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Kobayashi, Yumi

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Life cycle assessment of plant cell cultures

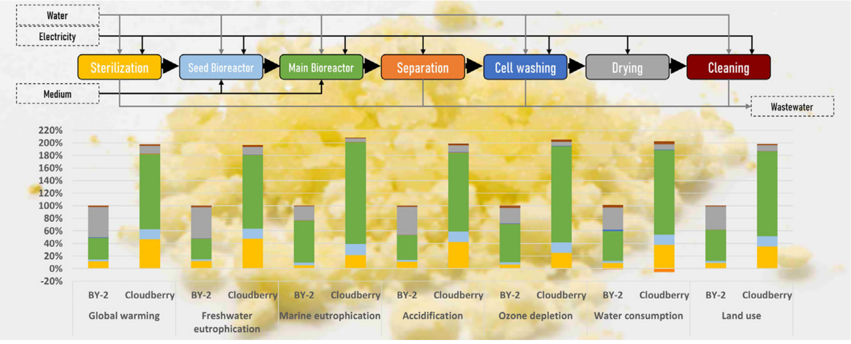
Yumi Kobayashi [a,b], Elviira Kärkkäinen [c], Suvi T. Häkkinen [c], Liisa Nohynek [c], Anneli Ritala [c], Heiko Rischer [c,*], Hanna L. Tuomisto [a,b,d]

[a] Department of Agricultural Sciences, Faculty of Agriculture and Forestry, University of Helsinki, P.O. Box 27, 00014 University of Helsinki, Finland
[b] Helsinki Institute of Sustainability Science (HELSUS), University of Helsinki, P.O. Box 4, 00014 University of Helsinki, Finland
[c] VTT Technical Research Centre of Finland Ltd., Tietotie 2, P.O. Box 1000, 02044 VTT, Espoo, Finland
[d] Natural Resources Institute Finland, P.O. Box 2, 00790 Helsinki, Finland

HIGHLIGHTS

- Environmental impacts of plant cell culture (PCC) production were assessed.
- High contributions of electricity (82–93%) for some impact categories were revealed.
- Optimization of bioreactor operation could reduce environmental impacts up to by 47%.
- Environmental impacts of PCC and microalgae products were comparable.
- Global warming potential of fresh PCC was close to that of heated greenhouse crops.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel food such as plant cell culture (PCC) is an important complementary asset for traditional agriculture to tackle global food insecurity. To evaluate environmental impacts of PCC, a life cycle assessment was applied to tobacco bright yellow-2 and cloudberry PCCs. Global warming potential (GWP), freshwater eutrophication potential (FEUP), marine eutrophication potential, terrestrial acidification potential (TAP), stratospheric ozone depletion, water consumption and land use were assessed. The results showed particularly high contributions (82–93%) of electricity consumption to GWP, FEUP and TAP. Sensitivity analysis indicated that using wind energy instead of the average Finnish electricity mix reduced the environmental impacts by 34–81%. Enhancement in the energy efficiency of bioreactor mixing processes and reduction in cultivation time also effectively improved the environmental performance (4–47% reduction of impacts). In comparison with other novel foods, the environmental impacts of the PCC products studied were mostly comparable to those of microalgae products but higher than those of microbial protein products produced by autotrophic hydrogen-oxidizing bacteria. Assayed fresh PCC products were similar or close to GWP of conventionally grown food products and, with technological advancements, can be highly competitive.

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* Corresponding author.
E-mail address: heiko.rischer@vtt.fi (H. Rischer).

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1. Introduction

Prior to the COVID-19 pandemic, 690 million people were already undernourished globally and preliminary projections suggest that the pandemic causes additional 83 to 132 million undernourished people (FAO et al., 2020). In addition, the whole food system is linked to a wide range of environmental issues including climate change, land-use change and water depletion (Ganivet, 2020; Springmann et al., 2018). Projections predict a population of 10 billion in 2050, which together with income growth causing dietary change toward higher consumption of animal products will increase the challenges of the food systems to provide sufficient, healthy and environmentally sustainable nutrition to all (Pourkheirandish et al., 2020; Tilman et al., 2011). To meet the hunger, food security and targets of the Sustainable Development Goals, not only energy but also dietary nutrient content must be considered.

Uncertainties in the future food production add another level of challenges around the food security. These are underpinned by issues such as productivity losses caused by the climate change and loss of natural resources, e.g., land degradation and water scarcity. The adverse impacts of climate change and consequences of agricultural practices have led to desertification and land degradation in many regions, negatively impacting crop yields in conventional farming systems (FAO, 2018; IPCC, 2019). While crop yields need to be increased to meet growing food demand, the yields of major crops plateaued in recent years and potential yield increase with conventional agricultural practices is limited (Roell and Zurbriggen, 2020). Instead of relying on the conventional food production system and improvements therein, novel technologies are being explored as a part of the solution. Cellular agriculture is based on industrial biotechnology using various host organisms to produce food, feed and materials (Rischer et al., 2020). Among these systems, plant cell culture (PCC) technology has been confirmed to produce highly nutritious biomass for food applications. PCCs from cloudberry, lingonberry and stoneberry were found to contain high dietary fiber (ca. 21–37%), starch (ca. 1%), sugar (ca. 18–34%) and protein (ca. 14–19%) with a balanced amino acid profile and good quality lipids (ca. 2%) (Nordlund et al., 2018).

