The contribution of enzymes and process chemicals to the life cycle of ethanol

Heather L MacLean$^{1,4}$ and Sabrina Spatari$^{2,3,5}$

1 Department of Civil Engineering and School of Public Policy and Governance, University of Toronto, 35 St George Street, Toronto, ON, M5S 1A4, Canada
2 Energy and Resources Group, University of California, 310 Barrows Hall, Berkeley, CA 94720-3050, USA
3 Civil, Architectural and Environmental Engineering, Drexel University, 3141 Chestnut Street, Philadelphia, PA 19104, USA

E-mail: hmaclean@ecf.utoronto.ca

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Abstract

Most life cycle studies of biofuels have not examined the impact of process chemicals and enzymes, both necessary inputs to biochemical production and which vary depending upon the technology platform (feedstock, pretreatment and hydrolysis system). We examine whether this omission is warranted for sugar-platform technologies. We develop life cycle (‘well-to-tank’) case studies for a corn dry-mill and for one ‘mature’ and two near-term lignocellulosic ethanol technologies. Process chemical and enzyme inputs contribute only 3% of fossil energy use and greenhouse gas (GHG) emissions for corn ethanol. Assuming considerable improvement compared to current enzyme performance, the inputs for the near-term lignocellulosic technologies studied are found to be responsible for 30%–40% of fossil energy use and 30%–35% of GHG emissions, not an insignificant fraction given that these models represent technology developers’ nth plant performance. Mature technologies which assume lower chemical and enzyme loadings, high enzyme specific activity and on-site production utilizing renewable energy would significantly improve performance. Although the lignocellulosic technologies modeled offer benefits over today’s corn ethanol through reducing life cycle fossil energy demand and GHG emissions by factors of three and six, achieving those performance levels requires continued research into and development of the manufacture of low dose, high specific activity enzyme systems. Realizing the benefits of low carbon fuels through biological conversion will otherwise not be possible. Tracking the technological performance of process conversion materials remains an important step in measuring the life cycle performance of biofuels.

Keywords: biofuels, lignocellulosic ethanol, life cycle assessment, greenhouse gas emissions, cellulolytic enzyme technology, cellulose recalcitrance

1. Introduction

Improving our understanding of the environmental performance of biofuels over their entire life cycles is critical to support technology development, biofuel expansion plans, and life cycle-based policies such as California’s low carbon fuel standard, as well as most importantly, the development of these fuels in a sustainable manner. Over the last two decades, environmental performance has been investigated using life cycle assessment (LCA) or, when referring to transportation fuels and vehicles, ‘well-to-wheel’ (WTW) approaches. The life cycle literature on biofuels has expanded greatly in recent years with the majority of studies and models citing improved performance, with respect to fossil
energy use and GHG emissions, of corn ethanol to a small extent and to a much greater extent, lignocellulosic ethanol compared to gasoline (e.g., [1]). While these studies have included activities throughout the life cycle, the studies have focused on ‘upstream’ agricultural and ‘downstream’ vehicle use activities, with less emphasis on the feedstock to ethanol conversion process. With few exceptions [2–5], studies have not considered the implications of the production and use of chemical and enzyme inputs to the conversion facility.

Ethanol production through biochemical processes utilizes chemicals for pretreatment, hydrolysis, and fermentation. Chemicals required vary depending on the specific processes employed. Enzymes (proteins which catalyze biochemical reactions) assist with liquefaction, saccharification, and fermentation and are also associated with other process benefits in ethanol production [6]. Enzymes have been used for over half a century in corn ethanol production and over the last 25 years their performance has improved and their costs have been reported to have fallen by 70% [6]. These enzymes (amylases) are common, relatively inexpensive and efficiently convert starch to sugar. The same is not the case for currently available enzymes that would be used in converting lignocellulose to ethanol. Lignocellulosic ethanol is undergoing vigorous development although it is still in the pre-commercial stage [7]. It has fewer food versus fuel issues and much larger production potential than ethanol from starch/sugar feedstocks and as well has been cited for its superior environmental performance compared to corn ethanol. However, lignocellulosic feedstock is more challenging than starch/sugar feedstock to convert to fermentable carbohydrates due to the former feedstock’s ‘recalcitrance’. The enzyme catalysts utilized in lignocellulosic ethanol production, ‘cellulase cocktails’, are composed of three groups of enzymes (endoglucanases, exoglucanases, and β-glucosidases) that assist in different mechanisms during hydrolysis. Cellulases are considered to be specialty products due to their small scale of production and that the industry is only a few decades old. Much improvement in cellulase performance is required [8] and their costs remain an impediment to the commercialization of lignocellulosic ethanol [9]. Research and development are ongoing to address these issues.

Corn ethanol, the biofuel produced in the largest quantity in the US, requires small amounts of chemical and enzyme inputs on a per liter (l) basis [10] and LCA analysts have excluded these from most studies. Often analysts employ cut-off criteria [11], to exclude process inputs that are not expected to change the outcome of the analysis. After a review of North American and European LCAs of lignocellulosic ethanol production, to our knowledge, with the exceptions we are aware of noted above, these studies have not included process chemical and enzyme inputs in spite of the higher (compared to corn ethanol) loadings expected to be required for near-term processes. Enzyme loading requirements for commercial lignocellulosic technologies as well as GHG emissions associated with enzyme production are uncertain at this time due, in part, to low specific activities (a measure of enzyme performance per unit of mass) of the cellulase cocktails available today and lack of LCA data on enzyme production.

