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Influence of cellulase enzyme production on the energetic–environmental performance of lignocellulosic ethanol

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ABSTRACT

Lignocellulosic ethanol is considered as a good alternative to increase the current amount of first generation ethanol produced, since it does not compete for land with food production. Biochemical technological routes have high potential to be adopted for lignocellulosic ethanol production, in which hydrolysis processes demand cellulase enzymes to convert cellulose and hemicellulose materials into fermentable sugars. Thus, an integral evaluation of lignocellulosic ethanol requires knowledge of the energetic–environmental costs of production of enzymes, which generally occurs off-site of biorefinery plants in specialized companies. The aims of this work are to assess the energetic–environmental costs of cellulase enzyme production and verify their influence on the lignocellulosic ethanol overall cost. For this, the production of industrial enzyme operating under submerged fermentation process was assessed by (i) emergy accounting, (ii) embodied energy analysis, (iii) and emission inventory. Monte Carlo simulation was used for uncertainty analysis. Results show that cellulase enzyme demands 4.06E14 seJ/kg_{enzyme} and 1664 MJ/kg_{enzyme} with a global warming potential of 21.90 $kgCO_{2\text{-eq}}/kg_{enzyme}$. These values are equivalent to 0.41%, 0.49%, and 0.02%, respectively, of the emergy, embodied energy, and global warming potential of lignocellulosic ethanol production, making their contribution to the overall energetic–environmental costs negligible when compared to other input resources of the ethanol production chain such as limestone and diesel.

1. Introduction

Liquid fuel for transportation purposes produced from vegetal biomass has received huge attention in recent years. This fuel type is generically referred to as biofuel. The International Energy Agency (2004) estimates that the presence of biofuel in the transportation sector (which currently consumes about 57% of the fossil energy used worldwide) will have increased from 1% to 7% by 2030. Such scenario displays an increase in consumption in 2004, of around 15.5 million ton of oil equivalent to an estimate of 146.7 million in 2030. According to Cerqueira Leite et al. (2009), Brazil contributed about 33% of the world's ethanol production in 2008, with potential role in fulfilling the future demand for ethanol biofuel. In this context, any strategy adopted for increasing biofuel production must be carefully assessed, for the sake of a net benefit on efficiency and socio-environmental damages reduction. This is especially true when agricultural systems (the vegetal biomass providers to ethanol production) are involved, which could lead to a fuel versus food competition.

Paramount efforts worldwide are being made focusing on technical issues regarding ethanol fuel production from vegetal biomass at lower energetic and economic costs compared to ethanol obtained directly from fermentable sugars. The former is labeled as second generation ethanol, or simply, lignocellulosic ethanol, the main processes involved for which being: the vegetal biomass receiving a thermo-chemical treatment, the resultant material being hydrolyzed, and finally ethanol being obtained from sugars fermentation and distillation. The main reason for supporting such technological route relies on the fact that the vegetal biomass used as raw material is inedible, and does not compete with food production. Furthermore, the conversion of a sub-product of agricultural production into fuel is attractive due to the low economic cost of the main raw material, and the potential reduction of greenhouse gases when compared to fossil fuel

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Nomenclature

AP	acidification potential
$CO_{2\text{-eq.}}$	carbon dioxide equivalents
GWP	global warming potential
PDF	probabilistic distribution function
seJ	solar emjoules
$SO_{2\text{-eq.}}$	sulfur dioxide equivalents
SmF	submerged fermentation process
SSC	solid state cultivation
UEV	unit emergy value

combustion. Wyman (2007) argues that lignocellulosic ethanol is finally being widely recognized as the only existing transport fuel with powerful economic, environmental, and strategic attributes.

The hydrolysis process used for lignocellulosic ethanol production usually demands enzymes. Enzymes are currently used for obtaining several different products, such as pharmaceuticals, chemicals, food-derivate products, and new applications are constantly in development. Due to modern biotechnology advances, enzymes today can be developed and used in processes where one has never thought they could be applied. Enzymes are effective catalysts and using them often results in significant reduction of water and energy demand, and increase of economical and environmental performance of production processes. By recognizing that the Earth is a system dependent on storage of ancient resources and is currently working over its carrying capacity, the enzyme technologies have potential as powerful alternatives to overcome the challenges that industries will have to face (Kirk et al., 2002). Under that perspective, Wyman (2007) emphasizes that lignocellulosic ethanol should be favored by policies regarding liquid fuel production, because it uses the knowledge–power from biotechnology to drastically reduce the production costs, besides all the other above mentioned advantages. As an example, comparing a conventional sugarcane Brazilian ethanol plant with a biorefinery scenario, Agostinho and Ortega (2013) found a similar value for energy return on investment (EROI) of around 4.5 for both systems, and a slightly better emergy efficiency for the biorefinery (59,900 vs. 72,700 seJ/J_{EtOH} for biorefinery and conventional plant respectively); Felix and Tilley (2009) reported an EROI of 2.62 and emergy efficiency of 110,000 seJ/J for switchgrass in USA, while for corn ethanol, Ulgiati et al. (2011) report values of 1.14 and 189,000 seJ/J for EROI and emergy efficiency, respectively. Concerning gas emission, MacLean and Spatari (2009) report a reduction of CO_2 released from 4847 to 2264 $gCO_{2\text{-eq.}}/kg_{enzyme}$ for corn and switchgrass ethanol, respectively.

Although recognizing that lignocellulosic ethanol production could lead to economic and environmental benefits, there are still some factors restricting its large scale production. According to Zhuang et al. (2007), the estimated economic cost of cellulase enzyme production ranges from 25% to 50% of total lignocellulosic ethanol production cost. Recently, Dias et al. (2012) estimated the influence of enzyme costs (0.11 USD/L_{EtOH}) as approximately 30% of the lignocellulosic ethanol production. A similar value was found by Hong et al. (2013), with an average enzyme cost of 0.12 USD/L_{EtOH}. Wyman (2007) highlights that more than just costly, the cellulase enzymatic activity is still slow and claims for enzymes with higher specific activity so as to increase the reaction and conversion ratios of vegetal biomass into sugars. In contrast, a critical discussion on the energetic and environmental aspects of cellulase production is rarely found in literature, and when it happens to be found (for instance Hong et al., 2013; MacLean and Spatari, 2009) only the direct energy demand and CO_2 emissions are focused on. In such context, some doubts arise: what are the embodied energy demand (i.e., direct and indirect energy) and the environmental load of the industrial production of cellulase enzyme in a global perspective? What is the influence of using cellulase enzyme on the energetic–environmental performance of lignocellulosic ethanol production?

