

# Influence of high gravity process conditions on the environmental impact of ethanol production from wheat straw

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## Abstract

Biofuel production processes at high gravity are currently under development. Most of these processes however use sugars or first generation feedstocks as substrate. This paper presents the results of a life cycle assessment (LCA) of the production of bio-ethanol at high gravity conditions from a second generation feedstock, namely, wheat straw. The LCA used lab results of a set of 36 process configurations in which dry matter content, enzyme preparation and loading, and process strategy were varied. The LCA results show that higher dry matter content leads to a higher environmental impact of the ethanol production, but this can be compensated by reducing the impact of enzyme production and use, and by polyethylene glycol addition at high dry matter content. The results also show that the renewable and non-renewable energy use resulting from the different process configurations ultimately determine their environmental impact.

*Keywords:* high gravity hydrolysis and fermentation, wheat straw, life cycle assessment, energy analysis

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## 1. Introduction

The development of sustainable processes for the production of biofuels is an ongoing effort. The bio-ethanol industry has taken initiatives to improve the energy and material efficiency of the existing bio-ethanol production technologies with the goal to make these more cost competitive. One way to reach this goal is to run the hydrolysis and fermentation processes at high gravity conditions, that is, at high concentrations of substrate. This results in a reduction of water use in the process and a higher concentration of ethanol in the fermentation broth. Consequently, a reduction in the energy needed during the downstream processing of the broth is achieved (Jørgensen et al., 2007). However, the development of such high gravity processes has mostly been done using sugars derived from starch or sucrose containing crops such as grains, corn, sugar cane or

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10 sugar beet ([Puligundla et al., 2011](#)). Due to concerns about the possible competition with food production, a  
11 hydrolysis and fermentation process using a second generation feedstock is a possible technology development  
12 pathway. [Koppram et al. \(2014\)](#) reviewed the challenges and perspectives regarding ligno-cellulosic ethanol  
13 production at high gravity conditions. The main challenges that need to be dealt with are a) high concentrations  
14 of inhibitory substances that are released during the pretreatment of the feedstock, b) high concentrations of  
15 sugars and ethanol which in themselves are inhibitory, and c) the high viscosity of the pretreated material in  
16 the hydrolysis and fermentation step which results in mixing and mass transfer limitations. These challenges  
17 are currently investigated for the production of ethanol from wheat straw ([Cannella et al., 2012](#); [Cannella and](#)  
18 [Jørgensen, 2014](#)) and the results of this research form the basis of the work presented in this paper.

19 One country that has pioneered the use of straw is Denmark. Besides its use for bedding and forage, approx.  
20 1.3 Mt of straw per year was used in Denmark on average for energy purposes during the period 2004-2008  
21 (which is approx. 25% of the available straw). Its resource potential has not yet been fully exploited (on  
22 average 38% was not used over that same period), due to economic restrictions such as the cost of handling  
23 and transportation of the straw ([Skøtt, 2011](#)). Furthermore, in the European Union agricultural residues such  
24 as wheat straw are readily available, but are not projected to either become more or less abundant until 2050  
25 ([Bentsen and Felby, 2012](#)). This may lead to an increase in the price of straw if demand increases. Another  
26 factor that limits the use of straw is the common practice to leave a certain amount of harvestable straw in the  
27 field. This is done in order to maintain the soil carbon pool, and to prevent greenhouse gas emissions from the  
28 soil ([Cherubini and Ulgiati, 2010](#)).

29 Life cycle assessment (LCA) is a technique for assessing the environmental aspects and potential impacts  
30 associated with a product throughout its life cycle ([International Organization of Standardization, 2006](#)).  
31 LCA has been applied extensively to assess second generation bio-ethanol production from different kinds of  
32 feedstocks. These assessments have generally been done using data that describe industrial-scale operations  
33 (for instance from the well-known NREL studies ([Aden et al., 2002](#); [Humbird et al., 2011](#))) and thus assess the  
34 bio-ethanol life cycle at a mature development stage. LCA can however also be used to assess the environmental  
35 impacts of a new process technology along its development path. [Shibasaki et al. \(2007\)](#) developed a method  
36 to employ LCA for the assessment of technologies that are at an early stage of development, and pointed out  
37 that scale-up effects cannot be neglected when such a technology is compared to a technology that already runs  
38 at an industrial scale. Besides scale issues, [Hillman and Sandén \(2008\)](#) also considered time aspects when  
39 performing an LCA of large-scale technical systems. They identified the shifting time frame and changes in  
40 the background system as issues that are given little attention. Considering the shifting time frame in LCA

41 through doing several assessments during a period of time instead of doing one assessment as a snapshot in  
42 time, for instance at the end of the projected development period, would give room for modelling technical  
43 development such as improved performance, and perhaps even a changed function of the product. For instance,  
44 bio-ethanol may over time be used as a building block chemical for the production of bio-polyethylene (Liptow  
45 and Tillman, 2012; Liptow et al., 2013) instead of as a fuel which would change the function of the ethanol  
46 production system, and may thus change the functional unit. Additionally, the background system may change  
47 over time due to changes in e.g. a country's energy mix, but may also change due to the scale at which a new  
48 technology is applied, e.g. increased land use due to increased biofuel production. Cherubini and Strømman  
49 (2011) pointed out in their review of LCA studies of bioenergy systems that most of them included global  
50 warming potential and energy use in the impact assessment. These energy analyses evaluated all energy inputs  
51 along the life cycle resulting in the cumulative primary energy demand of a product. Little mention was made  
52 of a more detailed view on these energy flows to evaluate, for instance, how much energy is contained in the  
53 main product and by-products. A minority of the reviewed studies included categories like acidification and  
54 eutrophication. As well, only few studies considered land use and land use change in their impact assessment  
55 due to the lack of a widely accepted impact assessment methodology.

56 The purpose of the life cycle assessment described in this paper was to assess the environmental impact of high  
57 gravity conditions during the hydrolysis and fermentation steps in an ethanol production process with wheat  
58 straw as the feedstock, at a very early development stage. Furthermore, the LCA was used to test the hypothesis  
59 that the production of second generation bio-ethanol at high gravity conditions is more environmentally friendly  
60 than at non-high gravity conditions. The results of the LCA are intended to help guide the development of the  
61 technology for high gravity hydrolysis and fermentation by providing technology developers, researchers and  
62 industry decision makers the environmental hotspots from an environmental life cycle point-of-view.

## 63 **2. Materials and methods**

### 64 *2.1. Description of the analyzed system*

65 A cradle-to-gate system, from the cultivation and harvesting of wheat straw (resource extraction) to the  
66 gate of the ethanol production plant, was defined for the analysis of a set of process configurations (see 2.2)  
67 (Fig. 1). The system was assumed to be situated in Kalundborg, Denmark.

68 It was assumed that enough straw is available nearby the plant and that it is cultivated and harvested at an  
69 average distance of 25 km from the plant. Also included in the upstream activities were the production of  
70 the enzymes, sodium hydroxide (needed to adjust the pH to 5.0) and polyethylene glycol (PEG), and the

71 production of fuel and electricity that are needed in all parts of the studied system. The model of the production  
72 of ethanol and co-products was based on the IBUS process technology developed by Inbicon (Fig. 2) (Larsen  
73 et al., 2012). In this continuous process the wheat straw is first prepared and then hydrothermally pretreated.  
74 The pretreated straw is then washed to separate the solubilized hemicellulose from the fibres. The solubilized  
75 hemicellulose is concentrated and leaves the process as the by-product C<sub>5</sub> molasses. Next, the pretreated fibres,  
76 now mainly consisting of cellulose and lignin, are enzymatically hydrolyzed and fermented to produce ethanol.  
77 During the downstream processing, ethanol is separated and purified up to 99.5%(v/v) using distillation and  
78 molecular sieves, and the solids in the bottom product of the distillation are dried. These solids consist mainly  
79 of lignin and are made into lignin pellets. These pellets were assumed to provide the energy demands of the  
80 process, and were exported if a surplus was generated. It should be noted that the non-digested cellulose was  
81 treated as a material loss (it is neither recycled nor incinerated). This was done in order to produce lignin  
82 pellets by-product with a high purity. The distribution of the produced ethanol to the pump and the use phase  
83 was excluded from the assessment because the focus is on the development of the new high gravity technology  
84 for ethanol production.