Overall, LCAs of biofuels have not transparently justified the exclusion of process chemicals and enzymes and many have failed to mention these inputs. Documentation of proposed approaches for implementing LCA to support low carbon (C) fuel standards in several jurisdictions do not discuss the potential importance of chemical and enzyme inputs into the conversion process [12, 13]. The focus on other aspects of the life cycle may be reasonable for current starch/sugar-derived biofuels but in the case of lignocellulosic biofuels a more detailed examination is justified since less is known about the performance of those technologies. Our research therefore aims to address the aforementioned gaps in the literature through developing more transparent life cycle models for lignocellulosic ethanol that identify the contributions of chemical and enzyme inputs and their associated global warming intensities. We examine select promising technologies since several are currently under development, emerging at pilot and demonstration scales [7, 14, 15], and show promise as low C transportation fuels. To attain this objective we;

1. quantify the life cycle energy use and GHG emissions associated with process chemicals and enzymes required to convert corn grain and lignocellulosic feedstocks (the latter for a set of conversion technologies) to ethanol;
2. determine the relative impacts of these inputs compared to those associated with the full life cycle of ethanol production,
3. compare our results with those in the published literature, and
4. discuss the potential of reducing impacts of chemicals and enzymes in the processes,

The results of the research are expected to inform LCA practitioners, the biofuel industry and policy-makers, particularly those developing climate change regulations and low carbon fuel standards.

2. Methods

We constructed life cycle inventory (LCI) models for selected corn and lignocellulosic ethanol conversion pathways assumed to be located in the US. These ‘case studies’ consist of combinations of feedstock, pretreatment/hydrolysis, and fermentation technologies and were chosen because they represent current (corn ethanol) and potential future (switchgrass-based lignocellulosic ethanol) technology combinations as described by Mosier et al [14] and Wyman et al [15]. Further details

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6 Among the lignocellulosic ethanol studies that have considered process chemical and enzyme inputs, only the European EUCAR [2] study disaggregated their results sufficiently to determine the contribution of these inputs to the life cycle (chemical and enzyme inputs represent 45%–62% of fuel production (often termed ‘well-to-tank’ (WTT) activities) GHG emissions for the lignocellulosic ethanol process modeled). EUCAR [2], however, did not disaggregate results sufficiently to determine the potentially important contribution of enzymes. Among the North American and European studies that include process chemical and enzyme inputs, [2–5] assumed the use of a single conversion technology, that based on a dilute acid pretreatment system under development at the National Renewable Energy Laboratory (NREL), although the specific technology parameters modeled varied based on the feedstock selected and the status of the technology assumed.

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on the feedstock, pretreatment and hydrolysis/fermentation technologies, including specifications of the lignocellulosic case studies, are provided in Spatari [16]. In this paper we summarize the modeling of the case studies but focus on the development of the process chemical and enzyme input aspects of each of the models. The paper examines the fuel production and distribution stages of the life cycle (well-to-tank, WTT) but as well discusses the WTT implications. The metrics studied are fossil energy consumption and GHG emissions. Each metric is normalized to the functional unit, one MJ of ethanol.

Corn ethanol case study. Corn ethanol is typically produced through dry-and wet-milling (biochemical) processes. All recent facilities constructed in the US utilize the former process and therefore it is selected for our study. A grinding pretreatment is followed by the application of a family of enzymes known as amylases to hydrolyze the starch, producing glucose which may then be fermented using commercially available yeast, specifically Saccharomyces cerevisiae (S. cerevisiae). The facilities produce ethanol and an animal feed co-product, most often, dried distillers’ grains with solubles (DDGS).

The dry-milling process from the GREET 1.7 model [17], which does not include process chemical or enzyme inputs, is the starting point of our model. We utilize the default pathways which assumes the use of natural gas as the primary process fuel. We then add cradle-to-gate modules for each of the process chemicals and enzymes to the model based on the required quantities for commercial dry-milling (taken from [10] due to actual industry data being proprietary) and fossil energy input and GHG emissions associated with the chemical and enzyme production. Our case study assumes that these inputs are purchased from industrial producers as is current practice and that existing methods of production are utilized. North American data were developed or utilized whenever possible, however, due to data availability, in a small number of cases data are from outside of North America. For example, data for the production of the enzymes are taken from a recent assessment by Novozymes [18] for their industrial scale manufacture in Denmark. Because enzyme manufacture is a specialty industry and no other data are available, we utilize the Danish data. Table 1 shows the chemical and enzyme loading specifications and data sources for the corn ethanol and other case studies and table A1 in the appendix shows the fossil energy input and GHG emissions (and data sources) associated with the production of the inputs for all case studies.