Most studies dealing with the economic, energetic, environmental, and technological aspects of lignocellulosic ethanol production, assume that the cellulase enzyme is produced on-site, within the ethanol plant boundaries (Felix and Tilley, 2009; Kazi et al., 2010; Mu et al., 2010). This approach is used due to the lack of precise information regarding off-site enzyme production. It could be justified by the low investment on material and energy demanded by enzyme production, however, quantitative information supporting this assumption is rarely available. Although acknowledging that enzyme production costs have decreased these last few years, there is still room for development, and the market indicates a tendency towards buying off-site instead of using on-site produced enzymes, at least in short and medium term (Menon and Rao, 2012). As discussed by Hong et al. (2013), the off-site production can be more economically competitive and environmentally effective than on-site production, since it can serve a large number of ethanol plants.

Some studies on the energetic–environmental aspects of lignocellulosic ethanol production considered off-site produced enzymes, albeit accounting them in monetary units (Dias et al., 2012). Notwithstanding, assumptions have to be made in order to account for enzymes in a biophysical basis (Agostinho and Ortega, 2013). The availability of intensity coefficients (of any kind) for cellulase enzyme could make those studies more precise, as with the other studies performed under the life cycle approach (Nielsen et al., 2007; Spatari et al., 2010; Hong et al., 2013).

The aim of this work is to assess the energetic–environmental performance of the industrial production of cellulase enzyme and to verify its pressure on the lignocellulosic ethanol production. To accomplish this, the following three methodologies are used: (i) emergy accounting, (ii) embodied energy analysis, and (iii) emissions inventory.

2. Methodology

2.1. System description and raw data source

There are two usual technological routes for producing enzymes: (i) submerged fermentation (SmF) and (ii) solid state cultivation (SSC). The SSC method, when compared to SmF, consumes less water and demands less direct energy, while co-producing less wastewater and providing highly concentrated enzymes. Those features result in a better cost–benefit relationship for the SSC method. On the other hand, the SSC method has disadvantages related to technical operational issues (for instance heat and mass transfer), thus limiting its application in large scale production. Also, a precise operational control is mandatory during in SSC process so as to avoid the cessation of enzymes production (Zhuang et al., 2007). Independently of the scale and enzyme's further application, the SmF method is the most frequently used one.

The production processes of cellulase enzyme through the SmF method are shown on Fig. 1, in which, according to Zhuang et al. (2007), the main steps are: (1) bacteria *Clostridium thermocellum* initial preparation and transfer from a freezer (−80 °C) to a sterilized shake-flash containing cellulose powder and nutrient-rich aqueous medium; (2) fermenting and transfer of the culture to the seed fermenter #1 (1.56 m^3) where a second fermentation occurs, consuming more cellulose and nutrients previously

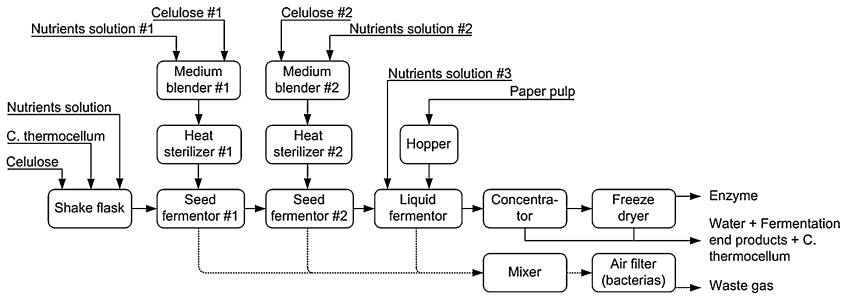

Fig. 1. Schematic representation of main process used for industrial cellulase enzyme production by SmF method.

prepared and sterilized by a blender; (3) culture transfer to the seed fermenter #2 (156.07 m^3) for a third fermentation process and volume increase by consuming larger amount of cellulose and nutrients; (4) finally, all-product transfer into a fourth fermentation process in the fermenter #3 (937.71 m^3), which uses nutrients and paper pulp as carbon source, since paper pulp is cheaper than cellulose. The downstream processes include a concentrator to remove the liquid part of the fermented product, and a freeze dryer used for removing residual liquids, purifying, and storing the cellulase enzyme produced. Nitrogen is added so as to guarantee an anaerobic environment. Water and energy (electricity) are used in all steps. Bacteria present in the gases formed by fermentation processes are retained in filters, preventing their release into the atmosphere.

The raw data used in this research are based mainly on the published works by Zhuang et al. (2007) and Wooley et al. (1999), but the works by Nielsen et al. (2007), Aberer et al. (2002) and Soetaert and Vandamme (2010) are also considered.

2.2. Scientific methods used for assessing the system's energetic–environmental performance

Among all scientific methodologies available to assess the energetic–environmental performance of any production system (for instance ecological footprint, life cycle assessment, materials flow accounting, and so on), three were chosen due to their importance in representing the current concerns about global scale efficiency, fossil fuel shortage, and climate change: (i) emergy accounting; (ii) embodied energy analysis; (iii) global warming potential. Additionally, most studies regarding liquid fuels production consider the energy efficiency, global warming potential, and renewability as indicators, such as the work by Agostinho and Ortega (2013) which is the main reference for our results comparison. All of these methodologies can be used for diagnosis and management by assessing potential improvement scenarios.

2.2.1. Emergy accounting

As opposed to the performance indicators focused on local scale, the global scale indicators cumulatively account for hidden process costs previously used to produce and make available all goods and services used locally. The robust systemic approach aligned with the capacity of accounting for all economic and natural resources recognizing the "quality" of energy, confers to emergy accounting (Odum, 1996) a higher eligibility than to other available tools (Agostinho and Pereira, 2013; Giannetti et al., 2013). Emergy is "the available energy of one kind previously used up, directly and indirectly, to make a service or product" (Odum, 1996). In accordance to the second law of thermodynamics, each transformation process degrades the available energy, while the "quality" of the remaining energy increases. Emergy is a scientific measure of wealth in terms of "resources" virtually embodied in goods and services. Further information on the emergy methodology, rules, and meanings, can be found in Odum (1996) and Brown and Ulgiati (2004).

Before one performs an emergy evaluation, the system under study must be represented by a detailed energy diagram using the symbols proposed by Odum (1996). All flows in the diagram are characterized in accordance to the quality of its source. Then, an emergy table can be generated in which all quantitative values of the identified energy and mass inflows are multiplied by their respective unit emergy values (UEV, measures of emergy intensity of a resource), resulting in flows of the same unit: solar emjoules (seJ). To estimate the emergy cost of producing cellulase enzyme, the UEV of enzyme is calculated here in seJ/kg_{enzyme}.

A sensitivity analysis of the UEV values taken from the literature was carried out by using the Monte Carlo simulation, and the detailed calculation procedure is shown in the Supplementary material section.

