The closed-loop plant-based low-gravity biorefinery based on recent developments in cellulosic nanotechnology - from seeds to construction components

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Introduction

Lignocellulosic plants are crucial for human survival, and it is a natural source of food and a source for fabrication of various materials, from building construction to packaging applications. Even though we are using mineral and fossil-based materials for many purposes in society, the anthropogenic impact on our climate call for a transition to biobased materials. The key for a future sustainable society is a circular economy, where components that we extract from nature can be reused, remanufactured, or recycled with minimal impact on our environment and the ecosystem. Central to this vision is the biorefining process, where all components of plants can be extracted and used as food or advanced materials while, at the same time, residues can be used directly as fuels or fertilizers. The same vision can also be seen as the key to maintaining any long-term sustainable life outside our planet, whether on a space station or another celestial body.

In recent years, there have been significant developments in extracting nanoscale cellulose fibers (termed nanocellulose) for use as a technical platform for a wide range of new biobased materials, including multiscale high-performance fibers, porous membranes, packaging thin films, bioplastics, and aerogels (2) with properties that can be tailored by various processing techniques. Simultaneously, by using new advanced characterization techniques enabled by spallation neutron sources, synchrotron x-rays, and free-electron lasers, we have, during the last years, gotten a much greater insight into biosynthesis processes at the molecular and supramolecular scales. The growing consensus is now that the elementary cellulose microfibril*, the smallest structural building block of cell walls in higher land plants, consists of 18 cellulose polymer chains and are synthesized by cellulose synthase (rosette) complexes (3-6). Several elementary microfibrils embedded in lignin and hemicellulose form composite that provides mechanical integrity to plant cells. The higher-order hierarchical structures of these cells have been tailored through millions of years of evolution to promote survival in their natural environment by optimizing stability and water transport (1) (see Fig 1).

One crucial hurdle of processing materials from nanocellulose has been that their properties strongly depend on both extraction methods and raw material. It appears that the same species and extraction method can result in different nanocellulose properties, where an important factor is that the conditions during plant growth can alter the structure and thus affect the resulting nanomaterials. However, it is difficult to precisely understand how the structure can be affected by external conditions

* Historically, the term "microfibril" has been used to describe the biosynthesized unit, even though it technically has smaller dimensions than the cellulose "nanofibrils" extracted from biomass.
since it is challenging to create a controlled environment for plant growth. This will be even more interesting to understand in extraterrestrial plant growth, where other stimuli (such as low gravity, visible and invisible radiation, and temperature gradient) might influence the formation of structures along the stem of a plant.

The two key questions that we would like to address with the proposed project in this white paper are:

- How will external stimuli (directional light and radiation, directional gravity, temperature and gradient, flow of air, nutrients, and water) affect the microstructure of plants?

- Can we control the plant cell-wall structure to maximize the usage of plants in a circular material loop in extraterrestrial environments?

**Why space?**

As we explore ways of surviving outside the Earth, we see it as crucial to study the influence of gravity, electromagnetic fields, cosmic radiation, flow, and temperature on plant growth in space stations and extraterrestrial environments on a fundamental level, down to the growth of single elementary microfibrils. We believe the unique environment at ISS is the only place where such a well-controlled study can be performed.

The proposed study will complement existing research being done in Veggie and the Advanced Plant Habitat (APH) on ISS. Current studies are focused on the possibilities of growing plants in space, with a particular focus on the sources for circular designs of food and nutrition. There are also studies targeting the lignin content of plants and the possibilities to grow plants engineered with less lignin for better nutrient absorption when ingested. We propose to continue along this line but ask more specific fundamental questions about plant microstructures during plant growth. As we believe that nanocellulose might be an essential platform for sustainable materials even when setting up research stations and possibly terraforming other celestial bodies (with no fossil coal sources), we see it is crucial to understand the properties the nanoscale material gets when extracting it from plants grown in any type of extraterrestrial environment. Specifically, we ask the following questions:

- What is the influence of the type of external stimulus on plant growth and the hierarchical structure of the plant cell wall? For example, provided all the necessities in terms of CO₂, light, temperature, humidity, pressure, nutrients, etc. Specifically, how is the structure affected by different external stimuli, e.g., flow and radiation, and does it impact the extraction and use of nanocellulose?

- What is the influence of directionality from external stimuli on plant growth? For example, in an environment where there is no directional preference through light and gravity, at what length scales does the structure of the plant cell wall differ from terrestrial growth?