Lignocellulosic ethanol case studies. Three ethanol production case studies are developed, all assume switchgrass as the feedstock. Two of the case studies were selected based on their promise for near/mid-term commercialization and the third, viewed as a ‘realistically optimistic’ mature technology, was selected in order to examine potential future performance of the industry. The selection of technologies was also driven by data availability due to the proprietary nature of most industry data. Figure A1 given in the supplementary information (available from stacks.iop.org/ERL/4/014001) and discussion following the figure illustrate the generic conversion steps for the biochemical conversion of lignocellulose to ethanol. The first case study, noted as DA–SSCF, uses a dilute acid pretreatment process developed by NREL in combination with simultaneous saccharification and co-fermentation (SSCF) hydrolysis and fermentation processes. The second, AFEX–SSCF, uses the ammonia fiber explosion (AFEX) pretreatment process developed by Bruce Dale and colleagues at Michigan State University, in combination with SSCF. Both pretreatment technologies are considered promising for commercial development according to Wyman et al [15]. The primary process chemicals utilized in the NREL dilute acid pretreatment system are sulfuric acid and lime and in the AFEX system, ammonia. These first two case studies

| Table 1. Chemical input specifications for ethanol conversion processes. (Notes: SSCF = simultaneous saccharification and co-fermentation, AFEX = ammonia fiber explosion, CBP = consolidated bioprocessing, diammonium phosphate ((NH₄)₂HPO₄) is abbreviated as DAP) |
|---------------------------------|------------------|------------------|
| Conversion process | Chemical | Chemical input (kg/dry metric ton feedstock) | Assumptions and data sources |
|---------------------|----------|---------------------------------|-----------------|
| **Starch-based technology:** | | | |
| Corn dry-milling | NH₃ | 2.1 | Chemical loading specifications taken from [10]. |
| | NaOH | 5.2 | |
| | CaO | 1.2 | |
| | Enzymes: | | |
| | alpha-amylase | 0.8 | |
| | gluco-amylase | 1.1 | |
| **Lignocellulosic technologies:** | | | |
| DA–SSCF | H₂SO₄ | 26.0 | Chemical loading specifications for H₂SO₄, cellulase and DAP taken from [19]; specifications for Ca(OH)₉ taken from [20]. |
| | Ca(OH)₉ | 29.0 | |
| | Cellulase | 9.2 | |
| | DAP | 1.9 | |
| AFEX–SSCF | NH₃ | 20.0 | Chemical loading specifications taken from [21, 22]. |
| | Cellulase | 9.6 | |
| | DAP | 2.2 | |
| Advanced AFEX–CBP | NH₃ | 8.1 | Chemical loading specifications taken from [23, 24]. |
| | DAP | 2.2 | |
are of near/mid-term lignocellulosic ethanol technologies operating as ‘nth’ plants as specified by the technology developers. We assume process chemicals are purchased from industrial producers (that they are produced using conventional feedstocks and energy supplies). For example, the ammonia is agricultural-grade produced via the Haber process using a natural gas feedstock, and is representative of ammonia sold in the US. Cradle-to-gate modules for each of the process chemicals and fossil energy input and GHG emissions associated with their production are included in the case studies (see table A1 given in the supplementary information (available from stacks.iop.org/ERL/4/014001)).

We develop an enzyme production model based on that specified by Sheehan et al [24], which was derived from an earlier material balance by Wooley et al [25], and is also applied in the process design by Aden et al [20]. The cellulases are assumed to be purchased from enzyme manufacturers who use submerged fermentation (SmF) technology, the current commercial process for cellulase production and the technology assumed by Aden et al [20] (for additional details on the SmF process see Zhuang et al [26]). The enzyme production facility is assumed to be co-located with the conversion facility (through licensing agreements with the enzyme producer). The cellulase cocktail (which includes endoglucanases, exoglucanases, and β-glucosidases) is produced in a unit operation that takes a fraction of the pretreated hydrolyzate (from ethanol conversion) along with other nutrients. The enzymes are produced using the fungus *Trichoderma reesei*, the dominant cellulase systems for industrial processing have come from this fungus (see [5, 20]). Process energy for the enzyme unit operation is assumed to be supplied by the US national electricity grid. The cellulase requirement for SSSF (and applied in the near-term lignocellulosic ethanol models) is modeled at 12 filter paper units (FPU)/g of cellulose as assumed in the nth plant designs by Aden et al [20] and Wooley et al [25] and a specific activity of 485 FPU/g cellulose is assumed based on NREL [19] and Eggeman [22]. Specific activity is the number of enzyme units contained in a given mass or volume of enzyme and determines the quantity of enzyme required to convert cellulose to glucose.

The final lignocellulosic ethanol case study models advanced AFEX–CBP, which is viewed as a mature technology. In our analysis AFEX–CBP represents the closest attainment of theoretical yields and highly advanced performance of biochemical conversion as envisioned by experts in metabolic engineering [27, 28]. It assumes an advanced switchgrass feedstock, higher in cellulose and hemicellulose composition than that used in the near-term models and an advanced AFEX pretreatment process in combination with consolidated bioprocessing (CBP), the latter a technology under development by Lee Lynd and colleagues at Dartmouth College. In CBP, enzyme production, hydrolysis, and fermentation are all assumed to be on-site (‘in situ’) and to utilize part of the biomass feedstock for process energy. We do not distinguish energy and GHGs associated with enzyme production in the CBP process as it all originates as energy from the biomass feedstock. As in the near-term case studies, process chemicals other than enzymes, are assumed to be purchased from industry and produced using conventional feedstocks and energy sources. The main chemical input to the advanced AFEX–CBP system is ammonia, however, the loading is less than half what it is for the near-term AFEX system due to recent experimental results reported by Sendich et al [29], who found that ammonia to dry biomass loadings of 0.3:1, reduced from 1:1 in the near-term model, do not compromise the sugar generation efficiency in pretreatment. The facility design data for the lignocellulosic ethanol conversion processes were provided by NREL [19], Eggeman [22] for Michigan State University, and Laser et al [23] for Dartmouth College, and were used to construct the LCI models. Further details on the technologies can be found in Spatari [16].