2.2.2. Embodied energy analysis

Energy analysis determines the direct and indirect energy required by a system to produce a specific good or service. Franzese et al. (2009) argues that embodied energy analysis is concerned with the availability and use of fossil fuel storage's, sometimes also referred to as commercial energy. Embodied energy accounts for fuels, electricity, fertilizers, chemicals in general, machinery, among others, in terms of oil equivalent. This methodology focuses on fossil energy shortage, therefore processes that do not use fossil energy are disregarded in the calculation procedure. For instance, all free-of-charge services provided by nature as well as human labor are not accounted for within the embodied energy analysis calculation framework.

The embodied energy of a good or service is calculated by summing energy system's input multiplied by their respective energy intensity factors. The final indicator shows the total amount of embodied energy demanded by the production system. One of most important indicators obtained from this methodology is the energy return on investment (EROI; the ratio of output by input energy), which is used when assessing a system's energy conversion efficiency. The embodied energy to produce 1 kg of cellulase enzyme is calculated in units of MJ/kg_{enzyme}. Detailed information regarding embodied energy analysis can be found in Slesser (1974) and Herendeen (1998).

A Monte Carlo analysis (item 2.3) was carried out to assess the different energy intensity factors available in the literature (see Supplementary material for calculation details).

2.2.3. Indirect emissions inventory

Emission inventory provides information regarding local and global emissions. Due to current concerns about global warming, such inventory is vital to any study concerning environmental

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issues. The present work focuses on indirect emissions, which are those released during the production of materials and energy later used by the industrial production of cellulase enzyme. These emissions usually occur away from the studied system, nevertheless they do contribute to the global environmental loading caused by this system.

In order to estimate the indirect emissions, all material and energy demanded during cellulase production system are converted into their oil equivalents (MJ) and then multiplied by the emission factors (g/MJ) published by USEPA (2008). The following emission factors are considered as: 6.70 E-4 g/MJ to hydrocarbons; 1.70 E-2 g/MJ to CO; 0.02 g/MJ to NO_x; 3.30 E-3 g/MJ to PM10 (particles size lesser than 10 microns); 8.30 E-5 g/MJ to CH_4; 3.70 E-4 g/MJ to N_2O; and 76.2 g/MJ to CO_2. Results correspond to the amount of gases released into the atmosphere per 1 kg of cellulase enzyme produced.

Additionally, the global warming potential (GWP) and the acidification potential (AP) indicators are estimated in units of $kgCO_{2\text{-eq.}}$ and $kgSO_{2\text{-eq.}}$ according to Jensen et al. (1997). The following intensity factors considered for a 20 year time-period being: (i) GWP includes $CO_2 = 1$, $CH_4 = 62$, $N_2O = 290$, CO = 1.6, and hydrocarbons = 3.1; (ii) AP includes $SO_2 = 1$, and $NO_x = 0.88$. Results consider 1 kg of cellulase enzyme as functional unit, and are compared with those of Agostinho and Ortega (2013).

2.3. Uncertainty analysis: Monte Carlo simulation

Parameter uncertainty analysis is frequently used in environmental assessments including energy and emission analysis, such as life cycle assessment (LCA), due to the inherent difficulties of data gathering and uncertainties related to the selection and calculations of the intensity factors. The use of uncertainty analysis under Monte Carlo simulation in emergy evaluations has been increasing lately so as to improve confidence in results and help the methodology become more accepted by the scientific community. Moreover, huge efforts are being made by some researchers (e.g., Raugei et al., 2014) to incorporate emergy accounting into LCA's commercial software, aiming to increase the amount of final indicators under different methods supporting decision makers. Monte Carlo approach is the most used one among uncertainty analyses due to its operational advantages. Li et al. (2011) have compared the stochastic (Monte Carlo) and analytic (Taylor and Variance methods) approaches when assessing uncertainty in emergy table-form models, and results showed similar results for all approaches.

Monte Carlo simulation is a stochastic model (i.e., it is based on multiple random iteration and not on mathematical equations) that basically randomly generates "*n*" successive samples further tested against a statistical model. The Monte Carlo simulation (i) establishes a probability distribution function (PDF) related to a random variable, and (ii) gets samples from that random variable through sufficient repetitions. So far there is no easy-to-be-used approach regarding the choice of the PDF, and different authors are assuming different PDFs for their studies. Campbell (2003), for instance, assumed a normal PDF while Li et al. (2011) and Ingwersen (2010) a log-normal, Brown et al. (2011) and Brown and Ulgiati (2010) a uniform distribution, and Acquaye et al. (2011) a Wakeby distribution. Hudson and Tilley (2013) discuss that a choice of a PDF makes a difference on the results, in which a normal and log-normal PDF can lead to higher final numbers than with other PDFs. Based on the maximum entropy theorem, Hayha et al. (2011) argue that a uniform PDF is a better choice when the model describing the initial parameters is unknown and only their minimum and maximum values can be estimated. Taking the above discussion into account, a uniform PDF is assumed for the description of the initial parameters in this work, even though recognizing that more work is necessary so one can understand the effects that different PDFs could have on results.

Regarding the sufficient repetitions for random sampling, a value of 10,000 was assumed for running the Monte Carlo simulation, which was performed by using a free-of-charge Microsoft Excel® add-in developed by Barreto and Howland (2006), as an alternative to commercial statistical software's that usually demand high computational power and cost.

3. Results and discussion

Fig. 2 shows the inputs and outputs of the main processes related to cellulase enzyme industrial production under submerged fermentation. All the internal processes – as described in Section 2.1 – include similar steps to those for regular fermentation in liquid environment, which are blending, heat transfer and fermentation. Concentrator and freeze dryers are used to assure the enzymes availability in medium/long time periods and their purity for specific later uses (for instance, pharmaceutical purposes). Fig. 2 also highlights all input materials, energy and labor needed for the industrial production of enzymes, including bacteria, carbon sources (cellulose and paper pulp), nutrients, energy (electricity), water, nitrogen, and pieces of equipment (stainless steel). All these input flows and their respective energy and emergy intensities are presented in Table 1. One important emergy input is "information", which represents all emergy demanded through years of scientific research as well as the emergy of the high quality staff involved. However, due to the operational difficulties in quantifying these values, they have been disregarded in this work for the time being.

Recognizing all potential uncertainty involving primary data for cellulase enzyme industrial production and also the intensity factors for energy and emergy methodologies available in the literature, a range of values were considered, instead of a representative and single chosen value for each system input (Table 1). Minimum and maximum parameters obtained from the scientific literature for each resource input underwent Monte Carlo simulation later on. The detailed calculation is available in the Supplementary material section.