## 85 2.2. Set-up of the LCA

86 The LCA was based on the results of a set of 33 laboratory experiments in which the key process variables  
87 (dry matter content, enzyme preparation and load, and process strategy) were varied. The rationale of the design  
88 of these experiments was to compare how the performance of the process (i.e. final ethanol yield) is affected by  
89 the introduction of novel commercial enzyme preparations. The two preparations that were compared were the  
90 novel Cellic CTec2 product and its predecessor, a 5:1 (weight) mix of Celluclast and Novozym 188 (henceforth  
91 called Celluclast), all from Novozymes A/S. The experimental design further involved:

- 92 ● testing the influence of dry matter content (DM) of the process. Previous studies have shown that initial  
93 dry matter content has a significant impact on the process performance. The design involved testing two  
94 conditions, 20 and 30% DM, which both are high dry matter conditions compared to most other studies,  
95 but cover the range which is regarded as industrially relevant.
- 96 ● running the experiments at 30% DM with the addition of polyethylene glycol (PEG, with an average  
97 molecular weight of 3000), with the goal to further increase the yield. It was expected that PEG addition  
98 would increase the yield because it lowers the interaction between lignin and enzyme, thereby reducing  
99 non-productive binding of the enzyme (Cannella and Jørgensen, 2014).
- 100 ● testing two different enzyme loads. Enzyme load is an important process variable that determines the  
101 commercial feasibility of cellulosic ethanol production. The loads of the two enzyme preparations were

102 5 and 7.5 FPU/g DM in order to compare the impact of enzyme load on the yield. These are reasonable  
103 loads from an industrial perspective.

- 104 • testing the two common process strategies applied in ethanol production from lignocellulose, separate  
105 hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF), along  
106 with the hybrid pre-saccharification and simultaneous fermentation (PSSF) strategy.

107 For a more detailed description of these experiments and their results, see [Cannella and Jørgensen \(2014\)](#).  
108 Additionally, for the sake of the LCA, a base case process configuration was defined for comparison purposes  
109 and lab experiments were run for it. In this configuration, the hydrolysis and fermentation were run at a more  
110 conventional dry matter content of 10%, while the enzyme preparation used was Cellic CTec2 at a load of 7.5  
111 FPU/g DM. All three process strategies (SHF, PSSF, SSF) were tested.

112 The functional unit in this assessment was 1 liter of ethanol produced from wheat straw by a process system  
113 that uses the high gravity hydrolysis and fermentation technology. An attributional approach was followed  
114 to carry out this LCA, because the purpose was to identify improvement possibilities in the technologies in  
115 development (and to compare those to a defined base case), and thus on what to focus in the development.

116 The life cycle impact assessment (LCIA) was carried out using the CML characterization method ([Guinée  
117 et al., 2002](#)). The following impact categories were selected for the evaluation of the high gravity process  
118 configurations:

- 119 • climate change/global warming potential (GWP)

120 The main goal of the use of biofuels is to reduce the use of fossil-based fuels and thus reducing their  
121 potential impact on global warming. In this assessment, biogenic greenhouse gas (GHG) emissions were  
122 assumed to be climate neutral, and thus only the environmental impact of fossil GHG emissions of the  
123 analyzed system were taken into account.

- 124 • eutrophication potential (EP)

125 The use of fertilizers during the cultivation of wheat can lead to increased eutrophication due to the  
126 emission of phosphorus and nitrogen.

- 127 • acidification potential (AP)

128 Combustion of fossil fuels and lignin pellets can lead to increased acidification due to the emission of  
129 SO<sub>2</sub>, NH<sub>3</sub> and NO<sub>x</sub>.

- 130 • photochemical ozone creation potential (POCP)

131 Combustion of fossil fuels and lignin pellets can lead to increased photochemical ozone creation due to  
132 the emission of volatile organic compounds (VOCs), CO and NO<sub>x</sub>.

133 As part of the assessment, an energy analysis of the product system was also carried out in order to assess energy  
134 use, both renewable (REU) and non-renewable (NREU). For the energy analysis, the ecoinvent cumulative  
135 energy demand method was used (Frischknecht et al., 2007). The environmental impact of the ethanol  
136 production may change significantly, for instance if the electricity fuel mix is (partly) based on renewable  
137 energy or if the energy needed in the production of the biofuel can be delivered by renewable resources.  
138 Sensitivity analyses were carried out to investigate changes in environmental impacts due to potential enzyme  
139 recycling (Fig. 2) and due to changes in the Danish energy mix over time.

140 A partitioning of the environmental burden based on the economic value of the main product (ethanol) and  
141 the by-products (C<sub>5</sub> molasses, lignin) was used (see Table 1) (Tillman, 2000). This was done according to the  
142 flow diagram of the Inbicon process includes two allocation points: one (allocation point A in Fig. 2) at the  
143 separation and wash stage where the C<sub>5</sub> molasses is separated from the fibres, and one (allocation point B in  
144 Fig. 2) where the ethanol and lignin pellets are separated from the remaining liquid. In the case of allocation  
145 point A, it was assumed that the price of the fibre flow out of the separation and wash stage was 50 €/tonne.  
146 Furthermore, straw is a by-product of wheat cultivation, which requires a partitioning of the environmental  
147 burden. Therefore, a third allocation point is at the wheat cultivation and harvesting process (allocation point C  
148 in Fig. 2). The ecoinvent process that is used to model this process, ‘wheat straw IP, at farm’, already  
149 allocated this burden based on the straw’s economic value: 7.5% of the environmental burden of the wheat  
150 cultivation and harvesting is allocated to the straw production (Nemecek and Kägi, 2007). A sensitivity analysis  
151 was carried out to investigate changes in the environmental impact allocated to ethanol due to varying product  
152 prices. In another analysis, the influence of applying system enlargement instead of economic allocation was  
153 investigated. When applying system enlargement, allocation is avoided, and the system under study is credited  
154 with the avoided emissions had its by-products been produced by an alternative process. In this analysis it  
155 was assumed that the lignin by-product is used to produce heat and replaced the production of an equivalent  
156 amount of heat from coal, and that the C<sub>5</sub> molasses replaced the production of molasses from sugar beet. The  
157 ecoinvent processes ‘heat, at hard coal industrial furnace 1-10MW’ and ‘molasses, from sugar beet, at sugar  
158 refinery’ (Nemecek and Kägi, 2007) were used to model these alternative processes, respectively.

### 159 2.3. Mass and energy balances and other data sources

160 The mass and energy balances for the process configurations (the foreground system, see Figs. 1 and  
161 2) were calculated based on the results of the lab experiments (see section 2.2), using a spreadsheet model.  
162 The main result of the experiments that tested the process configurations was the final ethanol yield after 168  
163 hours, expressed as a percentage of the maximum theoretical ethanol yield (Cannella and Jørgensen, 2014).

164 Furthermore, data on the usage of chemicals and enzyme in the experiments were gathered. It was assumed  
165 that these experimental lab data also apply to the industrial scale, i.e. the same yields and usage of enzyme and  
166 chemicals are assumed to apply. Next, the system was modeled at an industrial scale.