The switchgrass production data and farm to conversion facility transportation data for our case studies are taken from GREET 1.7 [17] due to this model being the most widely cited and transparent of the WTW models available for the US. The near-term and advanced AFEX–CBP case studies assume 2000 and 5000 dry metric ton per day facilities, respectively, with the latter assuming an associated larger transport radius modeled by Wu et al [30].

3. Results and discussion

3.1. Well-to-tank fossil energy consumption and GHG emissions

Figure 1 presents WTT results for fossil energy consumption and GHG emissions for the ethanol case studies. For transparency, the results do not include credits for co-product production although we discuss the implications of credits later in the paper. Similar to other studies, the results show the significantly better fossil energy and GHG emissions performance (on the order of 65%–70% improvement) of the lignocellulosic case studies compared to the corn ethanol case study. Additionally, the advanced AFEX–CBP model shows improved performance (40%–50% reduction in energy use and GHG emissions) compared to the near-term lignocellulosic ethanol case studies.

Figure 1 shows the contribution of chemical and enzyme inputs to WTT metrics. In the case of corn ethanol, these inputs make up a small fraction, only 3%, of both fossil energy input and GHG emissions. For the advanced AFEX–CBP technology, chemical and enzyme inputs are projected to make up 16% and 11% of fossil energy use and GHG emissions, respectively. However, these contributions on a MJ of ethanol produced basis are similar to those of the inputs for corn ethanol. The higher contribution percentages for the AFEX–CBP inputs make up a small fraction, only 3%, of both fossil energy and GHG emissions. For the advanced AFEX–CBP inputs to WTT metrics. In the case of corn ethanol, these inputs make up a small fraction, only 3%, of both fossil energy input and GHG emissions. For the advanced AFEX–CBP technology, chemical and enzyme inputs are projected to make up 16% and 11% of fossil energy use and GHG emissions, respectively. However, these contributions on a MJ of ethanol produced basis are similar to those of the inputs for corn ethanol. The higher contribution percentages for the AFEX–CBP technology are due to the overall improvement (six-fold reduction) in expected fossil energy and GHG emissions for this technology compared to that of corn ethanol. In the near-term lignocellulosic ethanol case studies, the production of chemical and enzyme inputs requires between 30% and 40% of fossil energy input, and is responsible for between 30% and 35% of GHG emissions (approximately 9 g CO₂ eq./MJ each out of a total of 29 and 27 g CO₂ eq./MJ for NREL–SSF and AFEX–SSF, respectively), which are not
insignificant, particularly given that these models represent technology developers’ estimates of nth plant performance. However, when comparing projected near-term lignocellulose-based technologies with today’s corn ethanol, fossil energy input and GHG emissions are lower by a factor of three for the former technologies.

3.2. Contribution of individual chemicals/enzymes to ethanol production: GHG emissions and comparison with literature

Table 2 shows the individual chemical and enzyme contributions to the GHG emissions associated with our four ethanol case studies (designated as Spatari and MacLean), and compares these with select pathways from literature, namely those from the Canadian GHEGenius 3.9c model by NRCan [4] and the European LBST model by EUCAR et al [2]. While NRCan [4] reports corn dry-mill and switchgrass SSCF models which we include in the comparison, EUCAR et al [2] does not. To facilitate a comparison of the pathways modeled in EUCAR (wood and wheat straw feedstocks in combination with SSCF), we include a wood (farmed hardwoods or softwoods) SSCF pathway from NRCan [4].

NRCan [4] derives chemical and enzyme input specifications from Aden et al [20] and Nielsen et al [18], both are assumed to be produced off-site. The EUCAR et al [2] WD–NREL–SSCF pathway derives chemical input specifications from Wooley et al [25], and the WS-Iogen-SSCF pathway takes input specifications from private industry (Iogen Corporation) and from Wooley et al [25]. The EUCAR et al [2] pathways assume on-site production of enzymes; therefore, all energy inputs and emissions associated with chemicals used for enzyme manufacture are aggregated into each chemical input, and because the facility uses steam and electricity produced on-site from the combustion of residues from the feedstock, those GHGs do not factor into the total GHG emissions. As documented by [2], only sulfuric acid inputs were specified by Iogen for the wheat straw model, and at a lower input ratio than in the wood model; EUCAR et al [2] assumed input of other chemicals listed as specified by [25], but in proportion to the lower sulfuric acid requirements. We cannot comment on enzyme manufacture by Iogen because public information is not available. With respect to the enzyme production assumptions of Wooley et al [25], our enzyme production module is based on that specified by [24], which was derived from the original material balance in [25], and therefore assumptions should be identical in the EUCAR et al study and in ours (Spatari and MacLean). However, the impact of the differing feedstocks assumed in the studies should be noted as woody biomass is higher in cellulose and slightly lower in xylan than switchgrass, furthermore, pretreatment and hydrolysis sugar yields vary between woody and herbaceous biomass. Both of these factors contribute to differences in overall ethanol yields between the two feedstocks, which impact life cycle metrics.

Table 2 shows that the production of the chemicals and enzymes required for our corn dry-mill, AFEX SSCF, DA SSCF and AFEX CBP case studies result in 1.6, 9.3, 9.8, and 4.1 g CO2 eq./MJ (53, 192, 194 and 35 g CO2 eq./l) of ethanol produced, respectively. Enzymes comprise the majority of chemical- and enzyme-related GHG emissions in the model of corn dry-milling. For our near-term lignocellulosic case studies, enzymes and ammonia (in similar proportions) are responsible for the majority of GHG emissions for the AFEX process; and enzymes and lime (also in similar proportions) for the NREL process. In the future, if mature AFEX CBP technology develops as proposed [23], ammonia would be the dominant chemical contributing to GHG emissions during ethanol conversion, although its contribution would be much smaller than it is in the near-term AFEX case study.