Table 1 shows that, among all system inputs, water corresponds to 98% (9870 kg_{water}/kg_{enzyme}) of total consumed material – excluding energy and monetary input flows. Reducing, reusing, and/or recycling water could lead to a significant reduction on some external impacts caused by enzyme production. For instance, such amount of water could be used elsewhere, such as in

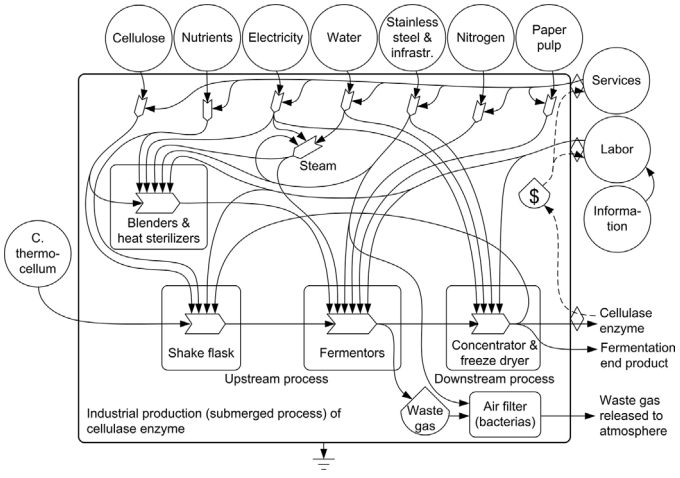

Fig. 2. Energy systems diagram of cellulase enzyme industrial production.

Table 1
Raw data, energy intensity and unit emergy values (UEV) to assess the industrial cellulase enzyme production under submerged fermentation processes.

Note	Input resource	Raw data values[a]			Energy intensity[b]			UEV[c]		
		Minimum	Maximum	Unit/kg_{enzyme}	Minimum	Maximum	MJ/unit	Minimum	Maximum	seJ/unit
Implementation phase										
#1	Concrete	3.72	3.72	kg	7.00E-2	2.72	kg	2.42E12	2.42E12	kg
#2	Sand	3.35	3.35	kg	2.00E-2	6.20E-1	kg	1.68E12	1.68E12	kg
#3	Bricks	2.60	2.60	kg	4.16	7.28	kg	3.66E12	3.66E12	kg
#4	Stainless steel	2.92E-3	2.92E-3	kg	7.90E1	8.18E1	kg	1.25E13	1.25E13	kg
#5	Services	3.82E1	3.82E1	USD	–	–	–	3.40E12	3.40E12	USD
Operational phase										
#6	Electricity	7.23E6	3.24E7	J	2.90E-6	4.17E-6	J	5.87E4	1.02E5	J
#7	Water (production)	1.85E3	1.85E3	L	2.37E-3	9.25E-3	L	4.03E8	1.39E9	L
#8	Water (refrigeration)	8.05E3	8.05E3	L	2.37E-3	9.25E-3	L	4.03E8	1.39E9	L
#9	Water steam	2.03E3	2.03E3	kg	–	–	–	–	–	–
#10	Paper pulp	9.16E1	9.16E1	kg	1.35E1	1.45E1	kg	9.80E11	1.54E12	kg
#11	Cellulose	5.05E-1	5.05E-1	kg	1.89E1	2.03E1	kg	1.37E12	2.16E12	kg
#12	Nutrients	–	–	–	–	–	–	–	–	–
	$(NH_4)\ 2SO_4$	1.15E-1	1.76	kg	6.06E1	6.06E1	kg	6.38E11	6.38E11	kg
	KH_2PO_4	1.64E-1	2.52	kg	8.90	8.90	kg	6.38E11	6.38E11	kg
	$MgSO_4{\cdot}7H_2O$	2.46E-2	3.78E-1	kg	1.00E1	1.00E1	kg	6.38E11	6.38E11	kg
	$CaCl_2{\cdot}2H_2O$	3.28E-2	5.04E-1	kg	9.24	9.24	kg	6.38E11	6.38E11	kg
	Tween 80	2.52E-1	2.52E-1	kg	–	–	–	6.38E11	6.38E11	kg
#13	*Clostridium thermocellum*	6.70E-10	6.70E-10	kg	–	–	–	–	–	–
#14	Nitrogen	4.87E-1	4.87E-1	kg	1.67	1.67	kg	9.88E11	7.04E12	kg
#15	Services	3.21E1	3.21E1	USD	–	–	–	3.40E12	3.40E12	USD
#16	Labor	3.70	3.70	USD	–	–	–	3.40E12	3.40E12	USD

[a] See Supplementary material for detailed calculation procedure.
[b] References for energy intensities can be found at Supplementary material.
[c] All unit emergy values (UEV) are presented without account for Labor & Services and referenced to an emergy baseline of 1.58E25 seJ/year (Odum et al., 2000). References for UEV can be found at Supplementary material.

agricultural production, industry, and/or for drinking; this is the opportunity cost of water use. On the other hand, water consumption has low influence on a system's total embodied energy (Section 3.1.2), as it represents about 5% of the total (Fig. 5). The huge difference between direct water consumption and its equivalence in energy terms is explained by the low amount of energy demanded in withdrawing water from rivers and lakes, but if such water were pumped from groundwater and/or demanded huge treatment efforts before usage, the energy demand would be higher. Although the concerns about water availability have not yet reached the same level of concern as for fossil fuel shortage and global warming, this important resource should be used with caution. Consuming 9870 kg of water to produce 1 kg of cellulase enzyme could be considered as a low cost–benefit operation, which raises doubts about the real importance of lignocellulosic ethanol against potential impact on water availability for other purposes. For example, assessing the water footprint of some food products, Mekonnen and Hoekstra (2011, 2012) estimated that vegetable production demands in average 322 kg_{water}/kg, fruits 967 kg_{water}/kg, cereals 1644 kg_{water}/kg, milk 1020 kg_{water}/kg, eggs 3265 kg_{water}/kg, and beef 15,415 kg_{water}/kg. These figures indicate that lignocellulosic ethanol production could potentially have an impact on food production, but instead of increasing competition for agricultural land as usually debated, its impact could be more significant on the water issues. Producing 1 L of lignocellulosic ethanol demands about 0.0169 g of cellulase (Agostinho and Ortega, 2013) resulting in about 0.21 kg_{water} from enzyme per kg_{EtOH}, which could be considered as an expressive value. Notwithstanding, it is worth it to say that water is also used during agricultural and industrial ethanol production. For comparison purposes, Gerbens-Leenes et al. (2009) provide water footprint values ranging from 1388 to 9812 L_{H2O} per L_{EtOH} obtained from sugar beet, potato, sugarcane, maize, cassava, barley, rye, paddy rice, and sorghum. The water issue is not the focus of this study and we recognize the fact that additional efforts are needed towards elucidating the water versus fuel debate.