167 The yield was used to calculate the amount of ethanol produced based on the amount of cellulose available.  
168 Several assumptions were made as well in order to calculate the mass and energy flows in the foreground  
169 system. With regards to the resource extraction, and production of auxiliary material and energy: 1) the  
170 material loss from the field until the wheat straw is prepared to enter the pretreatment process was 15%. This  
171 includes losses due to transportation, storage and preparation (Hurter, 2007); and 2) the water content of the  
172 harvested straw was 8%. With regards to the ethanol production process: 1) the hydrothermal pretreatment of  
173 the straw took place at  $p=15$  bar and  $T=195^{\circ}\text{C}$  with a residence time of 18.5 minutes; 2) the pretreated straw  
174 consisted of 55%(m/m) cellulose, 34%(m/m) lignin and 11%(m/m) other solid material; 3) the heat input for  
175 distillation was taken from Galbe et al. (2007) (see p. 319, Fig. 6); 4) the solids after distillation (Fig. 2) were  
176 assumed to be dried by pressing up to a dry matter content of 50%. These solids were then further dried up  
177 to 90% DM; and 5) it was assumed that lignin is burned with a 75% efficiency and the additional fossil fuel  
178 mix, if needed in the process configuration, with a 90% efficiency. The combustion of the lignin pellets was  
179 modeled using the ‘Combustion, dry wood residue, AP-42’ process from the US LCI database (NREL, 2012).  
180 This process was adjusted in order to account for the heating value of lignin (assumed to be 24 MJ/kg) and the  
181 combustion efficiency of lignin. The additional fossil fuel mix was assumed to be the Danish energy fossil fuel  
182 mix (Danish Energy Agency, 2011).

183 Besides the assumptions used and modeling choices made to calculate the mass and energy balances for the  
184 foreground system, several choices had to be made for modeling the background system (Fig. 1):

- 185 1) the cultivation and harvesting of the straw was modeled using the ecoinvent process ‘wheat straw IP, at  
186 farm’ (Nemecek and Kägi, 2007)). This model assumes that 25% of the harvestable straw is left in the  
187 field, and this is accounted for in the allocation (see section 2.2).
- 188 2) the production of NaOH was modeled using the ecoinvent process ‘sodium hydroxide, 50% in H<sub>2</sub>O,  
189 production mix, at plant’. The environmental impact of this process is allocated according to the masses  
190 of the different products (52.3% NaOH, 46.4% Cl<sub>2</sub> and 1.3% H<sub>2</sub>). Mass allocation was applied in this  
191 process because the amounts produced of the three chemicals can be clearly determined (Althaus et al.,  
192 2007). 7 g NaOH/kg DM of pretreated wheat straw was added in the experiments in order to adjust the  
193 pH to a level that is favorable to the hydrolysis and fermentation of the pretreated wheat straw.
- 194 3) the production of the Cellic CTec2 enzyme preparation was modeled using the life cycle inventory data

published by [Liptow et al. \(2013\)](#) (see p. 1077, Table 5). In the case of a load of 5 FPU/g DM of pretreated wheat straw, this was equal to 50 g enzyme preparation/kg DM of pretreated wheat straw. In the case of a load of 7.5 FPU/g DM of pretreated wheat straw, this was equal to 75 g enzyme preparation/kg DM of pretreated wheat straw.

- 4) the production of the Celluclast/Novozym 188 enzyme preparation was modeled by considering the production of Celluclast for both types of enzyme in this mix. It was modeled using the ‘Enzyme, Cellulase, Novozyme Celluclast’ process from the US LCI database ([NREL, 2012](#)). In the case of a load of 5 FPU/g DM of pretreated wheat straw, this was equal to 78 g enzyme preparation/kg DM of pretreated wheat straw. In the case of a load of 7.5 FPU/g DM of pretreated wheat straw, this was equal to 103 g enzyme preparation/kg DM of pretreated wheat straw.
- 5) the production of polyethylene glycol (PEG, average molecular weight of 3000 g/mol) was modeled by assuming it was produced from ethylene oxide via its interaction with water. Theecoinvent process ‘ethylene oxide, at plant’ was used to model this process (assuming that the impact of the polymerization of ethylene oxide to PEG is negligible) ([Althaus et al., 2007](#)). 10 g PEG/kg DM of pretreated wheat straw was added in the experiments that used PEG.
- 6) the fossil fuel mix used as additional fuel for the ethanol production process was assumed to be the Danish mix from 2009 and consists of 29% coal, 50% oil and 21% natural gas ([Danish Energy Agency, 2011](#)). This fuel is used for steam production. Changes in the fossil fuel mix over time were taken into account using sensitivity analysis.
- 7) the electricity used by the ethanol production process was produced in Denmark, and was modeled with theecoinvent process ‘electricity mix (DK)’.

The LCA software openLCA version 1.3 was used to model the complete ethanol production systems according to Fig. 1 (both the foreground and background systems) for the different process configurations, and to calculate their environmental impacts. The mass and energy balance results for the process configurations and the models used for describing the background system were thus integrated in this software.

### 3. Results and discussion

All process configurations run at high dry matter content (20 and 30% DM, respectively) and the base case configurations (at 10% DM) were analyzed in order to determine their environmental impact and energy use. The results are summarized in Fig. 3. This figure depicts the results for the four environmental impact categories analyzed, at the two different dry matter contents at which experiments were carried out and with

225 the two different enzyme preparations. Furthermore, the experiments at 30% DM content with an addition of  
226 PEG are shown in their own separate bar charts. Each bar chart contains the results for the different process  
227 strategies at the two different enzyme loads used and the base case experiments run at 10% DM. The yields of  
228 all experiments are shown above the bars in the charts showing the global warming potentials (GWP), and it is  
229 also depicted to what extent different stages in the life cycle contribute to the result. It should be noted that the  
230 three base case experiments (run with SHF, PSSF and SSF process strategies) all resulted in the same yield,  
231 and consequently had the same environmental impact. Therefore, in Fig. 3 only one bar is shown to represent  
232 these experiments. Most of the results discussion will revolve around Fig. 3.

233 There is no simple answer to the question whether high gravity hydrolysis and fermentation is environmentally  
234 preferable to hydrolysis and fermentation at the more conventional 10% DM conditions (Fig. 3). It is however  
235 clear that the configurations running at 30% DM without the addition of PEG are not preferable options.  
236 Furthermore, it is not directly obvious which process strategy (SSF, PSSF or SHF) is the better one. What can  
237 be seen at first glance is that the environmental impact is dominated by enzyme production, at all investigated  
238 process configurations and for both types of enzyme preparations. It is also clear that the acidification (AP)  
239 and photo-chemical ozone creation (POCP) potentials co-vary with the GWP. This is according to expectations  
240 because all these types of impacts are mostly a result of the significant use of non-renewable energy during  
241 the enzyme production and its resulting emissions of CO<sub>2</sub> (GWP), SO<sub>2</sub> (AP), and NO<sub>x</sub> and volatile organic  
242 compounds (POCP). Eutrophication potential (EP) on the other hand is caused by NO<sub>x</sub> emissions from  
243 combustion but also by leakage of fertilizers during the wheat cultivation phase.

244 In order to structure the analysis that follows, the causal diagrams depicted in Fig. 4 were constructed. Yield  
245 seems to be the factor which explains most of the results, and is hence given a central role in the diagrams.  
246 Yield is affected by the studied process variables, and yield consequently affects the non-renewable energy  
247 use (NREU) of the process configuration and its related emissions (Fig. 4a). Yield also affects wheat straw  
248 consumption (which consequently affects the renewable energy use (REU)) and hence eutrophication due to  
249 the cultivation of wheat (Fig. 4b). It should be noted that the 'process strategy' variable (see section 2.2) is not  
250 part of the constructed causal diagrams. When varying the process strategy (SHF, PSSF or SSF), keeping all  
251 other process variables constant, none of them is clearly preferable. Cannella and Jørgensen (2014) observed  
252 nevertheless that there is a difference in the preferred choice of process strategy between the two enzyme  
253 preparations (Cellic CTec2 or Celluclast) at 30% DM when considering yield: the use of Cellic CTec2 resulted  
254 in a higher yield when using SHF or PSSF, whereas the use of Celluclast resulted in a higher yield when using  
255 SSF or PSSF. For a more in-depth discussion on the influence of the process strategy on the yield, see Cannella

256 and Jørgensen (2014).