PCC technology is less affected by environmental conditions than conventional farming due to full containment. It also requires less materials and resources like fertilizers, pesticides, fossil fuels, water and land area (Daltoso and Melandri, 2011). However, the heterotrophic process is not entirely decoupled from agriculture and it is energy intensive, which contributes to environmental impacts. To the authors' knowledge, comprehensive environmental impact assessment has not been performed for PCC systems. Therefore, the main objective of this study was to apply life cycle assessment (LCA) to holistically assess the environmental impacts of PCC products and to seek potential targets for future environmental improvements of the PCC production systems. In order to realize the relative environmental performance of the PCC products, the LCA results were compared to other types of food products.

To fulfill the purposes of this study, heterotrophic PCCs of tobacco (*Nicotiana tabacum* L.) bright yellow-2 (BY-2) and cloudberry (*Rubus chamaemorus* L.) were studied. BY-2 is a well-established model system (Nagata et al., 1992) and has been widely used for plant biology studies (Nagata et al., 2004). The cell line is particularly known to achieve high productivity for heterologous protein expression (Häkkinen et al., 2018; Reuter et al., 2014; Ritala et al., 2014). Although reports on tobacco for food protein production are scarce (Wandelt et al., 1991), to realize the potential improvement of environmental performance, BY-2 cell culture was included in this study to represent a highly optimized and scalable PCC system. Cloudberry cell culture has a much shorter history and so far it has been produced in small batches for cosmetics applications (Nohynek et al., 2014). It has significant potential for food applications (Nordlund et al., 2018) and serves here as an example for a PCC with optimization potential in terms of growth rate.

2. Material and method

2.1. Plant cell culture systems

The pilot-scale PCC production systems at VTT Technical Research Center of Finland Ltd. (VTT) were examined in this study. The PCC production systems required a number of stages as shown in Fig. 1. For tobacco BY-2, 1 L seed was produced in shake flasks and used as an inoculum for the 30 L cultivation in a 40 L stirred tank fermenter that was then applied as a seed for the main 600 L fermentation (Reuter et al., 2014). The production of cloudberry cells has been described previously (Nohynek et al., 2014) and is also illustrated in Fig. 1. The experimental parameters and data for bioreactor operations for BY-2 and cloudberry PCCs are provided in Table S1 in the supporting information (SI).

After the main fermentation in both PCCs, cell suspension was drained out and cell biomass was separated by using a Larox filter press (model PF 0.1 H2, filtering surface area 0.1 m^2, Larox, Finland). The collected cell biomass was washed once with tap water which was treated with reverse osmosis (RO water) and sent through another separation round with a Larox filter press, which produced fresh PCC biomass. A freeze dryer (Christ epsilon 2-25DS, Christ, Germany) was used to obtain dry powder products.

The prepared media and bioreactors were sterilized at the beginning of the process and the bioreactors were cleaned by washing with water without chemical agents after each batch of PCC production. RO water was used for PCC production, steam and cleaning.

2.2. Life cycle assessment

Attributional LCA was conducted in accordance with ISO14040 and ISO14044 (ISO, 2006a; ISO, 2006b). OpenLCA 10.3 (GreenDelta, 2021) with ecoinvent 3.6 (Wernet et al., 2016) as background data was used for the calculations.

2.2.1. Goal and scope

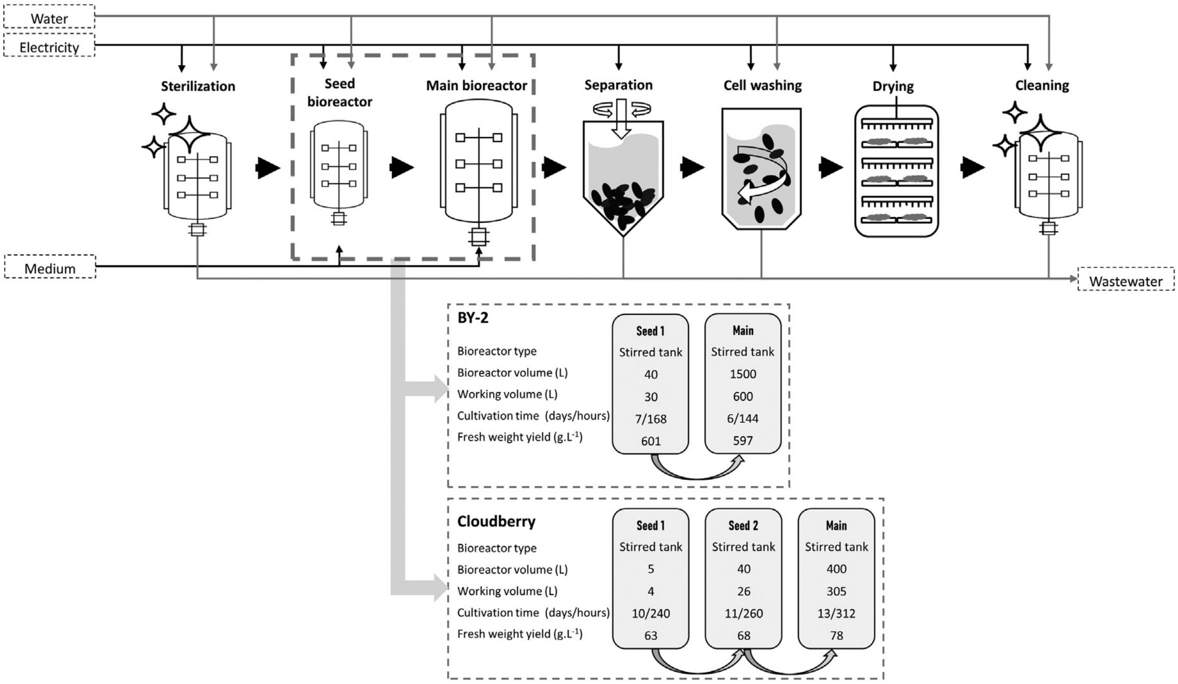
The main goals of this LCA were to evaluate the environmental performance of pilot-scale PCC production systems of tobacco BY-2 and cloudberry and to seek potential future improvements. The study was divided into three parts: 1) quantification of environmental impacts of current PCC production systems at a pilot scale (the baseline-case, hereafter); 2) identification of environmental hotspots of the PCC production systems; and 3) determination of potential approaches for efficient environmental impact improvements. The LCA results were compared to other novel food products and conventional foods for benchmarking. A functional unit of 1 kg of dried PCC biomass in the form of dried powder was selected for the analysis.