3.1. Biophysical costs related to cellulase enzyme production

3.1.1. Emergy accounting

The maximum and minimum values of raw data and unit emergy values (UEV) for all system resources were submitted to a sensitivity analysis through the Monte Carlo simulation. The resulting graphs (Fig. 3) clearly show which inputs have more influence on the total emergy of enzyme production. Fig. 3 shows the final result graph for a sensitivity analysis concerning emergy synthesis. "Services" (for both implementation and operation phases) and "Paper Pulp" are the two main system inputs influencing the total emergy of cellulase enzyme production, reaching about 90% of the total emergy value of the system. As

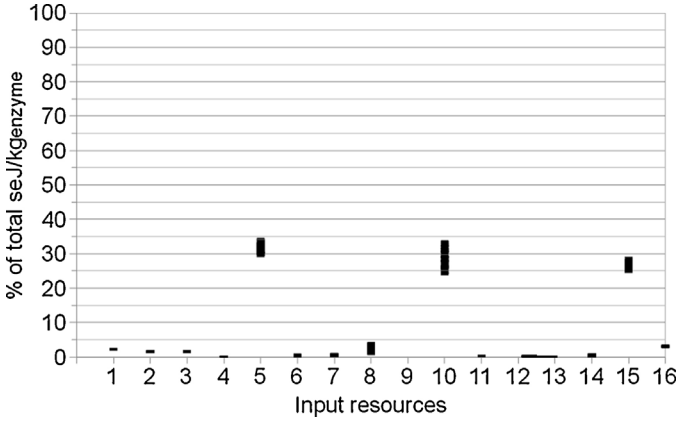

Fig. 3. Sensibility analysis for system's emergy resource inputs. Assumptions: uniform probability distribution function with 100 iterations for Monte Carlo analysis. Legend: 1, concrete; 2, sand; 3, bricks; 4, stainless steel; 5, services (implementation); 6, electricity; 7, water (production); 8, water (refrigeration); 9, water (steam); 10, paper pulp; 11, cellulose; 12a, (NH4) 2SO4; 12b, KH2PO4; 12c, MgSO4.7H2O; 12d, CaCl2.2H2O; 12e, Tween 80; 13, *Clostridium thermocellum*; 14, nitrogen; 15, services (operation); 16, labor.

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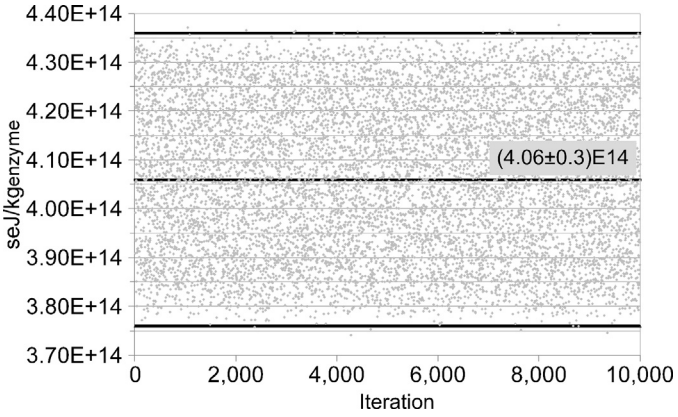

Fig. 4. Result of Monte Carlo simulation for emergy demanded by cellulase enzyme industrial production. Assumptions: uniform probability distribution function with 10,000 iterations. Results: average UEV of 4.06E14 seJ/kg_{enzyme} including Labor & Services; confidence interval for 95% of probability from 3.76 to 4.36E14 seJ/kg_{enzyme}; standard deviation of 1.52E13 seJ/kg_{enzyme}.

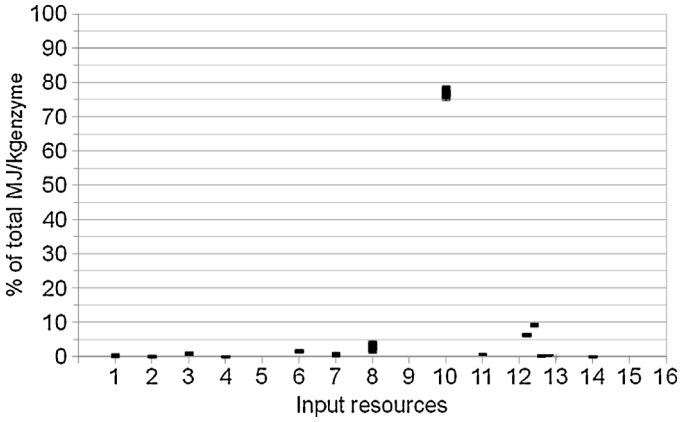

Fig. 5. Sensibility analysis for system's embodied energy resource inputs. Assumptions: uniform probability distribution function with 100 iterations for Monte Carlo analysis. Legend: 1, concrete; 2, sand; 3, bricks; 4, stainless steel; 5, services (implementation); 6, electricity; 7, water (production); 8, water (refrigeration); 9, water (steam); 10, paper pulp; 11, cellulose; 12a, (NH4) 2SO4; 12b, KH2PO4; 12c, MgSO4.7H2O; 12d, CaCl2.2H2O; 12e, Tween 80; 13, *Clostridium thermocellum*; 14, nitrogen; 15, services (operation); 16, labor.

frequently found in emergy evaluation of human-made processes, the "services" input item is the most relevant one on the total emergy demanded, which calls for calculation procedures both considering and not considering "services" input, due to the existing differences on the price elasticity of demand around the world.

Fig. 4 shows the total emergy of cellulase enzyme produced through a submerged method off-site of ethanol plant. The resulting value indicates that the production of 1 kg of cellulase enzyme requires (4.06 ± 0.30)E14 seJ/kg_{enzyme} considering the standard deviation obtained from Monte Carlo analysis. When disregarding Labor & Services from this total, a value of (1.55 ± 0.30)E14 seJ/kg_{enzyme} is obtained from Monte Carlo simulation. For a comparison, the UEV including Labor & Services for chemicals in general are 2.65E12 seJ/kg, 2.49E13 seJ/kg for herbicide/insecticide, and from 1.68 to 7.28E12 seJ/kg for fertilizers (see Agostinho and Ortega, 2013 for these UEVs reference details). The estimated UEV of cellulase enzyme rendered it a high quality product requiring high amounts of emergy for its production. In this sense, even if only a small amount of enzyme is required to produce lignocellulosic ethanol, it is recommendable to account for it within calculations of environmental impacts of lignocellulosic ethanol production.

Due to the lack of published scientific studies about the emergy performance of cellulase enzyme production, a comparative analysis among values was not hereby performed. The influence of cellulase enzyme on the lignocellulosic ethanol production is discussed in the following sections.