Although we see this project as crucial for creating a circular bioeconomy outside of our planet, the fundamental knowledge we gain will likely lead to significant advances in biomass utilization on Earth.
One example study

Central to the proposed project is the design of several small climate chambers with precise control of the stimulus we would like to study (see Fig 2). The starting point will be the design of the APH on ISS to create an environment for optimal growth conditions. We envision three different stages of this study, with different engineering requirements for the climate chamber:

- **Stage 1 (Fig 2a):** The first stage could likely be performed as parametric variations in the APH, where some of these might already have been done to some extent. Using optimal conditions as the starting point, systematic parametric variations of light conditions, temperature, humidity, pressure, and air composition can be performed.

- **Stage 2 (Fig 2b):** The second stage would require some minor design changes to the existing design to study the influence of certain conditions. For example, to study the effect of external flow, each climate chamber will be fitted with fans to create various sideways wind conditions. Another example would be to include a microwave generator with the possibility to control the frequency spectrum to mimic different levels of cosmic background radiation.

- **Stage 3 (Fig 2c):** The third stage will require substantial design changes to specifically study the influence of directionality in plant growth. Terrestrial growth relies on anchoring in the soil in one direction where water and nutrients are provided, while light is provided in the other direction. Light will be uniformly lit from all directions in the new climate chambers, and nutrients will be supplied through aqueous sprays.

The plants for the study will be chosen specifically for their usage as sustainable sources for advanced biobased materials and therefore be of a fast-growing species, for example, with bast fibers (flax, hemp, jute, etc.). Before experiments on the ISS, the same experiments will be performed on Earth, where presumably the only difference will be the presence of gravity. After a certain age or size, the plants are transported back for structural analysis and comparison with control plants grown on Earth. The plants will be characterized with a broad palette of techniques to understand the structure on all hierarchical levels, from single elementary microfibrils to the whole plant. The structural characterization methods will include:

- **From plant to cell:** Optical microscopy, scanning electron microscopy (SEM), X-ray or neutron tomographic microscopy (XTM/NTM).
Cell walls to fibril matrices: SEM, XTM, NTM, X-ray nanotomography, small/wide angle X-ray or neutron scattering (SAXS/WAXS/SANS/WANS).

Fibril level characterization: SAXS/WAXS/SANS/WANS, transmission electron microscopy (TEM), atomic force microscopy (AFM), nuclear magnetic resonance (NMR).

Additionally, the chemical composition of the plants will be determined in detail to study levels of cellulose, hemicelluloses, and lignin. Nanocellulose will be extracted from the plants and characterized both for individual fibril morphologies (using SEM or AFM) as well as a combination of rheometry and rheo-optics to determine dispersion properties for material processes. Finally, filaments will be spun from the nanocellulose with a detailed analysis of tensile properties. The process itself to make nanocellulose from the plants will be analyzed in detail to understand both the energy and water consumption. The residues from the nanocellulose production will further be characterized to understand the potential usage in other applications, e.g., biofuel or fertilizer.

What do we expect to learn?

By analyzing the structure of the same plant species depending on various growth conditions, we are likely to learn:

- The external conditions and stimuli that are beneficial for efficient production of high-value biobased nanomaterials. This knowledge will be applicable both for existing processes to advance terrestrial biorefining for sustainable circular bioeconomy, as well as understanding what is needed to adapt the concepts to extraterrestrial endeavors.

- Indirectly get a fundamental insight into how the plant adapts its growth strategy to external conditions and stimuli. This knowledge will be crucial in understanding the structural features that are purely genetic and thus provide essential input on how to engineer favorable structures genetically. Here, we would define “beneficial structures” as being structures that lead to superior assembled material properties from its nanoscale components as well as low energy/water consumption for extraction of the nanoscale material.

The proposing team

Our combined research groups consist of individuals with a strong background in the Characterization of cellulosic materials from fibril level to fabrication of new materials, including computational and experimental fluid mechanics, X-ray scattering techniques, polymer physics, and colloidal chemistry. Our current research projects include:

- Fundamental studies of cellulose nanofiber cross-sections using X-ray scattering techniques and development of new modeling approaches (4, 7-11).

- Nanomaterial fabrication through controlled alignment and assembly of cellulose nanofibers (CNFs) into filaments with tensile properties exceeding natural or synthetic biopolymeric materials (12-14).

- Fabrication of advanced and functional biocomposite nanomaterials (15, 16).

- Utilizing CNFs for nanofiltration in water purification applications (17) including development of CNF extraction through nitro-oxidation, where effluent can be utilized as fertilizer (18).
- Development of a novel rheo-optical flow-stop technique for Characterization of CNF dispersions (19-21).

- Development of in situ scanning SAXS in combination with simulations to study assembly and alignment processes of cellulose nanomaterials (22-25).

- Rheological Characterization and multiphase computational fluid dynamics to simulate processes of making materials from CNFs (26, 27).
References

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