### 257 3.1. Yield of process configuration

258 The yield is the main determinant of the environmental impact of a configuration. Keeping all process  
259 variables constant (see section 2.2), except for the process strategy, all environmental impacts studied will  
260 decrease with an increasing yield (Fig. 3).

261 Also, the renewable and non-renewable energy use (REU and NREU, respectively) vary with the yield. This  
262 results in a decreasing trend of the total extracted energy, as defined by Arvidsson et al. (2012) and calculated  
263 here as the sum of REU and NREU. The yield of the process configurations has a significant influence on  
264 the REU which indicates that the amount of wheat straw needed varies widely among the tested process  
265 configurations (from 5.5 kg up to 18.6 kg for the production of 1 liter of ethanol). The NREU varies to a lesser  
266 extent with the yields (from 25 MJ to 63 MJ per liter of ethanol) when compared to the REU, and depends  
267 mostly on the amount of added enzyme and polyethylene glycol (PEG). These two process variables (see  
268 section 2.2) greatly affect the yield and therefore the REU. It can be stated that generally the REU (straw use)  
269 decreases with increased NREU (enzyme load and PEG addition), which is in accordance with the analysis  
270 based on the causal diagram for eutrophication potential (Fig. 4b).

271 The yield of the base case configurations (10% DM, Cellic CTec2 at 7.5 FPU/g DM) is the highest of all  
272 configurations at 92% for each of the three process strategies tested (SHF, PSSF and SSF). However, for none  
273 of the impact categories does the base case configuration outperform all other configurations. This is explained  
274 by the use of additional fossil fuel by the base case configuration that is required for satisfying its energy  
275 demand (Figs. 3 and 5a). In other words, the energy contained in the lignin pellets produced by the base case  
276 configuration itself is not enough to meet its total energy needs which are higher because of its higher energy  
277 use during the distillation of the more dilute fermentation broth.

278 Of the process configurations running at high dry matter content (20 or 30% DM), those with the highest and  
279 lowest yields are both run at 30% DM. When comparing the base case configuration and the configuration with  
280 the highest yield (30% DM, PEG addition, Cellic CTec2, 7.5 FPU, PSSF with a yield of 89%) from an energy  
281 perspective, it becomes clear that these configurations differ due to the extra fossil fuel that is needed in the  
282 base case, and the addition of PEG and the small amount of lignin product in the highest yield configuration  
283 (Fig. 5b). When doing this comparison between the base case and the lowest yield configuration (30% DM,  
284 Celluclast, 5 FPU, SHF with a yield of 27%), the main difference is the renewable energy input needed to  
285 produce 1 l of ethanol (Fig. 5c). The product flow that contains most energy is the hemi-cellulose that is  
286 used to produce the C<sub>5</sub> molasses in the lowest yield configuration, whereas in the base case (and the highest

287 yield configuration) this energy flow is similar to the energy contained in 1 l of ethanol. As well, the energy  
288 contained in the lignin product is larger than in the highest yield configuration: 14% of the lignin that enters  
289 the downstream processing step leaves the system as lignin product, whereas in the highest yield configuration  
290 this is 3%.

291 It should be noted that the non-digested cellulose was treated as a material loss (see 2.1). If this cellulose was  
292 burned, it would replace a part of the extra fossil fuel that is needed in the base case configurations (Fig. 5a).  
293 In the case of the lowest yield configuration (Fig. 5c), an excess of energy would be produced if this cellulose  
294 was burned (assuming a combustion efficiency of 75%). This energy would then be another by-product of  
295 the process, and this would consequently change the environmental impact allocated to the ethanol that is  
296 produced.

### 297 3.2. *Enzyme load and preparation*

298 Another important determinant of the environmental impact of a configuration is the enzyme load. An  
299 increased enzyme load increases the yield for all process configurations (keeping all other process variables  
300 constant). However, this does not lead to a lower environmental impact: a higher enzyme load generally  
301 leads to higher global warming (GWP), acidification (AP) and photo-chemical ozone creation potentials  
302 (POCP) because of a higher non-renewable energy use (Fig. 4a). This is due to the large amount of fossil  
303 energy that is used during enzyme production (120 MJ and 52.1 MJ per kg of Cellic CTec2 and Celluclast  
304 enzyme preparations, respectively). There are two exceptions to this generalization: 1) the configurations  
305 running at 30% DM with PEG addition using Cellic CTec2 (Fig. 3c); and 2) the acidification potential of the  
306 configurations using Celluclast (Figs. 3d and 3e). In these cases, the impact of the increase in yield outweighs  
307 (or compensates) the impact of increased enzyme load and leads to a decreased (or equal) environmental  
308 impact. This demonstrates a trade-off between a reduced environmental impact due to increased yield, which  
309 is due to the increased enzyme load, and an increased environmental impact due to enzyme production. The  
310 causal link between enzyme load and eutrophication potential (EP) shows a different behavior (Fig. 4b). In  
311 this case, the impact of the increase in yield either compensates or, in some cases, outweighs the impact of  
312 increased enzyme load, thus leading to an equal or lower EP. Reasons for this different behavior are that 1) the  
313 enzyme production has a lower relative contribution to EP when compared to the other impact categories; and  
314 2) the increased enzyme load leads to decreased straw use, which consequently leads to a lower contribution  
315 of straw cultivation and harvesting to EP (Fig. 3). There is one exception to this behavior, the configuration  
316 running at 30% DM using Celluclast and the SHF process strategy (Fig. 3e), where the increase in yield is not  
317 great enough to compensate the impact of increased enzyme load.

318 Changing the type of enzyme preparation, from the Cellic CTec2 preparation to the Celluclast preparation,  
319 lowers the yield of all process configurations (keeping all other process variables constant). However, this does  
320 in general not lead to a higher GWP, AP and POCP (Fig. 4a). This is due to the lower fossil energy use during  
321 the Celluclast production when compared to the Cellic CTec2 production. The configurations running at an  
322 enzyme load of 5 and 7.5 FPU/g DM, both at 30% DM and using the SHF process strategy are two notable  
323 exceptions to this generalization. For these configurations all environmental impacts are higher because the  
324 decrease in yield is too great for the lower fossil energy use for Celluclast production to compensate. In the  
325 case of eutrophication potential, the causal links are the same as for the enzyme load (Fig. 4b).

### 326 3.3. *Dry matter content and PEG addition*

327 The dry matter content has a direct influence on the yield of a configuration: the higher the dry matter  
328 content, the lower the yield which consequently leads to a higher energy use (both REU and NREU) and thus  
329 to a higher environmental impact (keeping all other process variables equal) (Figs. 3 and 4). The addition of  
330 polyethylene glycol (PEG) has a direct positive influence on the yield and thus on the total energy use and the  
331 environmental impact of a configuration (compare Figs. 3b and 3c).

332 It should be noted that the production of PEG contributes little to all impact categories (Figs. 3c and 3f). The  
333 expected increase in the yield due to PEG addition is thus achieved without causing a significant environmental  
334 burden due to PEG production which is fossil-based. This is due to the small amount of PEG, 10 g per kg of  
335 dry matter, that was added to the experiments at 30% DM (Cannella and Jørgensen, 2014).