Compared to our case study of the corn ethanol pathway, NRCan [4] reports slightly higher GHG emissions due to chemical and enzyme inputs 4.1 g CO2 eq./MJ versus 1.6 g CO2 eq./MJ (87 versus 35 g CO2 eq./l). This difference primarily results from the higher contributions reported by NRCan related to ammonia and enzymes which are based on year 2003 operating data from a commercial alcohols plant in Chatham, Ontario, Canada [31]. It should be noted that this facility is not a standard dry-mill facility as it uses...
continuous fermentation, which results in additional inhibitory by-products, and therefore a lower ethanol yield compared to facilities using batch fermentation processes. While our enzyme loading assumption is the same as that of NRCan to facilities using batch fermentation processes. While our by-products, and therefore a lower ethanol yield compared to facilities using continuous fermentation processes. Our enzyme contribution is assumed to be 4 g CO2 eq., whereas in our near-term lignocellulosic case studies on a mass basis (1.7 g enzyme/kg feedstock), NRCan assumes a higher global warming intensity (GWI) factor for the production of alpha and gluco amylases as well as slightly lower ethanol yields.

Differences between the NRCan pathways and ours (and that of EUCAR et al [2]) are substantial in the cases of the near-term lignocellulosic ethanol pathways. NRCan reports emissions of 56 and 54 g CO2 eq./MJ associated with chemical and enzyme inputs to the switchgrass and wood SSCF pathways, respectively. These results are approximately six times greater than those corresponding to our case studies and 6–10 times greater than EUCAR et al [2] pathways. GHG emissions associated with the inputs of sulfuric acid and ammonia for the NRCan switchgrass model, as well as those associated with lime, sulfuric acid, and ammonia for their wood model are of the same order of magnitude as estimates of these chemicals’ contributions in our AFEX and DA case studies, respectively. These estimates are also generally the same order of magnitude as those of EUCAR et al [2]. Enzymes are by far the largest contributor to GHG emissions in the lignocellulosic pathways of NRCan [4]. The enzymes alone are reported to be responsible for approximately 50 g CO2 eq./MJ (more than 1000 g CO2 eq./t) of ethanol produced whereas in our near-term lignocellulosic case studies enzyme contributions are no more than 4 g CO2 eq./MJ (80 g CO2 eq./t). Although EUCAR et al [2] did not disaggregate their enzyme-GHG contributions, our comparison suggests they are on par with ours because we used the same data for chemical requirements.

The significant differences in the contributions of enzymes in the studies lead to our further analysis of enzyme parameters and ethanol conversion yields (see table 3). For the lignocellulosic pathways, compared to values in our case studies, NRCan assumes a higher enzyme loading, 41–45 g enzyme/kg feedstock based on a specific activity of 70 FPU/g cellulase compared to our loading of 9–10 g enzyme/kg feedstock (based on a much higher assumed specific activity, 485 FPU/g cellulase) as well as a higher GWI factor; 8000 g CO2 eq./kg enzyme compared to our value of 2300 g CO2 eq./kg enzyme. Data on enzyme manufacture applied in NRCan’s wood and switchgrass models are from (S&T)2 [32] which based their assumptions on the properties of cellulase cocktails they anticipated for the near-term and reflect those that are currently commercially available. Additionally, in citing an earlier version of Nielsen et al [18], (S&T)2 [32] selected a GWI factor on the higher end within a plausible expected range of 1000–10000 g CO2 eq./kg enzyme due to the higher costs of production for fungal-based enzymes like cellulases (although the GWI depends on the technology and energy inputs used to produce the enzymes and is not directly related to the cost of production). Nielsen et al [18] do not include a cellulase cocktail among the five enzymes studied, however, due to the lack of life cycle studies of enzymes and the fact that the cocktails are not now produced for commercial scale purposes, the assumption of utilizing a conservative value from this range may be justified. As well, there is still much uncertainty regarding the commercial scale production of these enzyme cocktails; their performance and associated GHG emissions. The decision by NRCan to base their enzyme production parameters on cellulases available today rather than on estimates of performance of enzymes anticipated for n-th plants is the primary source of the difference between their and our results, and the enzyme-related GHG emissions of NRCan being more than 13 times higher than those in our case studies.

### Table 2

| Model          | Spatari and MacLean | NRCan [4] | EUCAR et al [2] |
|---------------|---------------------|-----------|-----------------|
| Feedstock     | Process             |           |                 |
|               | Corn                | SG        | SG              | WD               | WS               |
|               | dry-mill            | SSCF      | SSCF            | SSCF             | SSCF             |
| Enzymes       | 1.1                 | 0.13      | 0.15            | 1.9              | 0.14             |
| Sulfuric acid | 0                   | 0.59      | 0.59            | 0.18             | 0.28             |
| Lime          | 0.17                | 0         | 0.4             | 0                | 2.8              |
| Ammonia       | 0.4                 | 5.4       | 0.22            | 2.0              | 4.3              |
| Nutrients     | 0                   | 0.21      | 0.22            | 0.05             | 0.03             |
| Yeast         | 0                   | 0         | 0.05            | 0.05             | 0                |
| Sodium hydroxide | 0               | 0         | 0.03            | 0                | 0.03             |
| CSL           | 0                   | 0         | 0.03            | 0.16             | 0.03             |
| Total GHG emissions | 1.6          | 9.3       | 9.8             | 1.9              | 4.1              |
|               | 9.3                 | 9.8       | 1.9             | 56               | 54               |