3.1.2. Embodied energy analysis

Raw data available in scientific literature about cellulase enzyme production shows variations, and a range of input values can be found therein. This is understandable as technological processes improve year by year, and there are different technological ways to obtain the same final product. Thus, the approach taken to embodied energy analysis method is the same as the one taken to emergy accounting, in which a range of values were considered so as to represent raw data and energy intensities and submitted to a Monte Carlo simulation. Fig. 5 shows that paper pulp (item 10) is the most important contributor to the total embodied energy of enzyme, reaching about 77% of total. The next most important inputs are the nutrients contributing with 10% of total embodied energy. Items 5, 15, and 16 (services and labor) are not considered by embodied energy analyses.

A simultaneous reading of Fig. 5 and Table 1 show that the implementation phase, which considers the infrastructure and equipment (items #1–#5), corresponds to less than 4.2% of the enzyme's total embodied energy. Such result was expected as materials and energy consumed during the operational phase are usually the most meaningful contributors, regardless of using economical or biophysical approaches for assessment. For the operational phase, the paper pulp and KH_2PO_4 items are the most important ones, reaching about 78% and 10%, respectively, of the total embodied energy. This highlights that attempts to reduce the use of paper pulp or having it replaced by other carbon sources with lower energy intensity could result in significant improvements for the system as a whole. A more precise control of all industrial processes during cellulose–enzyme conversion, the production of paper pulp in-situ, or working in a structure of clusters together with other industries are alternatives that could lead to a reduction in paper pulp demand and its energy intensity.

Fig. 6 shows the Monte Carlo results of 1664 ± 63 MJ/kg_{enzyme} of embodied energy to produce cellulase enzyme, for a confidence interval of 95%. The low standard deviation (≅3.6%), similar to that obtained through emergy evaluation (≅3.6%), indicates that the

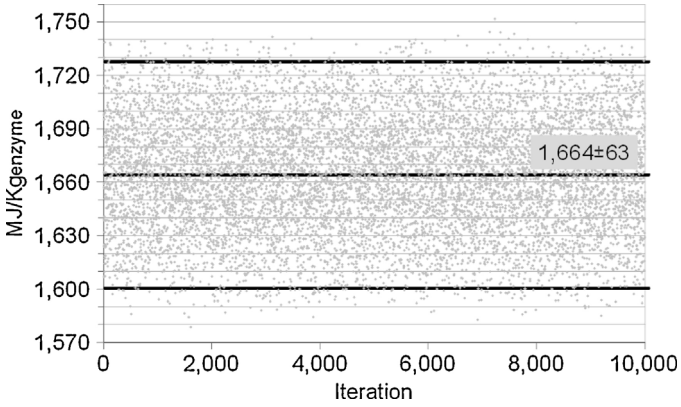

Fig. 6. Result of Monte Carlo simulation for embodied energy demanded by cellulase enzyme industrial production. Assumptions: uniform probability distribution function with 10,000 iterations. Results: average UEV of 1664 MJ/kg_{enzyme}; confidence interval for 95% of probability from 1601 to 1727 MJ/kg_{enzyme}; standard deviation of 32 MJ/kg_{enzyme}.

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Table 2
Average values of embodied energy for industrial production of cellulase enzyme under SmF process. Values estimated from Monte Carlo simulation.[a]

Note	Input item	Average values in MJ/kg_{enzyme}
Implementation phase		
#1	Concrete	5.19
#2	Sand	1.12
#3	Bricks	14.66
#4	Stainless steel	0.23
#5	Services[b]	–
Operational phase		
#6	Electricity	25.06
#7	Water (production)	11.12
#8	Water (refrigeration)	46.52
#9	Water steam[c]	–
#10	Paper pulp	1282.46
#11	Cellulose	9.90
#12	Nutrients	–
	(NH_4) $2SO_4$	106.90
	KH_2PO_4	152.71
	$MgSO_4 \cdot 7H_2O$	3.36
	$CaCl_2 \cdot 2H_2O$	5.04
	Tween 80	–
#13	*Clostridium thermocellum*[d]	–
#14	Nitrogen	0.81
#15	Services[b]	–
#16	Labor[b]	–

[a] Monte Carlo simulation by considering an uniform probabilistic distribution function under 10,000 iterations.
[b] Implementation and operational costs, and labor are not considered into embodied energy methodology.
[c] Water steam is not accounted because it is produced internally to the system boundaries. It was assumed that electricity is used to generate steam because none reference was found regarding different energy source for this purpose in the Zhuang et al. (2007) work.
[d] *Clostridium thermocellum* was not accounted due to its low initial demand and because it can be reused for next batches.

raw data used and intensity coefficients (for emergy or energy) have similar and good precision rate after 10,000 iterations.

According to Spatari et al. (2010), there is a high degree of variability among published works about energy demand and emissions resulting from enzymes production, in which values depend on the kind of enzyme produced and the industrial process involved. At the moment, it was not possible to find in scientific literature the energy demanded to produce cellulases, but some comparison can be drawn with figures from other kinds of enzymes production works. Studying the production of five different enzymes (for purposes of liquefaction, saccharification and crystallization of starch diminution; release of phytate bound phosphate; removal of proteins stains), Nielsen et al. (2007) estimated values ranging from 20 to 130 MJ/kg_{enzyme}, by considering the exclusive use of primary energy. Those figures mainly represent electricity and are 13–83 times lower than the value of 1664 MJ/kg_{enzyme} calculated in this work. Studying the industrial production of three different kinds of enzymes for pharmaceutical purposes, Kim et al. (2009) estimated a demand for nonrenewable energy ranging from 117 to 207 MJ/kg_{enzyme}. The phrase nonrenewable energy was not properly defined by the authors, however assuming that the phrase is related to electricity consumption, those values are 8 to 14 times lower than the value obtained herein. Aside from the methodological differences in the metrics used to assess the enzyme production, the industrial processes of the present work could be considered as more complete than those discussed above due to larger system boundaries (i.e., the window of attention), mainly in downstream processes, which could explain the differences among values obtained for the embodied energy demanded to produce enzymes.

3.1.3. Indirect emissions inventory

Table 2 shows, item by item, the values calculated for embodied energy of cellulase enzyme production. These numbers correspond to average values from the range diagramed in Fig. 5 obtained from the Monte Carlo simulation.

From the values of Table 2 expressed in MJ/kg_{enzyme}, the emissions inventory was elaborated by using the emission factors published by USEPA (2008). Fig. 7 shows that CO_2 is the main gas released indirectly, reaching about 99% of total gas release in mass units (21,892 gCO_2/kg_{enzyme}). This value is about 3800 times higher than that for NO_x release, which is followed by lower amounts of CO, SO_2, and PM10. The released amount of hydrocarbons, N_2O and CH_4, can be considered negligible under a 20-year time window of impact analysis when compared to other gases, which represent less than 0.32 g/kg_{enzyme}. However, an analysis comprising a longer time window could result in different figures because these gases remain in the atmosphere for long periods.