### 336 3.4. *Sensitivity and uncertainty analyses*

#### 337 3.4.1. *Mass and energy balances*

338 The additional use of fossil fuel by the base case configuration depends on the combustion efficiency of  
339 the fossil fuel and lignin combustion (assumed to be 90% and 75%, respectively). For instance, if the lignin  
340 combustion efficiency could be increased to 87%, then the base case configuration would not need additional  
341 fossil fuel. Avoiding additional fossil fuel use could also be achieved by increasing the energy efficiency of the  
342 pretreatment process step which needs a significant amount of energy (Fig. 5a). In this case, the pretreatment  
343 would need to be 29% more energy efficient in order to avoid additional fuel use in the base case configuration.  
344 These measures would lead to a reduction of approximately 14% of the GWP of the base case configuration.  
345 Such efficiency improvements would also change the environmental impact of the ethanol produced with  
346 the process configurations run at higher dry matter content. For instance, increasing the lignin combustion  
347 efficiency of the high gravity process configuration with the highest yield (Fig. 5b) to 87% would lead to an

348 increase in the amount of lignin by-product from 0.74 MJ to 4.3 MJ. Consequently, the GWP allocated to the  
349 produced ethanol would decrease by approx. 8%. For the high gravity process configuration with the lowest  
350 yield (Fig. 5c) the amount of lignin by-product would increase from 13.6 MJ to 23.6 MJ, and reduce the GWP  
351 allocated to ethanol by approx. 15%.

#### 352 *3.4.2. Allocation choices and system enlargement*

353 The impact results do not show a large sensitivity due to changes in the price of ethanol (allocation point  
354 B) or the pretreated fibre stream (allocation point A) (Fig. 2). Increasing the ethanol price from 600 €/tonne to  
355 825 €/tonne results in an increase of 0 to 6.5% in the impact results. Increasing the price of the pretreated  
356 fibre stream from 50 €/tonne to 86 €/tonne results in an increase of 0.4 to 10% in the impact results. The  
357 configurations with the lowest yields show the greatest sensitivity.

358 Wheat straw may be considered as a waste product. This implies that the environmental burdens of the  
359 cultivation and harvesting operations (see Fig. 1) are all allocated to the production of the wheat grain because  
360 the straw will have no economic value under such a consideration. In the case of GWP, AP and POCP, this  
361 leads to a reduction of the environmental impact of 11 to 22%, 12 to 23% and 2 to 4%, respectively. In the  
362 case of EP, the reduction is more significant and is 43 to 52% (see also Fig. 3). The configurations using the  
363 Celluclast enzyme cocktail show the greatest sensitivity.

364 System enlargement leads to different results for the environmental impact of a process configuration. To  
365 illustrate this, system enlargement was applied to the process configurations that are depicted in Fig. 5, and the  
366 results are shown in Fig. 6. The results show that, especially in the case of the process configurations with a  
367 lower yield, the amounts of the by-products that are produced have a significant influence on the environmental  
368 impact of a process configuration. However, for these three process configurations, the numerical results for  
369 GWP, AP and POCP are similar to those when applying economic allocation. The numerical results for EP  
370 show a larger discrepancy, where applying system enlargement results in a higher impact. It should be noted  
371 that these results strongly depend on the choice of alternative processes for the production of C<sub>5</sub> molasses and  
372 heat produced by lignin combustion when modeling the system enlargement.

#### 373 *3.4.3. Biogenic greenhouse gas emissions*

374 As stated in section 2.2, the impact that is caused by emissions of biogenic greenhouse gases (GHGs) was  
375 not included in the assessment. Hence, the benefits from CO<sub>2</sub> uptake during growth were not included either.  
376 However, had this been done it would change the results for the GWP of the analyzed process configurations to  
377 a certain extent. What follows is a short discussion of where the main biogenic GHG emissions occur in the

378 analyzed system from a cradle-to-gate and a cradle-to-grave perspective, and how the impact of these would  
379 manifest themselves.

380 From a cradle-to-gate perspective, biogenic CO<sub>2</sub> emission takes place during the fermentation: for every liter of  
381 ethanol that is produced from a C<sub>6</sub> sugar via fermentation, 0.75 kg of CO<sub>2</sub> is produced. Furthermore, biogenic  
382 CO<sub>2</sub> is emitted when burning lignin pellets to meet the process energy demands (approx. 0.1 kg CO<sub>2</sub> per MJ  
383 produced (AEA Technology, 2012)). When applying a cradle-to-grave perspective, by, for instance, using the  
384 produced ethanol as a transportation fuel and the C<sub>5</sub> molasses in cow feed production, biogenic CO<sub>2</sub> is also  
385 emitted during the combustion of the ethanol (1.5 kg of CO<sub>2</sub> per liter of ethanol, assuming perfect combustion),  
386 and emissions of biogenic CH<sub>4</sub> occur after consumption and digestion by cows of feed containing the C<sub>5</sub>  
387 molasses due to enteric fermentation (approx. 21 g CH<sub>4</sub> per kg of dry matter feed intake (Flysjö et al., 2011);  
388 C<sub>5</sub> molasses makes up a small percentage of this dry matter feed). Furthermore, excess lignin pellets that are  
389 produced and exported may be burned and thus emit biogenic CO<sub>2</sub>. All these emissions take place not long  
390 after the straw has been harvested.

391 The GWP of all these emissions is counteracted by the uptake of CO<sub>2</sub> due to the growth of wheat. Nevertheless,  
392 a certain amount of radiative forcing due to these emissions will occur because this uptake is not instantaneous.  
393 Therefore, incorporating the impact of biogenic CO<sub>2</sub> in the assessment would increase the GWP for all process  
394 configurations from both the cradle-to-gate and cradle-to-grave perspective. However, this will only be a  
395 marginal increase due to the fast growth rate of wheat when compared to, for instance, trees (Liptow et al.  
396 (2014), unpublished results). It should be noted that the impact of biogenic GHGs depends on the end-of-life  
397 stage of the ethanol and the by-products. For instance, if the produced ethanol were used as a precursor for  
398 bio-polyethylene (bio-PE) production, the carbon may be sequestered for a longer time compared to using  
399 ethanol as a transportation fuel, depending on what type of product the bio-PE is applied in. Furthermore, the  
400 methods used to assess the impact of biogenic greenhouse gas emissions are still in development, and may  
401 currently introduce additional uncertainty in the assessment results.

#### 402 3.4.4. Enzyme recycling

403 Because of the significant influence of the enzyme production and use, an analysis was done of the situation  
404 in which 25% of the activity of the enzyme is recycled. This value was chosen after consulting with an  
405 industrial project partner if this would be a feasible target. This was done for the base case configurations, and  
406 for the configurations running at high dry matter content with the highest and lowest yields. The analysis shows  
407 that improvements can be made in the environmental impact of the process configurations due to a reduction of  
408 the use of fresh enzyme preparation (Table 2). Other work already showed that an increase of enzyme activity

409 will also improve the environmental impact of the process in which the enzyme is used (Liptow et al., 2013).

#### 410 3.4.5. Changes in energy mix

411 The influence of the anticipated share of fossil fuel in the Danish energy mix over time (Lund and Mathiesen,  
412 2009; Danish Energy Agency, 2011) on the GWP of the enzyme production, and of the fossil fuel use for  
413 process energy, was analyzed. This was done for the base case configuration, and for the configurations  
414 running at high dry matter content with the highest and lowest yields (Table 3). The fossil fuel mix itself is  
415 also projected to change over time (largely replacing coal with natural gas, but maintaining oil use) and this  
416 was also taken into account. The results show that the total GWP of the configurations significantly decreases.  
417 Furthermore, the impact of the use of fossil fuel for process energy in the base case configuration does not  
418 change drastically (13%), while the impact of enzyme production decreases significantly (ca. 40%) (not shown  
419 in Table 3). Combining this result with the result of enzyme recycling (see section 3.4.4) indicates that the  
420 environmental impact of the process configurations can be improved significantly by having a more efficient  
421 enzyme use and a cleaner enzyme production.