Table 2. Contributions of individual chemical and enzyme inputs used in ethanol conversion to GHG emissions and comparison with estimates from literature (all values in g CO2 eq./MJ of ethanol). (Note: DA = dilute sulfuric acid; SG = switchgrass; WD = wood; WS = wheat straw; lime applied in the corn dry-milling process is CaO; lime applied in the sg NREL SSCF process is hydrated lime [Ca(OH)2]; CSL = corn steep liquor; for nutrient application, Spatari and MacLean case studies assume use of diammonium phosphate [(NH4)2HPO4] in lignocellulosic processes; NRCan [4] assumes use of phosphate nutrients (P2O5); and EUCAR [2] assumes use of ammonium sulfate [(NH4)2SO4]; all GHG emissions associated with enzyme manufacture in the EUCAR et al [2] case study are divided among the chemical inputs to the facility shown in the above table since enzymes are assumed manufactured on-site.)
Table 3. Comparison of GHG emissions related to enzyme inputs in current and near-term ethanol conversion processes. (Notes: SG = switchgrass; DA = dilute acid pretreatment systems; AFEX = ammonia fiber explosion pretreatment system; SSCF = simultaneous saccharification and co-fermentation; CBP = consolidated bioprocessing. Spatari and MacLean are pathways developed in this study. NRCan uses data from [29]. FPU = international filter paper unit. GWI = global warming intensity; N/A = not available. The lower heating value for ethanol is utilized to convert values from a /MJ basis to a /l basis and then values are rounded.)

| Model   | Spatari and MacLean | Spatari and MacLean | Spatari and MacLean | NRCan | NRCan | NRCan |
|---------|---------------------|---------------------|---------------------|--------|--------|--------|
| Feedstock Process | Corn dry-mill | SG AFEX SSCF | SG DA SSCF | Corn dry-mill | SG DA SSCF | WD DA SSCF |
| Ethanol yield: l/dry metric ton | 403 | 306 | 270 | 395 | 344 | 317 |
| Enzyme specific activity: FPU/g enzyme | N/A | 485 | 485 | N/A | 70 | 70 |
| Enzyme loading: g enzyme/kg feedstock (kg enzyme/l ethanol) | 1.7 (0.004) | 10 (0.03) | 9 (0.03) | 2.0 (0.005) | 45 (0.13) | 41 (0.13) |
| Enzyme GWI factor: g CO2 eq./kg enzyme | 4847 | 2264 | 2264 | 8000 | 8000 | 8000 |
| Enzyme production GWI: g CO2 eq./MJ ethanol | 1 (21) | 3 (71) | 4 (77) | 2 (40) | 49 (1040) | 49 (1040) |

factor of (S&T)² [32] (8000 g CO2 eq./kg enzyme instead of 2264 g CO2 eq./kg enzyme). Scenario 2 assumes the lower enzyme specific activity of (S&T)² [32] (70 FPU/g cellulase instead of 485 FPU/g cellulase as assumed in our case studies) and associated increased enzyme loading to 55 g enzyme/kg feedstock. Scenario 3 assumes both the low specific activity (70 FPU/g cellulase) and the high emissions factor (8000 g CO2 eq./kg enzyme). All other parameters were held constant at their original values. Figure 2 compares the results of this sensitivity analysis (the GHG emissions associated with the enzyme input) with our base case results (3 and 4 g CO2 eq./MJ for AFEX SSCF and DA SSCF, respectively) as well as the case for NRCan’s SG model (49 g CO2 eq./MJ). Scenario 1 (higher GWI factor) results in a modest increase in GHG emissions, to 12 and 13 g CO2 eq./MJ (for AFEX SSCF and DA SSCF, respectively) due to the higher GWI of the enzyme. Scenario 2 (lower specific activity), results in an increase in enzyme-related GHG emissions to 19 (AFEX SSCF) and 22 (NREL SSCF) g CO2 eq./MJ, more than five times the emissions of the base cases, and also higher than Scenario 1. As expected, Scenario 3 (higher GWI and lower specific activity) results in the highest emissions, 67 and 76 g CO2 eq./MJ for AFEX SSCF and NREL, respectively, 20 times greater than the base case. Moreover, Scenario 3 emissions are higher than those of NRCan [4] due to the lower ethanol yields in our case studies. Uncertainty in enzyme performance characteristics warrants further attention.