The CO_2 indirectly released plays an important role in the total emissions, usually corresponding to the largest part (in mass units) of the gases released. This result is in accordance to the work of Pereira and Ortega (2010) who estimated that 78% of all CO_2 released from first generation sugarcane ethanol fuel production (including agricultural, transport and industrial phases) occurs indirectly instead of directly. In fact, direct emissions resulting from fermentation processes of enzyme production are lower than indirect emissions. Thus, before labeling a biofuel as a low emission product, a careful assessment under a life cycle assessment approach must include not only the direct local emissions from fossil fuel burning, but also those related to all goods and services production used as inputs for the biofuel production.

Due to the high representativeness of CO_2 release during cellulase enzyme production, its item by item contribution is represented in Fig. 8. The KH_2PO_4 nutrient input is responsible for 53% of the indirect CO_2 release, followed by (NH_4) $2SO_4$ (37%), bricks (4%) and concrete (2%) used in the infrastructure. All other inputs for enzyme production correspond to less than 4% of total altogether. Intuitively, it was expected that inputs in the operational phase (electricity, paper pulp, water, and nutrients) would be the larger CO_2 emitters, however, they reached insignificant values as compared to nutrient inputs. This result can be explained by the calculation procedure, in which different percentages of fossil energy dependence were assumed by each system's input item, and by the Brazilian energy matrix. For nitrogen, cellulose, paper pulp and water it was assumed that

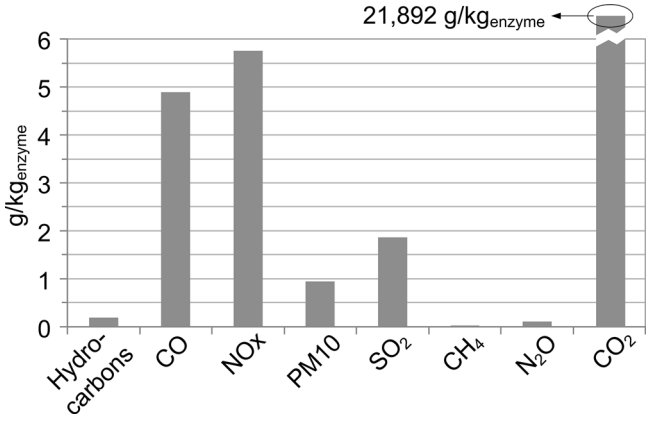

Fig. 7. Indirect released gases to produce 1 kg of industrial cellulase enzyme under submerged fermentation method.

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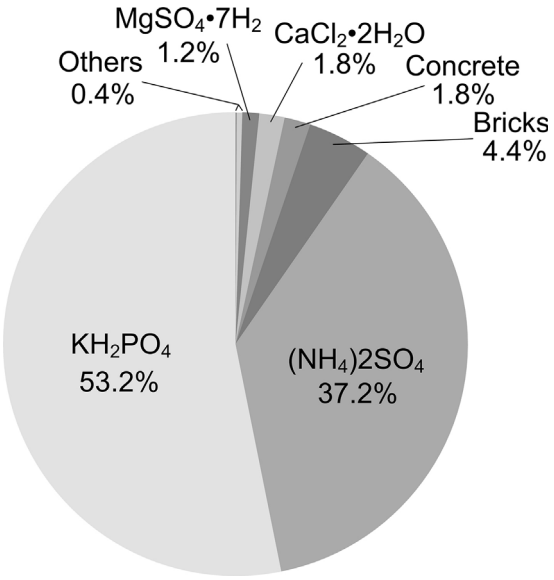

Fig. 8. Individual contribution on the total CO_2 indirectly released by resources consumed during industrial cellulase enzyme production under submerged fermentation process method.

100% of its embodied energy would come from electricity, considered as a zero CO_2 emitter, since it is produced from hydropower in Brazil. This assumption goes against the findings of Hallqvist (2012) and Rosa and Schaeffer (1995) according to which greenhouse gases are released from dams, however, more detailed data are needed in order to be included in our calculation procedure. Bricks contain 13% of their embodied energy derived from hydropower; while concrete, sand, stainless steel, and nutrients are totally dependent on fossil fuel (Boustead and Hancock, 1979).

The global warming potential (GWP) of cellulase enzyme production reached a value of 21.93 $kgCO_{2\text{-eq.}}/kg_{enzyme}$, basically composed of CO_2 (99%); this indicates that, when possible, a reduction or replacement of inputs considered as larger CO_2 emitters (e.g., KH_2PO_4 and $(NH_4)\,2SO_4$) could result in a significant reduction in GWP. For comparison, studying the cellulase enzyme on-site and off-site production of an ethanol plant, Hong et al. (2013) estimated values of 10.2 and 16.0 $kgCO_{2\text{-eq.}}/kg_{enzyme}$, respectively, by using dextrose as carbon source instead of paper pulp, while MacLean and Spatari (2009) report a value of 2.3 $kgCO_{2\text{-eq.}}/kg_{enzyme}$ estimated by considering other works and an on-site enzyme production. For enzymes production for pharmaceutical purposes, Kim et al. (2009) have found values ranging from 16 to 25 $kgCO_{2\text{-eq.}}/kg_{enzyme}$ obtained from a life cycle inventory database, and by assessing five different enzyme production processes – not including cellulases –, Nielsen et al. (2007) have found GWP values ranging from 1 to 10 $kgCO_{2\text{-eq.}}/kg_{enzyme}$. The differences among values found in literature for enzyme's GWP can be explained by the different industrial processes used and kind of enzymes produced, as well as to the methodological metrics used for assessment.

Regarding the acidification potential (AP) to produce cellulase enzyme, the value of 0.007 $kgSO_{2\text{-eq.}}/kg_{enzyme}$ was obtained in this work, which is similar to results of Nielsen et al. (2007) that obtained a range from 0.005 to 0.010 $kgSO_{2\text{-eq.}}/kg_{enzyme}$, but far from the range of 0.120–0.145 $kgSO_{2\text{-eq.}}/kg_{enzyme}$ estimated by Kim et al. (2009). The differences in the values found are reflections of different studied industrial processes, as well as the different methodological approaches used.