2.2.2. System boundaries

The following stages of the production systems were included in the system boundary of the LCA: seed culture, main fermentation, separation and washing of cells, drying, sterilization, and cleaning (Fig. 1). Electricity, water and chemical consumptions were taken into account, however, culture maintenance of the cell stock, inoculum generation for the first seed stage, material transport and materials used for equipment were excluded due to data unavailability and expected small impact contribution (Pietrzykowski et al., 2013). Electricity consumption of reverse osmosis and the treatment of reject water and other wastewater (Fig. 1) were also considered. Materials and works for construction and operations of facility buildings, as well as the land area used for the facility, were not included due to lack of data.

2.2.3. Life cycle inventory (LCI)

Foreground data were obtained from pilot-scale research at VTT and supplemented with theoretical calculations and published literature. The key inventory data and calculation details are provided in the SI (Section S3). Ecoinvent processes were used as background data,



BY-2	Seed 1	Main
Bioreactor type	Stirred tank	Stirred tank
Bioreactor volume (L)	40	1500
Working volume (L)	30	600
Cultivation time (days/hours)	7/168	6/144
Fresh weight yield ($g.L^{-1}$)	601	597

Cloudberry	Seed 1	Seed 2	Main
Bioreactor type	Stirred tank	Stirred tank	Stirred tank
Bioreactor volume (L)	5	40	400
Working volume (L)	4	26	305
Cultivation time (days/hours)	10/240	11/260	13/312
Fresh weight yield ($g.L^{-1}$)	63	68	78

Fig. 1. The plant cell culture production stages for dried products.
BY-2: tobacco (*Nicotiana tabacum* L.) bright yellow-2;
BY-2: Seed 1: Seed Bioreactor 1, (IF 40, New Brunswick Scientific, USA), Main: Main bioreactor (BioFlo PRO, New Brunswick Scientific, USA);
Cloudberry: Seed 1: Seed Bioreactor 1, (CT5 Biostat, Sartorius, Germany), Seed 2: Seed Bioreactor 2 (IF 40 New Brunswick Scientific, USA), Main: Main bioreactor (IF 400 New Brunswick Scientific, USA).

including production of chemicals, materials and utility. Some of the media ingredients were unavailable in the ecoinvent database and were thus substituted with substances that are similar in production processes or excluded when no substitute was found (Table S3). Since the amount of ingredients excluded were small (<0.3%) compared to the total medium, little impact on the results is expected.

2.3. Life cycle impact assessment (LCIA)

ReCiPe 2016 midpoint (H) (Huijbregts et al., 2017) was used as life cycle impact assessment (LCIA) method in this study. The ReCiPe method was selected as the characterization factors are representative for global scale and it provides a wide range of impact categories that are most relevant to energy intensive systems, such as PCC. We have chosen the following impact categories: global warming potential (GWP), freshwater eutrophication potential (FEUP), marine eutrophication potential (MEUP), terrestrial acidification potential (TAP), stratospheric ozone depletion (ODP), water consumption (WC) and land use (LU). These were selected by considering previous LCA studies of conventional and novel food products (Dekker et al., 2020; Smetana et al., 2017; Smetana et al., 2019; Poore and Nemecek, 2018), and for their relevance to electricity use and crop production (Thévenot et al., 2018), as impacts of these are expected to contribute to overall environmental performance.

2.4. Sensitivity analysis – environmental performance improvement potentials

Since the current PCC technology and particularly the processes investigated here are still in an early stage of development, there is potential to improve the system and its environmental performance. To assess the influence of material choice, technology selection, potential technological improvements and electricity sources to the environmental performance of the production system, several scenarios were considered as described below. The sensitivity analysis approach was applied, where individual variables were changed independently to determine their influence on the total impact. In addition, the scenario with the least environmental impacts, which is a combination of the best cases of all improvement potentials except for the electricity source (the best-case, hereafter), was investigated for each electricity source.

2.4.1. Electricity sources

The Finnish electricity grid mix available in ecoinvent v3.6 was used in the baseline-case. However, the grid mix can change in the future. We have considered a projected future electricity grid mix of Finland in the year 2050 (FI2050), hypothetical 100% photovoltaic (PV-FI) and 100% wind energy (Wind-FI) scenarios. The FI2050 scenario was constructed based on the 'Growth' scenario used in a report of the Ministry of Employment and the Economy of Finland (Vapaavuori et al., 2014). The contributions of each electricity source are shown in Table S4. Although the technologies of electricity generation likely advance in the future, the ecoinvent processes based on current technologies were used for each electricity source to construct a future energy mix. Similarly, the contributions of different scales and technologies among the same energy sources, e.g., monocrystalline and polycrystalline silicon for photovoltaic (PV) technology, were assumed to be unchanged from those of the ecoinvent process. An exception to this is heat and power co-generation (CHP). The ecoinvent process includes fossil-fuel based CHP, however, for the FI2050 scenario, only non-fossil-based CHP, i.e. biogas and wood chip, were considered.

