's scalability and market penetration.

One notable achievement has been the submission and subsequent progression of a patent application, which has successfully navigated the approval processes in several countries. This patent is crucial for protecting the innovative aspects of the CLEO technology and forms a key component of the project's intellectual property strategy (*Cabrera, 2022, Cabrera, O.Y., 2022. Lignin Composition WO2022/117391.*).

However, the year 2023 brought to light significant challenges in sourcing competitively priced biomass feedstocks (low-sulfure lignin and sustainably produces ethanol or methanol), due to increased market competition for these materials. This has necessitated a broader investigation into alternative uses of lignin beyond biofuel applications, as part of a strategic pivot to ensure the sustainability of the CLEO project amidst fluctuating market conditions.

Although not all planned activities for WP6 were executed, the project has made meaningful advances in understanding the sustainability and economic aspects of CLEO production. The ongoing business development activities under WP7 continue to adapt to emerging market conditions, focusing on enhancing the techno-economic models to better capture the commercial landscape and potential of CLEO technology. This strategic approach is aimed at ensuring that CLEO can achieve commercial viability and contribute to a more sustainable maritime sector.

Commercial Results Obtained:

The commercial outcomes of the CLEO project have been mixed, reflecting the challenges inherent in pioneering new technologies in competitive markets. While significant technological advancements were achieved, translating these into commercial success has been tempered by external market conditions and internal project challenges.

Despite initial high expectations, the commercial viability of CLEO biofuel has been constrained primarily by the high cost and limited availability of essential raw materials, such as low-sulphur lignin and sustainable alcohols. These costs have remained prohibitive, preventing the project from achieving a competitive edge in the current market for marine fuels. The fluctuating prices and availability of these materials have made it unfavourable to establish a consistent and economically viable production process.

Additionally, the relatively small current market for sustainable ethanol and methanol has limited the potential for immediate large-scale commercialization of CLEO biofuel. The project's results, while promising in a technical sense, have thus far not translated into increased turnover or market share. However, the project has successfully established a framework and a proof of concept that could potentially be scaled if market conditions become more favourable. It should be noted that to produce CLEO sufficient for just one commercial ship or container vessel, an amount of ethanol equivalent to the entire annual output of a full-scale second-generation (2G) ethanol plant is required, illustrating the vast quantities needed for such applications.

In summary, while the CLEO project has laid important groundwork and demonstrated the feasibility of lignin-based marine biofuels, the expected commercial results have not yet been realized due to external market pressures and the nascent stage of biofuel technologies in the maritime sector. While the technology for the time being is on hold, the project partners remain committed to monitoring market developments and exploring strategic opportunities to leverage the project's results in the future.

Target Group and Added Value:

The primary target group for CLEO biofuel comprises maritime operators and fuel suppliers looking to diversify their energy sources and reduce their environmental footprint. Despite the limited commercial results to date, the project offers potential added value in terms of environmental benefits and alignment with global decarbonization goals.

The added value of CLEO biofuel lies in its potential to provide a sustainable alternative to conventional marine fuels, contributing to reduced greenhouse gas emissions and enhanced energy security. This is particularly relevant for shipping companies and regulatory bodies focused on complying with stricter emissions regulations in the maritime sector.

However, the commercial uptake of CLEO biofuel has been slower than anticipated, largely due to the high production costs and the current small market for sustainable biofuels. While the technology holds promise, the economic viability remains a significant barrier, limiting the immediate target group to entities capable of investing in long-term sustainability projects or those located in regions with strong support for green maritime technologies.

Dissemination of Project Results:

The dissemination strategy for the CLEO project has been carefully managed to balance the need for competitive edge with the commitment to contribute to the scientific community. While public and commercial disclosures have been limited to protect the project's competitive advantages, there has been robust engagement within the academic and scientific communities.

Scientific Community Engagement:

The project team has actively participated in several academic conferences, presenting findings and engaging with peers to foster collaboration and gain insights that could refine the project's direction and outcomes. These interactions have been crucial in keeping the scientific community informed of the ongoing research and technological developments within the CLEO project.

List of conference presentations:

- Motoki Terauchi, presentation at SAE - CO2 Reduction for Transportation Systems Conference - The road to decarbonization, Torino, Italy, June 12-13, 2024.
- Motoki Terauchi, presentation at JSAE- Annual Congress, Yokohama, Japan, May 22-24, 2024.
- Tor Ivan Simonsen, IGNs PhD and Postdoc Conference 2022 (Best oral presentation award)
- Tor Ivan Simonsen, IGNs PhD and Postdoc Conference 2023 (Best poster award)
- Tor Ivan Simonsen Visualize your Science Conference (May 11, 2023)

List of popular science outreach:

- Nyt dansk vanilje-brændstof kan gøre fremtidens fragtskibe grønne, Tor Ivan Simonsen in Videnskab.dk, 2023, https://videnskab.dk/teknologi/nyt-dansk-vanilje-braendstof-kan-goere-fremtidens-fragtskibe-groenne/
- Verdens Fragtskibe Udleder Mere CO2 End Tyskland. I En Kælder På Frederiksberg Udvikler Tor Fremtidens Klimaneutrale Skibsbrændsel, Tor Ivan Simonsen in collaboration with Anders Seneca Bang in Verdensmaal.dk, 2023, https://www.verdensmaal.org/nyheder/verdens-fragtskibe-udleder-mere-co2-end-tyskland.-i

Publication of Research Findings:

A significant aspect of the project's dissemination efforts has been the publication of research papers. The team has successfully published multiple papers detailing various aspects of the technology, from biofuel formulation to the optimization of lignin solvation processes. Additional manuscripts are in the final stages of preparation or under review, which will further document the scientific achievements of the project. These publications serve not only to share knowledge but also to establish a foundation for future research in sustainable marine biofuels.

List of published scientific papers and submitted manuscripts:

- *Kumar S, Risbo J, Kirkensgaard JJK, Orozco YC, 2022, LEO and LiMO Fuels: Structural and Rheological Characterization of Solvolytically Fractionated Lignin Dispersed in Alcohols. ACS Sustainable Chem. Eng. 2022, 10, 39, 13156–13164*
- *Simonsen, T. I., Kumar, S., Djajadi, D. T., Kirkensgaard, J. J. K., Risbo, J., Thomsen, S. T., and Orozco, Y. O., 2024. Characterization and Structural Analysis of Alcohol-Fractionated Lignin Biofuels Processed at Ambient Temperature. Heliyon. Status: Under review.*
- *Terauchi, M., Simonsen, T., Mortensen, S., Schramm, J., & Ivarsson, A., 2024. Experimental Study of Lignin Fuels for CI Engines. SAE International.*
- *Simonsen, T. I., Djajadi, D. T., Montanvert H., Sgarzi, M., Gigli, M., Thomsen, S. T., Orozco, Y. O., and Crestini, C., 2024. Improving the production efficiency and sustainability of lignin-alcohol fuel processed at ambient temperature. Bioresource Technology*
- *Terauchi, M., et al., Experimental Study of Lignin Fuels for Marine Engines, JSAE Technical Paper 20245285, 2024.*
- *Simonsen, T. I., Djajadi, D. T., Ponzecchi, A., Crestini, C., Gigli, M., Sgarzi, M. and Thomsen, S. T., 2024. Lignin Molecular Weight Estimation by Dispersion Analysis. Macromolecular Rapid Communications. Status: Under review.*
- *Simonsen, T. I., Djajadi, D. T., Thomsen, S. T., 2024. Enhanced Alcohol Solubility of Lignin-Rich Residues from Wheat Straw Hydrothermally Pretreated with Varied Temperature and Additives. Bioresource Technology Reports. Status: Under review.*

PhD Research and Theses:

Two PhD candidates have been integral to the research activities, contributing to both the project's outcomes and the broader academic discourse. One thesis has been submitted and is scheduled for defence on October 10th, 2024 (Title: Lignin-Alcohol Dispersions - A Promising Marine Biofuel, Author: Tor Ivan Simonsen, UCPH-IGN, 2024). While another is expected to be submitted by the end of 2024 (Title: Experimental Assessment of Sustainable Lignin Fuels for Marine Engines, Author: Motoki Terauchi, DTU Construct). These theses encapsulate comprehensive studies conducted as part of the project and are anticipated to contribute significantly to the field, enhancing understanding and application of biofuel technologies in the maritime sector.

In conclusion, although public and commercial disclosures have been restrained to maintain a competitive advantage, the project's scientific results have been widely disseminated through scholarly articles, conference presentations, and PhD research. This strategic approach ensures that while the project retains its commercial potential, it also contributes valuably to the scientific community and supports ongoing advancements in sustainable energy solutions.

6. Utilisation of project results

Technological and Commercial Utilization:

The CLEO project has developed significant technological advancements in the production of sustainable biofuels for the maritime sector. These results are anticipated to be utilized by our consortium partners, including academic institutions and industry players like APMM and MAN, who have actively contributed to refining the biofuel's composition and testing its application in engines, and to establishing a market outlook. The project's outcomes have potential applications in enhancing the sustainability profiles of marine operators and fuel suppliers seeking renewable alternatives to reduce their carbon footprint.

To the best of our knowledge, our project has successfully produced the largest batch of lignin-based fuel ever recorded on a global scale. This milestone represents a significant advancement in the field of sustainable biofuels. By scaling up production to the ton scale, we have not only demonstrated the feasibility of large-scale lignin fuel production but also set a new benchmark for the industry.

In terms of commercial utilization, the groundwork laid by the CLEO project is poised to facilitate the future commercialization of lignin-based biofuels. Although current market conditions, characterized by high costs for key raw materials like low-sulfur lignin and sustainable alcohols, have impeded immediate commercial viability, the project partners remain committed to advancing this technology. They expect that with favorable developments in raw material costs and increased market demand for greener fuel options, there will be a viable path to market entry.