#### 422 3.5. Comparison with other relevant studies

423 The environmental impact of the tested process configurations were compared with those published in two  
424 other studies (Wang et al., 2013; Borrion et al., 2012). These studies were chosen because they also evaluated  
425 bioethanol production from wheat straw. Both studies were based on a plant design with a capacity of 2000  
426 metric tonnes per day of wheat straw, whereas the current study used raw lab data to perform the LCA of the  
427 experimental process configurations without scale-up considerations. It should be noted that no other studies  
428 were found that evaluated the influence of high gravity conditions on the environmental impact of second  
429 generation bioethanol production.

430 Wang et al. (2013) studied the environmental sustainability of bioethanol production from wheat straw in the  
431 UK using, among others, liquid hot water pretreatment. This study was set up as a 'well-to-wheel' LCA and the  
432 functional unit was 'to drive 1 km in a flexible-fuel vehicle'. The ethanol production process was based on the  
433 NREL corn stover-to-ethanol model (Humbird et al., 2011). The enzyme preparation for hydrolysis was Cellic  
434 CTec at a loading of 10 FPU/g glucan (approx. 3.3 FPU/g DM). The fermentation process was carried out using  
435 *Zymomonas mobilis* capable of fermenting both C<sub>5</sub> and C<sub>6</sub> monomer sugars. There was thus no C<sub>5</sub> by-product  
436 stream. Instead, surplus electricity was the by-product which was credited with avoided emissions of average  
437 electricity generation. They found that enzyme production is a main contributor due to its energy-intensive  
438 nature. Furthermore, the ethanol production process (not including straw cultivation and harvesting) emitted

439 4.52 kg CO<sub>2</sub> per l ethanol. This however included biogenic CO<sub>2</sub> emissions of approximately 2.8 kg/l ethanol  
440 from burning the distillation bottom product (Wang et al. (2013), p. 721, Fig. 4b). Therefore, approximately 1.7  
441 kg CO<sub>2,eq</sub> per l ethanol were emitted, assuming climate neutrality of the biogenic carbon. This is comparable  
442 to the results for the base case and most of the process configurations running at 30% DM with PEG addition  
443 (Figs. 3c and 3f), as well as most of the process configurations running at 20% DM (Figs. 3a and 3d).  
444 Borrion et al. (2012) did a LCA of bio-ethanol production from wheat straw and used an older version of the  
445 NREL corn stover-to-ethanol model (Aden et al., 2002) to describe the ethanol production process. The wheat  
446 straw was pretreated using dilute sulfuric acid hydrolysis. This study did not specify the enzyme preparation  
447 that was used. This was also a ‘well-to-wheel’ LCA and the functional unit was the amount of fuel needed to  
448 drive 1 km with a small passenger car. This study found a GWP of 3.3 kg CO<sub>2,eq</sub> per l ethanol when considering  
449 the ‘well-to-gate’, and a GWP of 2.6 kg kg CO<sub>2,eq</sub> per l of ethanol without straw cultivation and harvesting  
450 (Borrion et al. (2012), see p. 14, Table 5). Like Wang et al. (2013), this study also pointed out the significant  
451 contribution of the enzyme production. Furthermore, the global warming potential of the ethanol production  
452 (not including straw cultivation and harvesting) is comparable to or slightly higher than most of the process  
453 configurations run at 30% DM (Figs. 3b and 3e).

#### 454 **4. Conclusion**

455 Higher dry matter content leads to a higher environmental impact of ethanol production. Nevertheless, the  
456 results indicate that reduction of the impact of enzyme production and use, and the use of PEG at 30% DM can  
457 compensate this increase. The results also show that the renewable and non-renewable energy use resulting  
458 from the different process configurations ultimately determine their environmental impact. Future development  
459 work may focus on determining the process conditions at which the environmental impact is minimized by  
460 finding the optimal dry matter content for the hydrolysis and fermentation, and decreased enzyme use while  
461 maintaining a high yield.

#### 462 **Supplementary material**

463 Supplementary material is available as an electronic annex.

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**Table captions****Table 1**

Energy content and price for the feedstock (wheat straw) and the products (ethanol, C<sub>5</sub> molasses and lignin) of the process

**Table 2**

Reduction in the environmental impacts due to enzyme recycling for the: a. base case (10% DM, 7.5 FPU, all process strategies, yield = 92%); b. high gravity process configuration with the highest yield (30% DM, PEG addition, Cellic CTec2, 7.5 FPU, PSSF, yield = 89%); c. high gravity process configuration with the lowest yield (30% DM, Celluclast, 5 FPU, SHF, yield = 27%).

**Table 3**

Global warming potentials (GWP) (in kg CO<sub>2,eq</sub> per liter of ethanol produced) due to projected changes in the Danish energy mix for the: a. base case (10% DM, 7.5 FPU, all process strategies, yield = 92%); b. high gravity process configuration with the highest yield (30% DM, PEG addition, Cellic CTec2, 7.5 FPU, PSSF, yield = 89%); c. high gravity process configuration with the lowest yield (30% DM, Celluclast, 5 FPU, SHF, yield = 27%).

**Fig. 1.** Cradle-to-gate system for the evaluation of the high gravity process configurations. The parts of the flow chart with the dashed lines have not been included in the assessment.

**Fig. 2.** Flow diagram of the Inbicon process (adjusted from [Larsen et al. \(2012\)](#)). In bold, the products are indicated; in italics, several flows are specified. Three allocation points applied in the LCA are indicated.

**Fig. 3.** Life cycle impact assessment results for the process and the base case configurations. Figs. 3a to 3c show the results for the Cellic CTec2 enzyme preparations; Figs. 3d to 3f show the results for the Celluclast enzyme preparations. The numbers above the bars in the GWP charts are the ethanol yields of the configurations.

**Fig. 4.** Effects of the process variables on: a. global warming potential (GWP), acidification potential (AP) and photo-chemical ozone creation potential (POCP); b. eutrophication potential (EP). A '+'-sign declares a positive causal link, e.g. if the enzyme load increases, then the yield increases. A '-'-sign declares a negative causal link, e.g. if DM content increases, then yield decreases. In red, the main differences between the diagrams are indicated.

**Fig. 5.** Sankey diagrams of the energy flows in the: a. base case (yield = 92%) (10% DM, 7.5 FPU, all process strategies); b. high gravity process configuration with the highest yield (yield = 89%) (30% DM, PEG addition, Cellic CTec2, 7.5 FPU, PSSF); c. high gravity process configuration with the lowest yield (yield = 27%) (30% DM, Celluclast, 5 FPU, SHF). The flows through the main process, and the amounts of renewable and non-renewable fuel used are distinguished.

**Fig. 6.** Results of the LCA when applying system enlargement for the: a. base case (10% DM, 7.5 FPU, all process strategies); b. high gravity process configuration with the highest yield (30% DM, PEG addition, Cellic CTec2, 7.5 FPU, PSSF); c. high gravity process configuration with the lowest yield (30% DM, Celluclast, 5 FPU, SHF).

Table 1

	Energy content [MJ/kg]	Price [€/tonne]
Wheat straw	17.2	40 <sup>1</sup>
Ethanol	29.7	600 <sup>2</sup>
Lignin	24	300 <sup>3</sup>
C <sub>5</sub> molasses	18	86 <sup>4</sup>

<sup>1</sup> Price of wheat straw fluctuates significantly; €40 per delivered tonne is assumed (Ekman et al., 2013).

<sup>2</sup> <http://www.nasdaq.com/markets/ethanol.aspx>

<sup>3</sup> Assumed to be sold as an alternative renewable fuel (Qin, 2009).

<sup>4</sup> See Morgen (2006).