2.4.2. PCC production system optimization potentials

2.4.2.1. Cultivation time. Energy intensive operation of bioreactors is expected to largely contribute to the environmental impacts of the system. Hence, reduction in cultivation time may effectively improve environmental performance. The cultivation time could depend on the selection of cell lines (Eibl et al., 2018). For example, the theoretical fresh biomass doubling time of BY-2 in the main cultivation was 13.3 h whereas for cloudberry it was 45.2 h. Selection of different types of bioreactor and impeller, and adjustment of medium composition and physical parameters such as temperature, also influence productivity (Murthy et al., 2014; Ochoa-Villarreal et al., 2015; Gubser et al., 2021; Eibl et al., 2018). Moreover, inoculum optimization, for example the growth time of each seed fermentation can affect the growth time of final fermentation by shortening the lag-phase at the beginning of the cultivation. Here, we have applied 25%, 50% and 75% theoretical reduction of total cultivation time to determine its impacts to the total environmental performance of the PCC production. This proportionally reduces electricity consumption of bioreactor operation while assuming that the changes in environmental impacts caused by other modifications are minimal.

2.4.2.2. Mixing energy. Electricity consumption for mixing is the largest in the bioreactor operation in the PCC production system. It is known that the mixing power requirement per unit volume of medium decreases with increased total volume. Therefore, upscaling of the production system from pilot scale to production scale reduces mixing energy. For instance, for a 300 L bioreactor, about 40% reduction in power consumption can be expected by scaling up to 5000 L and close to 80% reduction with 1,000,000 L bioreactors (Benz, 2008). The LCA results were recalculated with 40%, 60% and 80% less mixing energy while assuming similar yields for larger bioreactors to realize the influence of mixing energy efficiency.

2.4.2.3. Drying technology. A freeze dryer was used in our PCC production systems, however, there are other drying technologies which require lower electricity. A spray dryer and drum dryer, which are applicable for food production, were considered as alternatives. The electricity consumption is around 2.4 kWh.kg-1 of evaporated water for freeze dryers (Smetana et al., 2017) while they are about 1.1 and 0.9 kWh.kg-1 of evaporated water for spray dryers and drum dryers, respectively (Fasaei et al., 2018). The influence of different technologies to the product's nutritional contents has not been considered due to data unavailability.

2.4.2.4. Feedstock sucrose source. Sugarcane was selected as the source of sucrose in the baseline-case. The environmental impacts of an alternative source of sucrose, sugar beet, were also investigated. Ecoinvent processes for ‘the rest of the world’ were used for both alternatives. Although sugar beet can be produced locally, the Finland-specific process was unavailable.

2.5. Benchmarking

Dry powder of PCC-derived biomass was chosen for the environmental impact assessment in this study, however, the fresh PCC biomass (90–98% water content) can also be marketed in the form of fresh products for different end uses. For fresh products, comparisons with other fresh food such as vegetables can be sensible while the dry powder products should be compared with other dry products. For these reasons, benchmarking was conducted against various types of food products to have a glance at the relative state of environmental performance of pilot-scale production of PCCs. Although there are a number of LCA studies of conventional agricultural products, because of the discrepancy in approaches among LCIA methods for most of the impact categories, comparisons with conventional food products were performed only for GWP where the characterization factors were commonly taken from IPCC reports (IPCC, 2001; IPCC, 2007; IPCC, 2013). The baseline and best-case LCA results were recalculated for the production system without the drying stage based on per kg of fresh product to enable comparisons with conventional fresh food products. Due to the difference not only in LCA methods, but also in farming practices and geographical regions for each crop, we have considered data for broader categories of crops provided in meta-analysis studies (Clune et al., 2017; Poore and Nemecek, 2018) for the comparison. The categories include berries, fruits, tree nuts, seeds and greenhouse-grown vegetables. The impacts of dry PCC product were compared to other novel dry food products: microalgae (Smetana et al., 2017) and microbial protein (Järviö et al., 2021). ReCiPe method was used in this study and the microbial protein study (Järviö et al., 2021), but as IMPACT 2002+ was used for most of the impact categories in the microalgae study (Smetana et al., 2017), the impacts of PCCs were calculated also with IMPACT 2002+.

3. Results and discussion

3.1. Analysis of the baseline-case for PCCs

The results of the baseline-case scenario of PCCs showed 97–108% higher environmental impacts for cloudberry than BY-2 for all the impact categories considered (Fig. 2), numerical results are provided in Tables S13–S17. This is mostly due to higher impacts during the bioreactor operations and sterilization phases of cloudberry PCC production. In contrast, higher impacts in BY-2 production were observed for the drying phases due to its relatively high water content.