Economic Impact and Market Entry:

So far, the project has not directly led to increased turnover, exports, employment, or significant private investments due to the preliminary stage of the technology and market constraints. However, project partners are attentive on the future economic developments. Under certain circumstances, successful commercialization and increased turnover and exports might be achievable, particularly in regions aggressively pursuing maritime decarbonization. Additionally, the scaling up of production and broader adoption of CLEO biofuel can potentially drive employment and attract private investments in green technologies.

Competitive Market Situation: The market for sustainable marine fuels is becoming increasingly competitive with several alternatives such as LNG, hydrogen, and other biofuels gaining traction. Main competitors include established biofuel producers and new entrants developing synthetic fuels. Especially green methanol is rapidly gaining traction as a marine fuel due to its potential to significantly reduce greenhouse gas emissions from ships. As a renewable fuel, green methanol can be produced from sustainable resources such as biomass and biogenic CO_2 with green H_2, making it a compelling alternative to traditional fossil fuels. Its adoption in the maritime industry is driven by the increasing regulatory pressures to lower emissions and the sector's commitment to achieving international climate goals. Additionally, methanol's relatively straightforward integration into existing engine technologies and fuel infrastructure, compared to other alternative fuels like ammonia or hydrogen, offers a practical and immediate pathway for reducing the environmental impact of shipping operations. These advantages position green methanol as a key player in the maritime industry's transition towards more sustainable fuel solutions.

Despite these alternatives, CLEO biofuel offers a distinct advantage due to its potential for higher energy security and reduced dependency on fossil fuels, aligning with global energy policy objectives to decrease maritime emissions.

Barriers and Strategies for Market Entry:

Entry barriers include the high cost of raw materials, stringent regulatory standards, and the nascent infrastructure for biofuel production and distribution. To overcome these barriers, the project partners plan to continue advocating for policy support to lower the costs of sustainable feedstocks and enhance fuel infrastructure. Additionally, ongoing R&D efforts aim to improve the efficiency and cost-effectiveness of the production process, making CLEO more competitive.

Contribution to Energy Policy Objectives:

The CLEO project contributes directly to energy policy objectives by providing a renewable fuel alternative that enhances energy security and reduces reliance on imported fossil fuels. In times of geopolitical instability or fluctuating oil prices, CLEO biofuel could offer a more stable and sustainable fuel option for the maritime industry.

Ph.D. Involvement and Dissemination:

Two Ph.D. candidates have been integral to the project, contributing to both the technological and analytical advancements. Their research findings have been disseminated through teaching activities, conference presentations, and are encapsulated in their forthcoming thesis defenses. These educational activities not only spread knowledge about the project's findings but also inspire future research and development in the field of sustainable energy.

7. Project conclusion and perspective

Project Conclusions: The CLEO project has made significant strides toward developing a sustainable biofuel for the maritime sector. While it has demonstrated the technical feasibility of producing and using a lignin-based biofuel at a pilot scale, the project also highlighted substantial challenges in scaling and commercialization due to the high cost of raw materials and limited market readiness for sustainable fuels.

Next Steps for the Developed Technology: The immediate next steps for the CLEO technology involve continued research and development to optimize production processes and reduce costs. This includes exploring alternative, more cost-effective lignin sources and further refining the biofuel's formulation to improve its economic viability and market competitiveness. Additionally, efforts will be made to strengthen partnerships with industry stakeholders to facilitate pilot projects that could showcase the biofuel's practical applications in real-world maritime settings.

Future Development Perspectives: The insights gained from the CLEO project are poised to significantly influence future developments in the field of renewable marine fuels. By demonstrating the potential of lignin-based biofuels, the project contributes to the broader discourse on sustainable energy solutions in the maritime industry, which is under increasing pressure to reduce its environmental footprint.

Long-term, the project's outcomes could help pave the way for greater acceptance and adoption of biofuels, influencing policy decisions and encouraging further investments in renewable energy technologies. The methodologies and knowledge developed can also be applied in other sectors looking to transition away from fossil fuels, potentially leading to broader environmental and economic impacts.

Moreover, the technological advancements and challenges documented throughout the project provide valuable lessons for similar future initiatives, highlighting the importance of continuous innovation and adaptation in the face of evolving market and regulatory landscapes.

8. Appendices

- https://videnskab.dk/teknologi/nyt-dansk-vanilje-braendstof-kan-goere-fremtidens-fragtskibe-groenne/
- https://www.verdensmaal.org/nyheder/verdens-fragtskibe-udleder-mere-co2-end-tyskland.-i
- *https://pubs.acs.org/doi/10.1021/acssuschemeng.2c04017?ref=PDF*
- *https://saemobilus.sae.org/papers/experimental-study-lignin-fuels-ci-engines-2024-37-0022*
- *https://pubmed.ncbi.nlm.nih.gov/39032534/*
- *https://tech.jsae.or.jp/paperinfo/en/content/p202401.285/*