3.4. Well-to-wheel analysis of the ethanol case studies

Examining the full life cycle (WTW) and not just WTT activities is necessary to understand progress that could be made toward the overall goal of improving the environmental performance of light-duty vehicle (LDV) transportation. We briefly discuss the implications for WTW results of including chemical and enzyme inputs in the case studies examined. Assuming the use of E100 (neat ethanol) in a LDV as specified by [35], for the near-term lignocellulosic ethanol case studies, these inputs would be responsible for 26–28 g CO2 eq./km driven of the WTW’s 76–84 g CO2 eq./km (33%–35%). For the mature technology case study, these inputs would be responsible for 5 g CO2 eq./km out of the WTW’s 42 g

Figure 2. Scenario analysis varying enzyme specific activity and loading. Notes: in the base scenario we assume an enzyme loading, specific activity, and enzyme GWI factor of 9–10 g enzyme/kg feedstock, 485 FPU/g cellulase, and 2300 g CO2 eq./kg enzyme, respectively. The three scenarios examine the following: Scenario 1 assumes enzyme loading, specific activity, and enzyme GWI factor of 9–10 g enzyme/kg feedstock and 485 FPU/g cellulase, 8000 g CO2 eq./kg enzyme, respectively; Scenario 2 assumes 55 g enzyme/kg feedstock, 70 FPU/g cellulase, and 2300 g CO2 eq./kg enzyme, respectively; Scenario 3 assumes 55 g enzyme/kg feedstock, 70 FPU/g cellulase, and 8000 g CO2 eq./kg enzyme, respectively; sg = switchgrass; AFEX = ammonia fiber explosion; DA = dilute acid; SSCF = simultaneous saccharification and co-fermentation.
CO₂ eq./km (13%). Still, comparing these WTW figures to a functionally equivalent gasoline vehicle (252 g CO₂ eq./km), the total WTW GHG emission reduction is on the order of 65%–85% for near-term and mature technologies. Vehicles able to use up to 85% ethanol by volume (E85) are sold today in the US, but not those able to use E100. The above calculations make simplifying assumptions so as to not complicate the results by introducing emissions associated with the gasoline blending component required for E85. A fuel consumption of 13.4 l ethanol/100 km is assumed for the E100 and in-use GHG emissions factors as in [35]. As ethanol-optimized engines are not in commercial production, Spatari et al [35] did not take into consideration ethanol’s higher octane value compared to that of gasoline, which could potentially result in greater efficiency of an ethanol- versus a gasoline-optimized engine.

The above calculations illustrate that biofuel conversion process requirements can be significant contributors to near-term lignocellulosic ethanol WTW GHG emissions; life cycle evaluations must take into consideration specific technology requirements for each specific biofuel conversion technology employed, whether sugar or thermochemically based. However, the high WTW GHG emissions of gasolinefueled LDVs and potential for much lower emissions of lignocellulosic ethanol vehicles have lessened attention to ethanol production process inputs. In addition, the majority of ethanol WTW studies have identified significant fossil energy and GHG emissions benefits associated with the production of co-products of corn and lignocellulosic ethanol through including associated credits. Our WTT results did not include co-product credits. In the case of lignocellulosic ethanol, when an electricity co-product credit is included, which assumes that excess electricity can be sold to the grid, the impacts of process chemicals and enzymes (if they have been included) have often been shown within an aggregate energy use or emissions value representing ethanol conversion. This value is often negative (beneficial) due in large part to the magnitude of the co-product credit and therefore, again, likely for this reason, little attention has been given to the process inputs. The large credit results from the common assumption in US models that electricity production through a primarily fossil fuel-based mix (such as the US national grid) is displaced by the biobased electricity.

4. Enzyme technology impacts on the ethanol life cycle

Making reasonable estimates of enzyme performance to include in life cycle models is challenging due to the lignocellulosic ethanol processes not being at commercial scale, the ongoing development of enzymes, and the proprietary nature of the data involved. Enzyme specific activities vary from manufacturer to manufacturer (and until recently, manufacturers had not published the specific activities of the cellulases they were developing) and dosages used in hydrolysis also vary among laboratories testing different substrates. Although the specific activity assumed in our case studies is considerably higher than those of cellulases available currently, the most recent NREL design [20] specifies an even higher specific activity (600 FPU/g cellulase); as well, T. reesei cellulases with activities reported in this range have been documented by others (e.g., [33]). On the other hand, Genencor’s [34] recently announced ‘next-generation’ cellulase cocktail, Accelerase 1000, is described by the manufacturer to be an important formulation for scaling up lignocellulosic biomass processing, and, in spite of advances made with this formulation, it requires a loading of 0.1–0.5 g enzyme/g cellulose (approximately 32–160 g enzyme/kg feedstock); high in both cost and GHG emissions if produced using current conventional energy inputs. An additional complicating factor in predicting enzyme impacts is that a further mixture of enzymes, such as one including xylanases may be necessary for improving hemicellulose sugar yields; these have not been included in any LCA study of lignocellulosic ethanol to date. Lastly, there are tradeoffs between enzyme and solvent/chemical use in ethanol production; increased solvent use during pretreatment decreases structural and compositional obstacles to hydrolysis, therefore potentially lowering enzyme requirements. Research and development are ongoing to; examine beneficial tradeoffs between enzyme and chemical use, improve enzyme production processes, increase enzyme specific activity, locate new highly active enzymes, and investigate the potential for enzyme recycle.