The contributions of electricity consumption to the total impact were particularly high for the categories of GWP, FEUP and TAP, where the electricity consumption was responsible for 83–94% of the total impacts (Table S11). In these categories, the higher electricity use of bioreactor operations and sterilization led to higher impacts for the cloudberry culture. Although the total electricity requirements per batch for these phases were higher in BY-2, it required less electricity per functional unit because of its higher yield. On the other hand, while the same drying method was used for both PCCs, the electricity consumption was considerably higher for BY-2 due to its greater water content (97.3%) of fresh biomass compared to cloudberry water content (89.7%). For BY-2, it is crucial to optimize the bioreactor cultivation and harvest immediately when the stationary stage is reached to avoid further water uptake (Reuter et al., 2014). In general, the high electricity requirement of potential technological solutions complementing agriculture in future food security is a sustainability burden. Concerning the direct electricity consumption, bioreactor mixing is the highest electricity consumer (64%) followed by sterilization (26%) in the case of cloudberry (Fig. S2); while for BY-2, drying used the highest electricity (53%) followed by bioreactor mixing (29%) and sterilization (13%). Due to the dominating contribution of electricity use in many of the impact categories, efforts to reduce electricity consumption in these energy intensive phases and to use renewable energy sources could efficiently mitigate the environmental impacts.

While electricity was also an important contributor for WC and LU, water consumption associated with feedstock sucrose production was another notable contributor (23–30%) (Table S11). Feedstock sucrose production was also a major contributor for MEUP for both PCCs (56–58%). For ODP, sucrose (14–15%) and ammonium nitrate (26–28%) used in the media were notable contributors in addition to electricity consumption. Although the concentrations of sucrose and ammonium nitrate in the media were identical for the two PCCs, the higher biomass productivity of BY-2 again had an advantage in impacts per functional unit.

3.2. Analysis of potential environmental improvements – sensitivity analysis

3.2.1. Electricity mix

The change in electricity sources showed the strongest influence to the environmental performance of both PCCs among the potential improvements examined (Fig. 3). Use of wind energy achieved the greatest reduction in the categories of GWP, MEUP, TAP, WC and LU, and considerable impact reduction trends were observed in all impact categories ranging from 34 to 37% for MEUP to 81% for GWP. Although the PV showed the least reduction in most impact categories, the reductions were still considerable, ranging from 12 to 13% for FEUP to 67–70% for LU. The reduction rates for FI2050 were between wind energy and PV in all categories except for FEUP, ODP and LU. In case of FEUP, FI2050 performed the best while it had no considerable influence on ODP. LU increased (250%) due to the increased use of wood-chip based CHP, which causes considerably larger LU per unit of electricity generated compared to other electricity production technologies (Table S15).

Uncertainty in projecting the future electricity grid mix is unavoidable due to many different factors including economic growth, technological

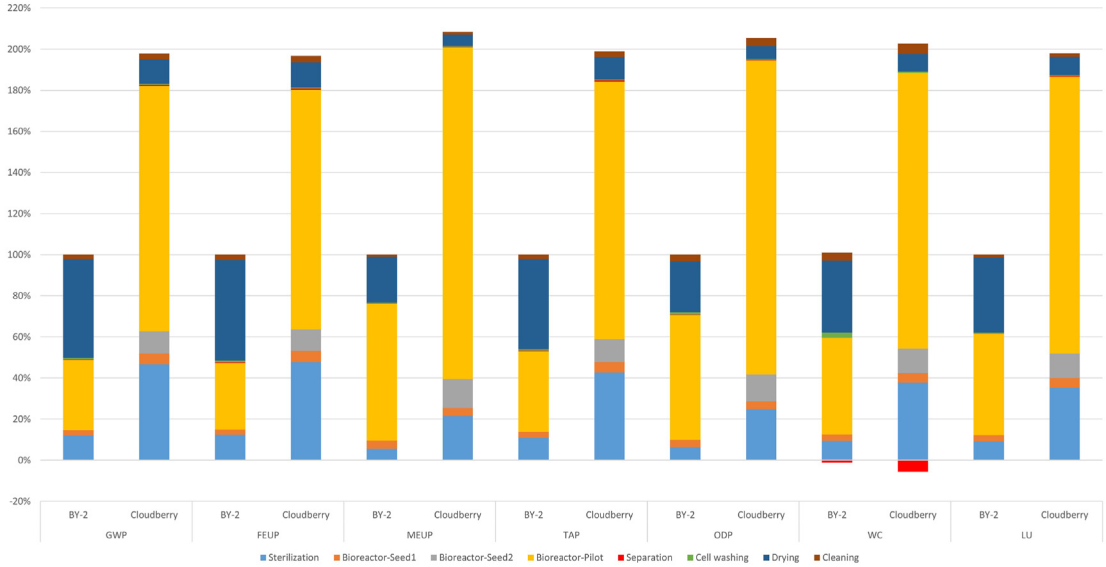


Fig. 2. Environmental impacts of cloudberry PCC relative to Tobacco BY-2 PCC in the baseline-case scenario.
BY-2: tobacco (*Nicotiana tabacum* L.) bright yellow-2, GWP: Global warming potential, FEUP: Freshwater eutrophication potential, MEUP: Marine eutrophication potential, TAP: Terrestrial acidification potential, ODP: Stratospheric ozone depletion potential, WC: Water consumption, LU: Land use.

advancement, and public acceptance. We have considered one of several future scenarios, acknowledging its uncertainty. We have considered the scenario for contributions of each technology to overall electricity production, however, the future improvements of each technology were not taken into account as mentioned earlier. For example, CHP technologies and PV cell efficiency for solar energy will likely be improved in the future, which would reduce environmental impacts per unit electricity production. Also, while carbon capture and storage (CCS) was not considered in the FI2050 electricity scenario, it could be a part of low carbon electricity solution (Sipilä et al., 2012). If these additional potential improvements were included, increased impact reductions can be expected.

