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Short Communication

Changes in carbon footprint when integrating production of filamentous fungi in 1st generation ethanol plants

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ABSTRACT

Integrating the cultivation of edible filamentous fungi in the thin stillage from ethanol production is presently being considered. This integration can increase the ethanol yield while simultaneously producing a new value-added protein-rich biomass that can be used for animal feed. This study uses life cycle assessment to determine the change in greenhouse gas (GHG) emissions when integrating the cultivation of filamentous fungi in ethanol production. The result shows that the integration performs better than the current scenario when the fungal biomass is used as cattle feed for system expansion and when energy allocation is used. It performs worse if the biomass is used as fish feed. Hence, integrating the cultivation of filamentous fungi in 1st generation ethanol plants combined with proper use of the fungi can lead to a reduction of GHG emissions which, considering the number of existing ethanol plants, can have a significant global impact.

1. Introduction

Production of 1st generation bioethanol is well established, with USA and Brazil being the dominant producers. European production of ethanol is mostly based on wheat grains or sugar beet (RFA, 2014). Research and development at these facilities should, among other things, focus on minimising the dependence on oil price fluctuations. A common strategy is to study the potential of using process side-streams to save energy, to optimise production or to increase the diversity of products.

Irrespective of the feedstock, distillation of ethanol leaves a stream (called stillage when using grains as feedstock and vinasse when sugar substrates are used) which is normally used in animal feed products. Stillage and vinasse contain all nutrients (carbon, nitrogen, minerals, etc.) that the yeast did not consume. Such streams have a large potential for further use since they contain 10–16% solids (Nitayavardhana and Khanal, 2010).

Filamentous fungi have been investigated for production of value-added products from the nutrients in these side-streams. These products include fungal biomass for fish feed, ethanol (Ferreira et al., 2014; Nitayavardhana and Khanal, 2010) and single-cell oil (Mitra et al., 2012). A common aspect of these studies is that the filamentous fungi that used are the same as those traditionally used for production of human food. This means that the fungal biomass can be regained as single product safe for consumption, or that the stillage that contains this biomass can be used to produce animal feed. It has also been suggested that removing nutrients from the stillage enables easier evaporation and drying during production of the animal feed, which can potentially save energy (Lennartsson et al., 2014).

An example of using filamentous fungi is the production of ethanol and biomass for feed from thin stillage using *Neurospora intermedia*. A techno-economic analysis of thin stillage valorisation using *N. intermedia* has been done previously (Rajendran et al., 2016). It was shown that this integration could lead to a 2.5% decrease in the overall energy consumption of the plant and a 4% increase in the ethanol production.

One main argument for using biofuels instead of fossil fuels is the reduction in GHG emissions, and several ethanol production processes have been analysed using LCA (e.g. Börjesson et al., 2010; Luo et al., 2009). However, no previous LCAs have focused on the potential benefits of integrating the cultivation of filamentous fungi in ethanol production plants. The environmental impact of such products has also gained importance from a market perspective, with the development of policies and standards, such as the European Union (EU) Renewable Energy Directive (RED), that promote the use of renewable fuels (Directive 2009/28/EC). These policies are expected to increase the market for biofuels in the EU (Magar et al., 2011). This contribution presents the first insights into the environmental impact when integrating cultivation of filamentous fungi in ethanol production plants, which is a technology that can potentially be applied to all bioethanol facilities in the world.

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2. Material and methods

2.1. Goal and scope definition

Attributional LCA was used to determine the changes in GHG emissions when integrating cultivation of the filamentous fungus *Neurospora intermedia* in an ethanol production plant. In terms of the ethanol product the LCA is cradle to distillery (the use of the ethanol is the same whether the fungi is included or not), whereas post-distillery use of the fungal biomass has been included in some scenarios. Therefore, the LCA was performed considering two ethanol production technologies: (i) the conventional production technique, currently taking place in any ethanol plant using grains as substrate (called the Base Scenario) and (ii) the same process but where cultivation of filamentous fungi is integrated by fermentation of the thin stillage (Scenario A). The LCA is limited to the climate change category, since global warming is one of the main drivers of biofuels promotion (Directive 2015/1513/EC; Gnansounou et al., 2009). The calculations were done using the SimaPro v.8.3 software (PRé Sustainability: Amersfoort, The Netherlands). The functional unit is 1.0 MJ of energy in the ethanol fuel that is produced at the distillery, and is based on the lower heating value (LHV) of 26.81 MJ/kg.