In focusing our analysis on process chemicals and enzymes applied in the conversion stage of selected ethanol production processes, we address a gap in scientific knowledge associated with the environmental implications of ethanol. We note key limitations of our study. Although we use the best available data at the time of the study, we include only a limited set of conversion systems and technology improvements may have occurred since the time of data collection. Technologies not studied may have lower (or higher) chemical and enzyme inputs which could affect the conclusions. For example, some pretreatment methods (e.g., hot water, autohydrolysis and organosolv) are expected to require no or very low chemical inputs. There is significant uncertainty surrounding chemical loadings and enzyme specific activities, loadings and production to be used for anticipated commercial ethanol facilities. Our analysis did not examine the potential of production of enzymes and chemicals utilizing alternative technologies under development or utilizing low carbon energy sources for enzyme production. Zhuang et al [26] examine costs for an alternative enzyme production technology that uses solid state cultivation (SSC) as opposed to submerged fermentation (SmF), the technology assumed in [20] and in our near-term case studies. SSC is a technology widely used, for example to produce fermentative foods, but is not used currently at commercial scale for enzyme production. Based on their cost estimates, enzymes produced via SSC are estimated to have a significantly lower production cost ($15.67/kg in 2004 US $) compared to those produced by the SmF method ($40.36/kg in 2004 US $). Both values

7 The SSC production method is expected to use less energy and emit lower GHG emissions than the SmF process because it produces a more concentrated enzyme in a non-aqueous medium, thus reducing product refining costs. However, the authors note several technical challenges to be overcome in the process. How the technologies will develop in the future is uncertain. Results should be interpreted with these considerations in mind.
are well below the market price of $90/kg cited by [26]. Combining the enzyme costs from Zhuang et al with the enzyme load parameters from our AFEX SSCF case study and the GHGenius [4] SG SSCF literature case, the enzyme costs (in $/l of ethanol) are estimated as: 2.82, 1.27, and 0.49 for AFEX SSCF and 11.70, 5.25 and 2.04 for GHGenius SG SSCF for the market price, SmF, and SSC technologies, respectively. No matter which of the enzyme cost estimates is assumed, all are prohibitively high and all are higher than NREL’s target ethanol production cost of $1.07/gal ($0.28/l) [20] and costs projected by [36]. For additional details of the cost data, see table A2 given in the supplementary information (available from stacks.iop.org/ERL/4/014001).

Most widely cited life cycle models of biofuels have not inventoried enzyme or chemical inputs, but have instead assumed they would be used in sufficiently small quantities so that they would not significantly impact life cycle metrics. This is a reasonable assumption for corn dry-milling as fossil energy use and GHG emissions associated with these inputs are found to be small in comparison with those associated with the process fuels currently utilized in these facilities. Considering the high costs associated with high enzyme and chemical loadings, the assumption may also be reasonable for lignocellulosic processes as these will not be commercialized unless industry cost targets are met. Mature technologies which have low input chemical requirements and utilize on-site (in situ) enzyme production would result in low GHG emissions (assuming necessary advances for these technologies are realized). In contrast to the corn and mature lignocellulosic ethanol pathways, for near-term technologies, we find that chemical and enzyme inputs could contribute significantly to life cycle fossil energy use, GHG emissions, and cost. Enzymes are the most significant input to the conversion processes modeled, due to both the loadings required and the fossil energy use and GHGs emitted during enzyme production. For the case studies examined, even with advances expected for nth plants, chemical and enzyme inputs may require between 30% and 40% of fossil energy and account for 30%–35% of GHG emissions. The difference between the enzyme contributions reported in our case studies (approximately 4 g CO₂ eq./MJ ethanol) which are based on nth plant values and those of (S&T)² [32] (approximately 50 g CO₂ eq./MJ ethanol) which assume current enzyme characteristics is notable and warrants further attention. This difference points to the fact that bioethanol conversion technology and material inputs are not minor components of the life cycle given the state of these technologies. As we have described, pretreatment chemical requirements and enzyme loadings depend upon the conversion process selected. For lignocellulose-based feedstocks, some technologies offer lower inputs than others, but all require chemicals and enzymes at this time. These inputs should be inventoried as part of biofuel and low C fuel LCA standards. In order to do so, improved data are needed from enzyme producers on enzyme performance and production characteristics, as well as insights related to expected industry technological developments. This information must be made available to LCA analysts while respecting industry competitiveness, potentially through blending of various manufacturers’ data.

A future research step that would add to our findings is a detailed stochastic analysis of enzyme usage for different biomass substrates. Such an analysis needs to consider feedstock species and composition, pretreatment technology used, and probability distributions that adequately capture enzyme specific activities, loads, and GWI. Those probability distributions should be informed by laboratory (and larger, ideally) scale measurements in order to understand the variability among different technologies and feedstocks. At this time publicly available information only is sufficient to inform a scenario analysis of GHG sensitivity to enzyme variables (figure 2). The results of our analysis reinforce what researchers already know about necessary technological advances for making low C bioethanol. What we find in addition is that the technologies themselves need much improvement in their overall GWI if lignocellulose-based fuels are to in fact become low C fuels.

It is challenging to predict the impact of process chemicals and enzymes in future commercial applications. With the dynamic nature of current investigative research in lignocellulose-to-ethanol conversion, inputs are likely to decline through technology development. Looking across the entire life cycle, it may be more straightforward to achieve reductions of impacts of the conversion process than those for example, related to feedstock production. A conversion facility is a relatively controlled setting compared to fields where biomass is grown. The latter involves many complex natural and industrial process parameters and as well, spatial variation with different climates. Therefore, impacts related to chemicals and enzymes may have less uncertainty and reductions may be achieved more readily. On the other hand, those improvements have yet to be made, introducing another component of uncertainty, and tested at pilot scale.

In spite of the impact of process chemical and enzyme inputs required for converting lignocellulose to ethanol, the WTW performance of the fuel is expected to be significantly better than that of corn ethanol and crude-oil-derived gasoline/diesel as specified in projected Aspen nth plant facility designs. The difficulty is that those process designs have not yet been shown to perform as such. Enzyme production technology improvements combined with lower chemical loadings and increased recovery rates are critical, however, for improving the environmental and cost performance of biochemical conversion processes.

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