3.2.2. Cultivation time and mixing energy

It is obvious that reduction of cultivation time would reduce the electricity consumption during bioreactor operation phases. Because of the higher contributions of the bioreactor operation phases of cloudberry, the cultivation time reduction had greater influence on the cloudberry production system (Fig. 3). This can be more clearly observed for GWP, FEUP and TAP, where the contribution of electricity consumption is higher. In these categories, reduction of cultivation time by 25%, 50% and 75% reduced the total impact for BY-2 by 7%, 13–15% and 20–23%, and by 14–16%, 28–31% and 41–47% for cloudberry, respectively. Due to the high degree of environmental impact improvement potentials, it may be worth considering improvement of productivity by pursuing higher performance PCC lines, genetic improvements and optimizing media composition and other cultivation parameters. Cultivation strategies for maintaining optimal dissolved oxygen, possible feeding models, better suited impeller and/or different bioreactor types should also be considered.

Since the mixing energy dominates the electricity consumption of the bioreactor operations, the impact reduction trends were similar to those of cultivation time optimization. As the contributions of electricity consumption to MEUP and ODP were small compared to other impact categories, electricity saving by improving cultivation time or mixing efficiency had smaller influence on MEUP and ODP reduction.

3.2.3. Drying technologies

As spray dryer and drum dryer require less electricity than a freeze dryer, their application reduced the associated impacts (Fig. 3). While impacts of BY-2 were substantially reduced (12–31%), limited reduction (<1–4% reduction) was observed for cloudberry due to the smaller contribution of drying phase.

3.2.4. Feedstock sucrose source

Besides WC and LU, the difference in environmental impacts between sugarcane and sugar beets was minor (Fig. 3). Choosing sugar beets would reduce 18% and 20% for the BY-2 and cloudberry, respectively, for WC and 18% for LU.

3.2.5. Combinations of all optimization potentials

If the best case of each system improvement potential can be achieved, the impacts would be reduced by 29–64% depending on the impact category even with the current electricity mix (Fig. 4). In both PCCs, the greatest reduction can be expected in GWP, FEUP, WP and LU followed by TAP with any electricity source, except the case of FI2050 where LU increased 33% and 21% for BY-2 and cloudberry, respectively, due to the increased share of wood-chip based CHP as mentioned earlier. While the reduction is smaller for MEUP and ODP, it is still considerable (around 30%).

3.3. Benchmarking

The comparison of results among different LCA studies is complex due to the variation in method choices such as functional unit, system boundaries, impact category selection and LCIA methods (Clune et al., 2017; Laurent et al., 2020). Differences in assumptions used, level of detail in inventory data, as well as regional specifics, such as electricity grid mix, transport mode and distance, and waste treatment methods, add more complications to the study comparisons. In addition, since the suitable markets for PCC products remain uncertain, food products relevant for comparisons cannot be clearly defined. Because of these limitations, the comparisons should be recognized as rough indications and for more precise comparisons, consistency in LCA methods and scenario assumptions need to be achieved.

The environmental impacts of dry and fresh PCC products along with those of other novel and traditional food products are shown in Table S12 and key nutritional values of dried PCC, microalgae and microbial protein products are provided in Table S2. PCC products contain a balanced and bioavailable nutrient composition of amino acids, carbohydrates and lipids

(Nordlund et al., 2018). Additionally, there are health-promoting factors such as vitamins and secondary metabolites (Suvanto et al., 2017; Rischer et al., 2022). Microalgae may constitute a similar compositional matrix, but they are cultivated in various ways, autotrophically and heterotrophically, leading to a broad range of environmental impacts (Smetana et al., 2017). The impacts of the baseline-case PCC products mostly fall in or close to the range of microalgae products except for MEUP, which is smaller for the PCC products. Although some of the impacts of the best-case PCC products are much lower, it should be noted that the impacts of microalgae can also be improved with cleaner energy and future technological advancements.

Comparison of PCCs with microbial cellular agriculture systems is challenging. These systems generally aim to produce specific nutritional compound categories, such as protein rather than a broad portfolio of compounds or biomass (Sillman et al., 2019). Disregarding heterologous expression, i.e. genetically engineered strains, there are reference cases of single-cell protein productions (Ritala et al., 2017), but environmental impact studies are limited. In comparison with the autotrophic system, impacts of heterotrophic PCC products are considerably higher for most of the impact categories. For GWP and FEUP, the best-case of PCC products may be in the range of their Finland average energy mix scenario. While PCC products are still more impacting than their hydro power scenario, the values are in the same order of magnitude for GWP.

While the study location of the microbial protein production was Finland (Järviö et al., 2021), the microalgae production was assumed to take place in Germany (Smetana et al., 2017). Finnish data were used in this study when available, including that for electricity generation. As shown earlier, the majority of the impacts were caused by electricity consumption and drastically affected by the type of electricity source, which can be considerably different depending on the chosen location. Since the assumed scales of microbial protein and microalgae production systems were much larger than the PCC production systems studied here, they may have gained advantages by economies of scale. These differences should be kept in mind when comparing different LCA studies.

Fresh BY-2 cell culture is already within or close to the range of GWP of conventional products even for the baseline-case and it can be highly competitive in the best-case. In contrast, the baseline-case for cloudberry cell cultures causes two to seven times higher GWP than the maximum values of the field grown fresh products. While GWP of cloudberry cell cultures is still higher than that of products grown in heated greenhouses, cloudberry cell cultures cause only 15% higher than the maximum value of the greenhouse grown products. The impacts of the best-case scenario fall within the range of all fresh products selected here.