Table 2

Process configuration	Reduction of environmental impacts			
	GWP	EP	AP	POCP
Base case	18 %	11 %	15 %	23 %
Highest yield	21 %	12 %	17 %	24 %
Lowest yield	18 %	9 %	9 %	23 %

Table 3

Process configuration	Fossil share in energy mix		
	80 %	67 %	50 %
Base case	2.6	2.3	1.7
Highest yield	2.3	2.0	1.5
Lowest yield	3.1	2.6	2.0

Figure 1

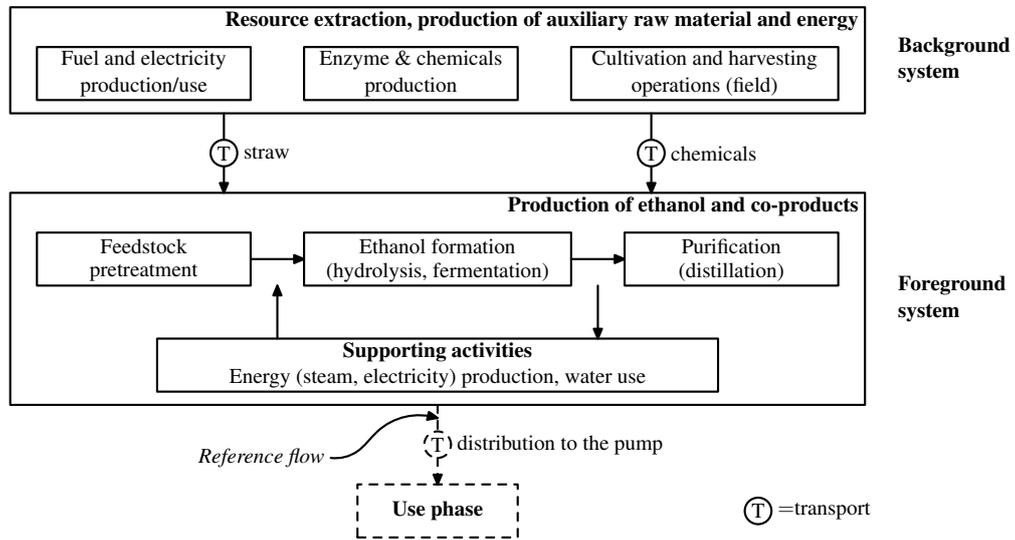


Figure 2

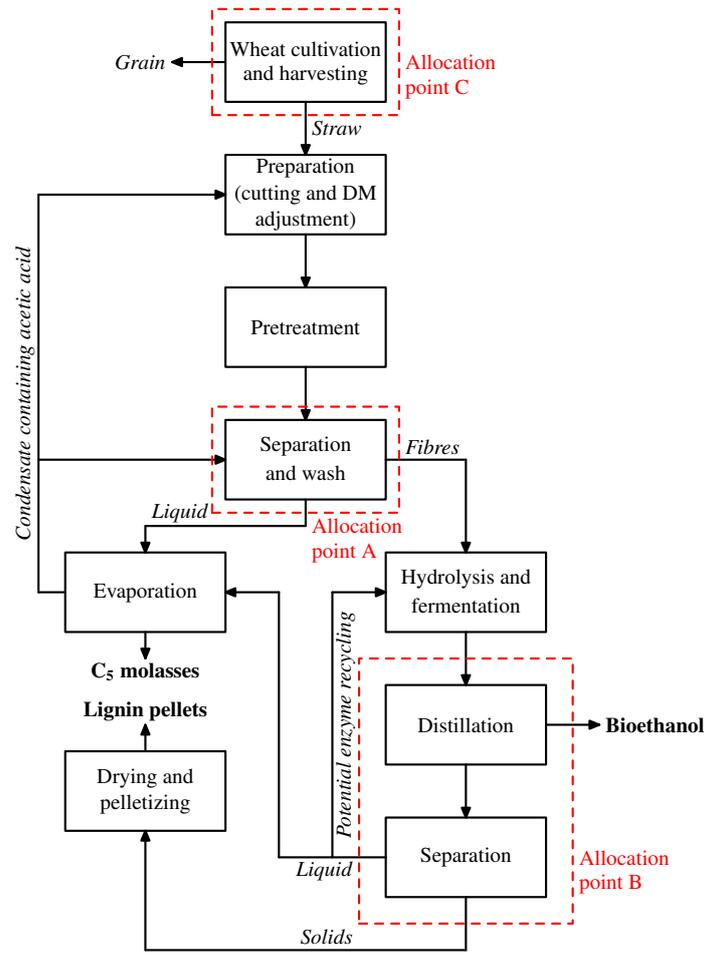
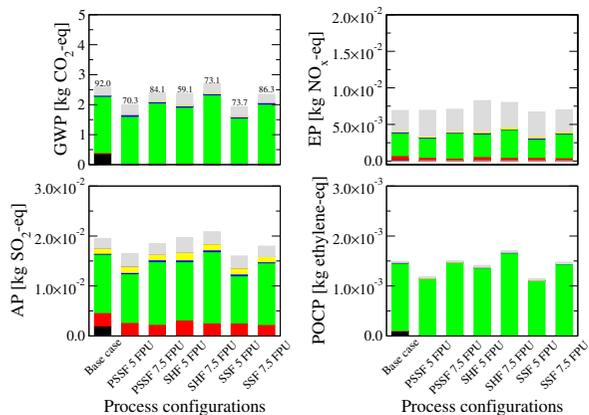
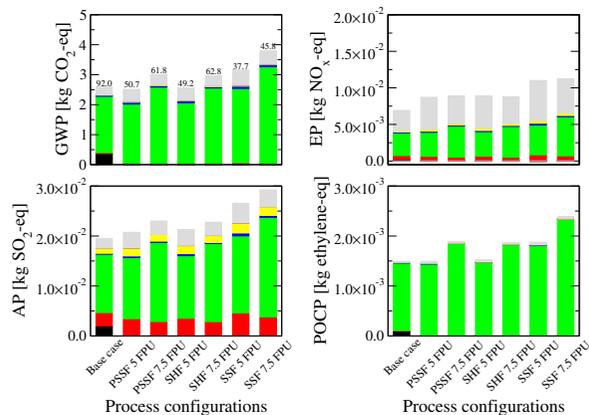