2.2. Systems description

2.2.1. Base Scenario

The Base Scenario considered in this study is a common process employed in ethanol production plants that use grains as feedstock. The process has previously been simulated with Aspen Plus® (v.8.4) (Aspentech: Burlington, MA, USA) based on data provided by a typical ethanol plant that uses grains as feedstock (Rajendran et al., 2016). After transportation to the plant, the grains are dry-milled to produce coarse flour which is subsequently mixed with water. A liquefaction process then occurs where starch is converted to dextrins with the help of α-amylase. Ethanol and CO_2 are produced under simultaneous saccharification and fermentation with baker's yeast and glucoamylase (that converts the dextrins to glucose). The remaining medium, known as whole stillage, exits through the bottom of the distillation column and undergoes a centrifugation step (in a decanter) giving rise to a liquid fraction (thin stillage) and a solid fraction (wet distiller's grains). The thin stillage goes through a series of evaporations to produce a syrup that is combined with the wet distiller's grains in the dryer to produce dried distiller's grains with solubles (DDGS). DDGS is sold as animal feed. The condensate from the series of evaporations is recycled as process water. The Base Scenario is illustrated in Fig. 1.

2.2.2. Integrating the cultivation of N. intermedia (Scenario A)

The fungus *N. intermedia* is cultivated in the thin stillage that comes from the centrifugation and that is described above for the Base Scenario. The cultivation is done in a bubble column bioreactor for production of ethanol and fungal biomass (Ferreira et al., 2015), and is carried out under continuous mode at a dilution rate of 0.1 h^{-1}, at 35 °C, and under aerobic conditions through sparging of the bioreactor with sterile air at 0.5 vvm (volume of air per volume of liquid per minute). During cultivation, 5 g/L of ethanol, 7.9 g/L of CO_2 and 4 g/L of fungal biomass containing 51.4% (g/g) protein are produced (Ferreira et al., 2015). The biomass is subsequently separated and dried together with the DDGS or it is dried as a separate product. Part of the liquid remaining after biomass separation (85%) is directed to the evaporators after which the ethanol is re-circulated in the process similarly to the condensate described in the Base Scenario, and the remaining part of the liquid (15%) is sent directly back to the process (Fig. 1).

2.3. System boundaries and allocation

As illustrated in Fig. 1, the life cycle consists in the cultivation of wheat, transportation to the distillery and the fuel production. It does not include the fuel distribution and the end-use in vehicles (which is the same for the Base Scenario and Scenario A).

Although ethanol is the major product from the distillery, other valuable products are also manufactured, such as DDGS and fungal biomass. This study presents the results from the LCA using two different methodologies for the partition of the environmental impacts between ethanol and the co-products, i.e., system expansion and energy allocation.

For the system expansion the DDGS is used as cattle feed in the Base Scenario. Two scenarios are studied when integrating the cultivation of *N. intermedia* in the process. In the first scenario, Scenario A-1, the DDGS is used for cattle feed and the fungal biomass is used as fish feed. In the second scenario, Scenario A-2, the DDGS and fungal biomass are combined and used as cattle feed. The alternative products that are considered for cattle feed are soybean and barley, similar as in Börjesson et al. (2010), and for fish feed they are fishmeal and fish oil. The use of fungi biomass has been proved as a viable replacement for fish feed due to its composition rich in protein and a favourable composition of amino acids (Edebo, 2009). The biochemical composition of the by-products and replacement products are presented in Table 1. The mass of alternative products that is needed to replace the DDGS and fungal biomass was determined from their energy and crude protein content.

Partitioning via the allocation method is done using the products energy content, as mentioned above the energy for ethanol is 26.8 MJ/kg and as shown in Table 1, the energy content for DDGS and fungi biomass are 16.1 and 15.7 MJ/kg respectively.

2.4. Life cycle inventory

2.4.1. Wheat production

The data for wheat production was derived from Ahlgren et al. (2011). That study describes the GHG emissions from crop production in Sweden within the context of the EU sustainability criteria for biofuels (Directive 2009/28/EC). The cultivation of wheat includes the production of fertilizers and pesticides, fields operation and direct and indirect nitrous oxide emissions. The results from Ahlgren et al. (2011) are presented at a county level and, for this study, average values for resources used and emissions for production of winter wheat in all Swedish counties were used.

2.4.2. Ethanol production

Data for the integration of filamentous fungi in the ethanol production plant (Scenario A) were obtained from lab-scale experiments (Ferreira et al., 2015) of up to 20 L cultivation volumes as well as from process modelling of the ethanol plant (Rajendran et al., 2016) using Aspen Plus® (v.8.4) (Aspentech: Burlington, MA, USA).

The energy requirements were taken from industry data and from the process modelling. The electricity and steam are produced in a combined heat and power plant using wood chips and secondary wood fuels.

The consumption of chemicals such as enzymes, phosphoric acid, yeast, nutritional salts, sulfuric acid, sodium hydroxide and hydrogen peroxide, was retrieved from an ethanol company where the production is based on grains. The chemicals used in the process were similar for both scenarios with the exception of sodium hydroxide, which has a larger consumption in Scenario A. The emissions from the production of these chemicals were retrieved from EcoInvent v.3.1 database (Wernet et al., 2016), Finnveden et al. (1994) and Bernesson (2004).

2.4.3. Indirect land use change (iLUC)

The iLUC was assessed for the wheat production and for the