Compared to the products exclusively grown with the conventional farming practice, high-tech food production systems such as PCC may have advantages in the impact categories of water consumption and land use. As concerns about land availability for farming are growing (FAO, 2017), the ability to produce nutritious food in smaller areas can provide a crucial advantage. While we have used the impact category of water consumption provided in the ReCiPe method, there are diverse methods to assess water-use impacts, hence it is not straightforward to compare different products in different studies. In fact, the preferable environmental impact assessment method of water use is still under debate to this day (Karlsson Potter and Röös, 2021). Inclusion of comparative analysis of land use and

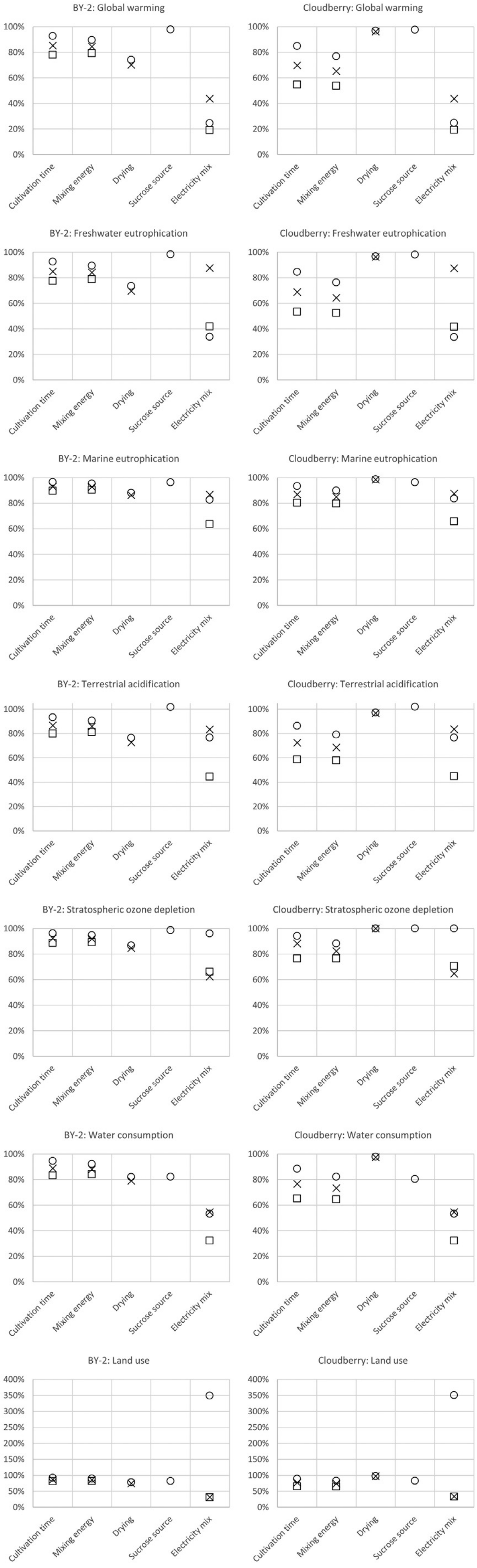


Fig. 3. Environmental impacts of improvement scenario relative to the baseline-case.
BY-2: tobacco (*Nicotiana tabacum* L.) bright yellow-2;
Theoretical cultivation time: ○ – 25% reduction, × - 50% reduction, □ – 75% reduction;
Mixing energy: ○ – 40% reduction, × - 60% reduction, □ – 80% reduction;
Drying: ○ – Spray dryer, × - Drum dryer;
Feedstock sucrose source: ○ – sugar beets;
Electricity mix: ○ - Projected Finnish electricity mix in year 2050 (FI2050), × – Photovoltaic, □ – Wind energy.