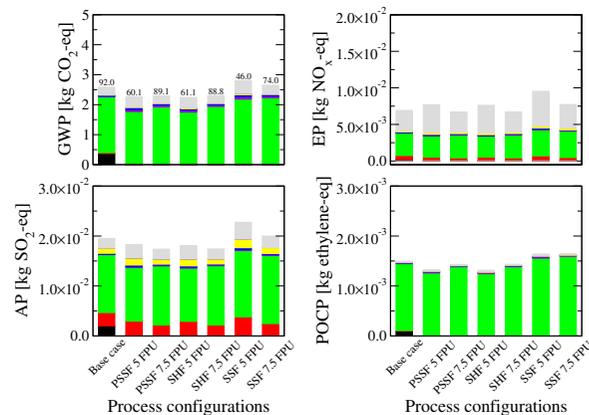
Figure 3



(a) Cellic CTec 2, 20% DM

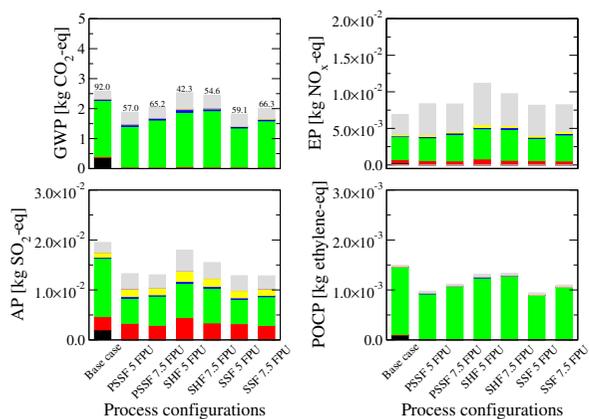


(b) Cellic CTec2, 30% DM

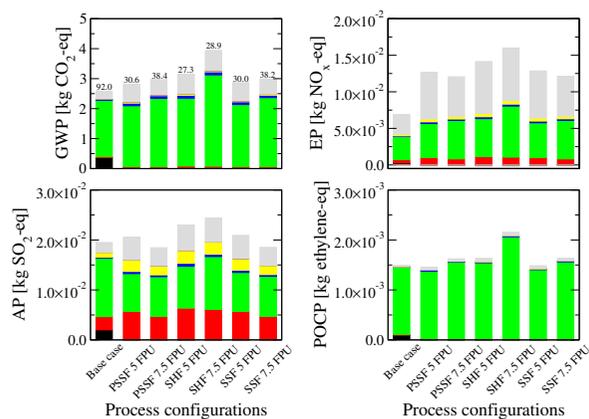


(c) Cellic CTec2, 30% DM with PEG addition

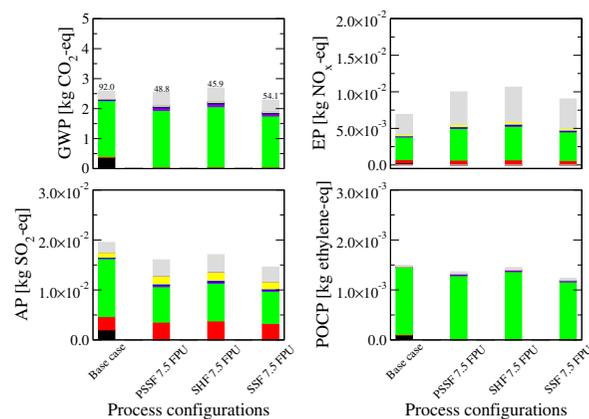
3



(d) Celluclast, 20% DM



(e) Celluclast, 30% DM



(f) Celluclast, 30% DM with PEG addition

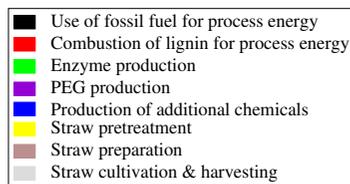
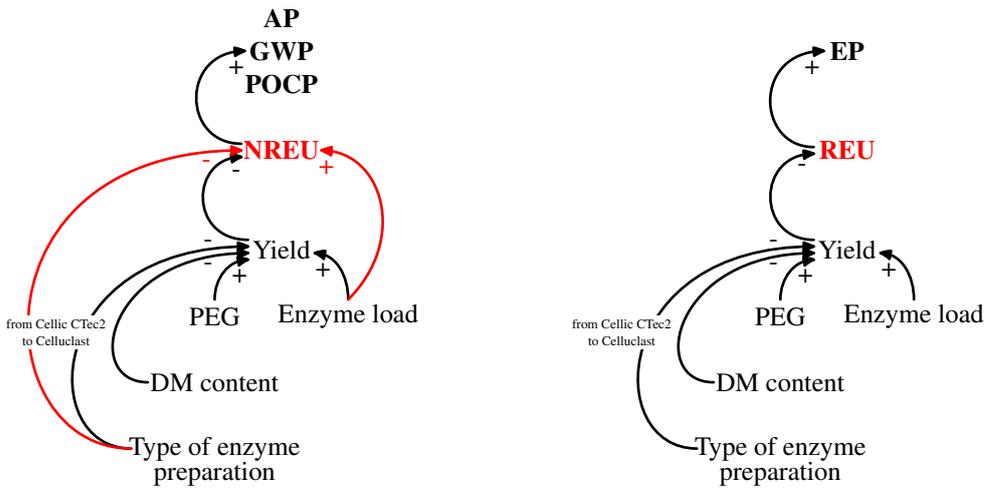


Figure 4



(a) Causal diagram for GWP, AP and POCP

(b) Causal diagram for EP

Figure 5

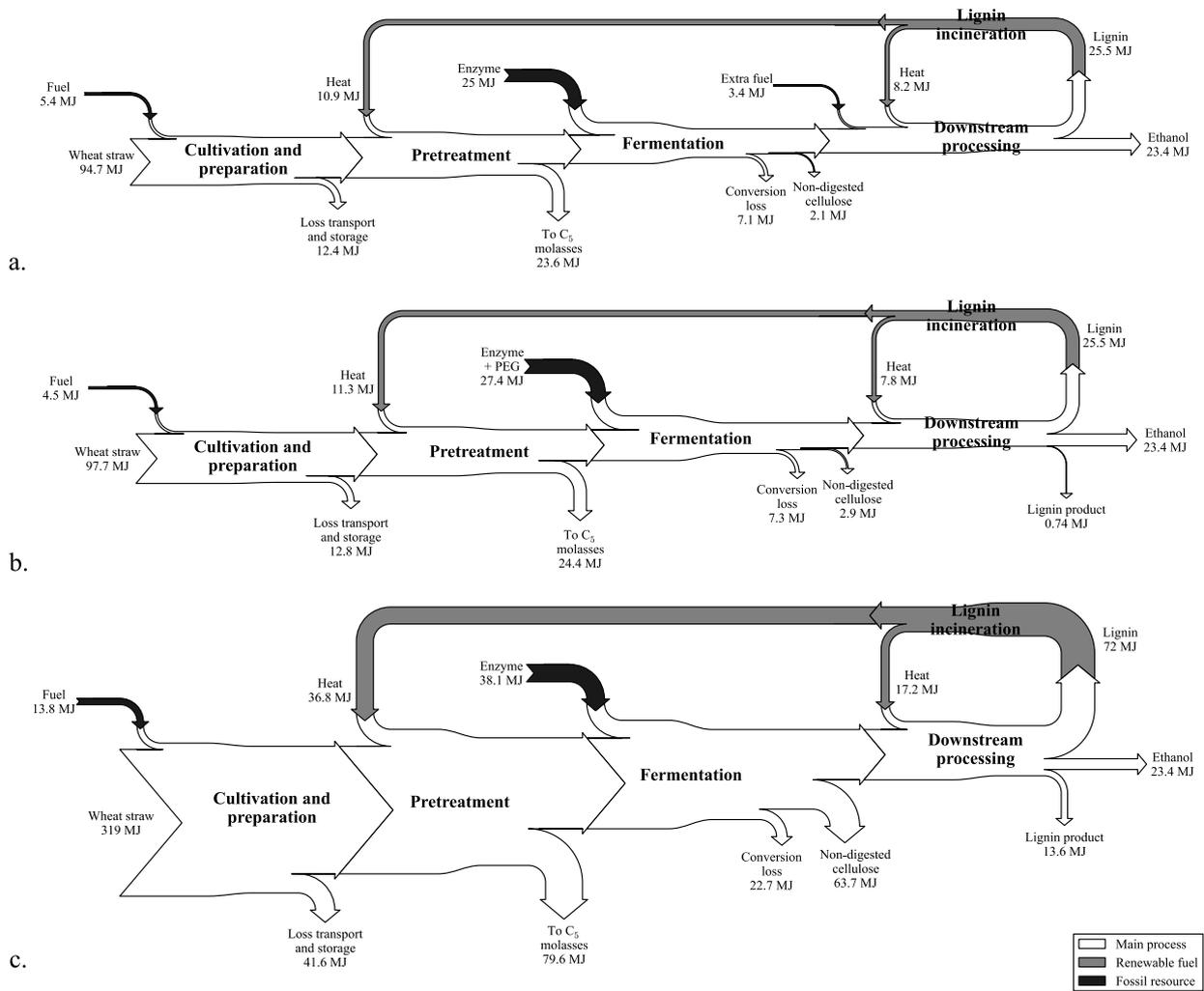


Figure 6