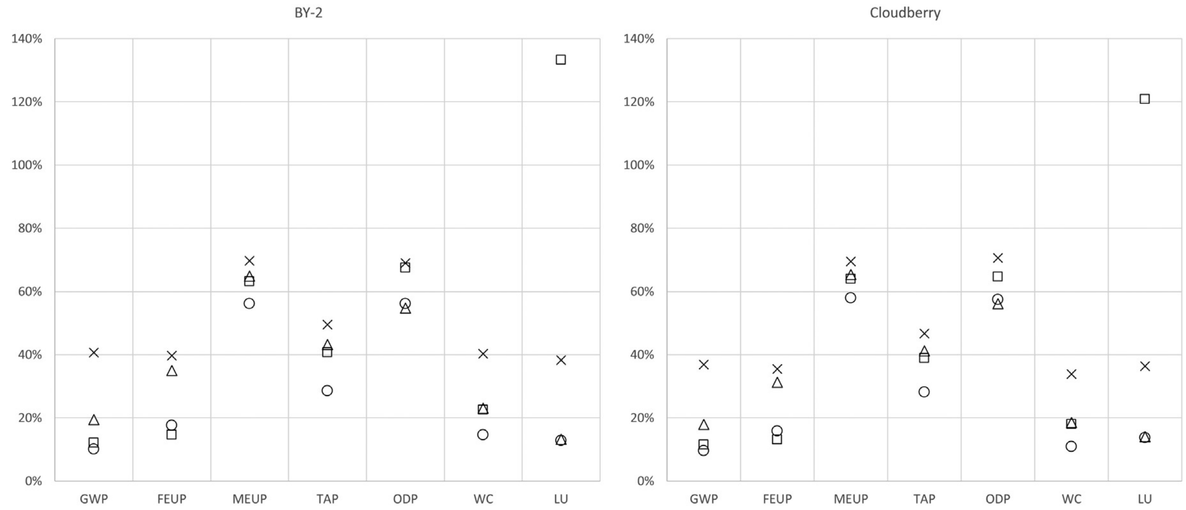


Fig. 4. Environmental impacts of the best-case scenario with each electricity source relative to the baseline-case.
BY-2: tobacco (*Nicotiana tabacum* L.) bright yellow-2, GWP: Global warming potential, FEUP: Freshwater eutrophication potential, MEUP: Marine eutrophication potential, TAP: Terrestrial acidification potential, ODP: Stratospheric ozone depletion potential, WC: Water consumption, LU: Land use,
×: Current Finnish electricity mix, □: Projected Finnish electricity mix in year 2050 (FI2050), Δ: Photovoltaic, ○: Wind energy.

water use between PCC and conventionally grown products may help to realize the environmental benefits of PCCs.

One of the advantages of a PCC production system compared to conventional farming is that it does not require pesticides nor herbicides (Eibl et al., 2018). Therefore, toxicity related impacts might be lower in PCC systems. Although these impact categories were not included in this study, they may also be relevant indicators for comparative studies. It must be emphasized that contained PCC production facilitates closed loop processes such as medium recycling (Lowrey et al., 2016) and therefore further, technology-enabled, minimization of water and mineral use is foreseen. The dependency on sucrose, which is produced by conventional agriculture, as a carbon source can be at least partly reduced by the economic utilization of otherwise wasted food-grade side streams (Häkkinen et al., 2020).

Another advantage is that PCC can be produced year-round regardless of climate and season. As such, products can be locally produced and the impact related to product transport and related food loss can be minimized. Since our study comparisons were made for impacts up to farm/factory gates, these potential benefits of PCC were not counted. When the system boundary of the study is extended to retailors or end consumers, PCC may gain considerable environmental advantages.

3.4. Consideration of different functional units

A mass based functional unit was used as it is considered to be sufficient for the main purpose of this study (Heller et al., 2013). However, different functional units could be more suitable depending on the aim of a study. For comparative LCA of food products, quality based functional units that consider the nutrient content may better serve the purpose (Schau and Fet, 2008). The consideration of the quality of nutrients such as digestibility could further improve the comparisons of food products (Sonesson et al., 2017).

When comparing to food products which are high in certain nutrients, such as protein, use of a functional unit that takes only a specific nutrient into account may be disadvantageous for nutritiously well-balanced products such as PCCs. Food products high in protein may lack dietary fiber content, for example. The novel food products considered for benchmarking are high protein products aiming to replace conventional animal proteins or to fulfill increased protein demand in the future. The characteristics of nutritional contents of PCC also differ from conventional fresh products, including berries (Nordlund et al., 2018). As such use of mass-based functional units are likely not ideal for comparison among different food products. The benchmarking of this study should be understood with these limitations in mind. Since the selection of functional units affect the environmental preference of products and systems (Dourmad et al., 2014), they should be carefully selected according to the study aim.

3.5. Economic and social aspects

This paper focused only on the environmental aspect of PCC, however, for more comprehensive sustainability assessment, economic and social aspects also need to be assessed with appropriate tools, such as life cycle costing (LCC) and social life cycle assessment (SLCA). The use of PCC products often encounter an economic burden due to requirements of specialized medium and their low multiplication rate (Häkkinen et al., 2018). To be marketed as food products, cost efficiency is an important factor. Cost reduction while also reducing environmental impacts together with regulatory approval as novel food constitute the biggest challenges that PCC production systems need to overcome.

A potential advantage of PCCs is its controlled and stable supply. Yield variation of field grown berries between years and geographical locations are enormous (Tahvanainen et al., 2019). A combination of soil and climate conditions is usually identified as the explanation for the yearly variations (Krebs et al., 2009). Climate change is expected to increase these variations (Gornall et al., 2010). The climate and location independent production system of PCCs could accomplish stability in supply chain and reduce price fluctuations.

A social challenge could be the consumer acceptance of PCC as food. Foods are consumed for multiple purposes, which include not only provision of nutrition but also other quality aspects, such as taste and aesthetics, as well as for defining one's culture where food can offer emotional and psychological values (Heller et al., 2013). Simply providing high quality nutrition with lower environmental impacts at a competitive price may not attract consumers despite official regulatory approval.

3.6. Implications

PCC products potentially play an important role in complementing agriculture in the future food security for their regional and seasonal independency and the balanced nutrition among other advantages. However, due to energy intensive operation, the production system at the current state causes substantial environmental impacts. As shown in this study, the potential of environmental impact reduction for PCC production is enormous. This study may serve as a guide for effective environmental performance improvements of PCC production systems and to aid in the search for a sustainable path to food security.

CRediT authorship contribution statement

Yumi Kobayashi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Elviira Kärkkäinen:** Methodology, Investigation, Data curation, Writing – review & editing. **Suvi T. Häkkinen:** Investigation, Data curation, Writing – review & editing. **Liisa Nohynek:** Investigation, Data curation, Writing – review & editing. **Anneli Ritala:** Investigation, Data curation, Writing – review & editing. **Heiko Rischer:** Conceptualization, Investigation, Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing. **Hanna L. Tuomisto:** Conceptualization, Methodology, Investigation, Validation, Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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