3.2. Influence analysis of cellulase enzyme production on the energetic–environmental performance of lignocellulosic ethanol

Evaluating a scenario of lignocellulosic ethanol production in Brazil, Agostinho and Ortega (2013) report an average demand of 0.0169 g of cellulase enzyme in producing 1 L of ethanol. Such enzyme demand could be reduced by using more effective enzymes to convert cellulose and hemicelulose materials into fermentable sugars, wherein Wyman (2007) states that such goal could be reached with the decisive help from genetic engineering and selection. Considering the 0.0169 g/L_{EtOH} as the coefficient and after careful verification of results obtained in this work for the energetic–environmental performance for cellulase enzyme production, Table 3 could be elaborated. It summarizes the results obtained from using two different functional units: (i) per 1 kg of cellulase enzyme; (ii) per 1 L of lignocellulosic ethanol. While the former can be useful as a reference value for future works when making comparisons among different cellulase enzyme production processes, the latter is important when assessing the influence of cellulase enzyme on the energetic–environmental performance of lignocellulosic ethanol production.

4. Emergy accounting

Agostinho and Ortega (2013) estimated an emergy demand of 1.75E12 seJ to produce 1 kg of lignocellulosic ethanol (or 1.40E12 seJ/L_{EtOH}). Values in Table 3 indicates a demand of 4.06E14 seJ/kg_{enzyme} or 6.86E9 seJ_{enzyme}/L_{EtOH}. Table 4 shows that cellulase enzyme production contributes about 0.49% of the total emergy demand to produce 1 L of ethanol, a value which can be considered negligible when compared to other input resources in the ethanol production chain, such as limestone and diesel, which contribute 14 and 13%, respectively, to the total ethanol emergy demand. Table 4 also shows the contribution of cellulase enzyme production to the lignocellulosic ethanol considering the works of Felix and Tilley (2009) and Coppola et al. (2009), which vary from 0.06 to 0.26%, also deemed negligible in all cases. Table 4 also includes first generation ethanol production systems for a gross comparison, since in this case specific enzymes – not cellulases – are used to convert starch into sugars. Results indicate that enzyme influence on the emergy for first generation ethanol production can also be considered as negligible, with contributions ranging from 0.07 to 0.36%.

5. Embodied energy

Considering the 1664 MJ/kg_{enzyme} figure obtained in this work (Table 3), the use of cellulase enzyme in the lignocellulosic ethanol production systems studied by Agostinho and Ortega (2013) results in a negligible influence of 0.41% (Table 4). This table also shows that similar results are obtained by considering other published works as reference, in which the enzyme contributions are lower than 0.06%.

Table 3
Summarized results of energetic–environmental load in producing 1 kg of cellulase enzyme and its correspondent load in producing 1 L of lignocellulosic ethanol.

Amount	Units per kg_{enzyme}	Amount	Units per L_{EtOH}[a]
4.06E14	seJ_{enzyme}/kg_{enzyme}	6.86E9	seJ_{enzyme}/L_{EtOH}
1,664	MJ_{enzyme}/kg_{enzyme}	2.81E-2	MJ_{enzyme}/L_{EtOH}
21.93	$kgCO_{2\text{-eq.enzyme}}/kg_{enzyme}$	3.71E-4	$kgCO_{2\text{-eq.enzyme}}/L_{EtOH}$
7.00E-3	$kgSO_{2\text{-eq.enzyme}}/kg_{enzyme}$	1.18E-7	$kgSO_{2\text{-eq.enzyme}}/L_{EtOH}$

[a] Conversion factors: 0.0169 g_{enzyme}/L_{EtOH} and 0.8 kg_{EtOH}/L_{EtOH}.

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Table 4
Influence of using cellulase enzyme on the energetic–environmental performance of ethanol production by considering the three biophysical methodologies used in this work.[a]

Methodology/reference	Raw resource and kind of ethanol	Resources demand or impact in producing 1 kg of ethanol fuel	Enzyme contribution (in %) on ethanol fuel production
Emergy accounting			
Agostinho and Ortega (2013)	Sugarcane (lignocellulosic)	1.75E12 seJ	
This work (Table 3)			0.49[e]
Felix and Tilley (2009)	Switchgrass (lignocellulosic)	3.25E12 seJ	
This work (Table 3)			0.26[e]
Coppola et al. (2009)[f]	Wheat straw (lignocellulosic)	7.82E12 seJ 1.32E13 seJ	
This work (Table 3)			0.11[e]
This work (Table 3)			0.06[e]
Yang et al. (2011)[g]	Cassava (starch)	2.41E12 seJ	
This work (Table 3)			0.36[e]
Liao et al. (2011)	Corn (starch)	9.82E12 seJ	
This work (Table 3)			0.09[e]
Liu et al. (2012)[h]	Rice (starch)	1.15E13 seJ	
This work (Table 3)			0.07[e]
Embodied energy			
Agostinho and Ortega (2013)	Sugarcane (lignocellulosic)	6.86 MJ	
This work (Table 3)			0.41
Nielsen et al. (2007)			<0.01–0.04[b]
Kim et al. (2009)			0.04–0.06[c]
Vink et al. (2003)			0.02[d]
Global warming potential			
Agostinho and Ortega (2013)	Sugarcane (lignocellulosic)	2.03 $kgCO_{2\text{-eq.}}$	
This work (Table 3)			0.02[i]
Kim et al. (2009)			0.02–0.03[j]
MacLean and Spatari (2009)			5.02[k]
Dunn et al. (2012)			6.7[l]

[a] 0.0169 g_{enzyme}/L_{EtOH} × 1.25 L_{EtOH}/kg_{EtOH} × 1 kg/1000 g × 4.06E14 seJ/kg_{enzyme} = 2.29E9 seJ_{enzyme}/kg_{EtOH}.
[b] From 20 to 130 MJ_{enzyme}/kg_{enzyme} (Nielsen et al., 2007) × 0.0169 g_{enzyme}/L_{EtOH} × 1.25 L_{EtOH}/kg_{EtOH} × 1 kg/1000 g = from 2.75E-3 to 4.37E-4 MJ_{enzyme}/kg_{EtOH}.
[c] From 117 to 207 MJ_{enzyme}/kg_{enzyme} (Kim et al., 2009) × 0.0169 g_{enzyme}/L_{EtOH} × 1.25 L_{EtOH}/kg_{EtOH} × 1 kg/1000 g = from 2.47E-3 to 4.37E-3 MJ_{enzyme}/kg_{EtOH}.
[d] 82 MJ_{enzyme}/kg_{enzyme} (Vink et al., 2003) × 0.0169 g_{enzyme}/L_{EtOH} × 1.25 L_{EtOH}/kg_{EtOH} × 1 kg/1000 g = 1.73E-3 MJ_{enzyme}/kg_{EtOH}.
[e] 6.86E9 seJ_{enzyme}/L_{ETOH} (Table 3) × 1.25 L_{EtOH}/kg_{EtOH} = 8.59E9 seJ_{enzyme}/kg_{EtOH}.
[f] Scenario SC, G2 recycled input: 2.67E5 seJ/J_{EtOH} × 4186 J/kcal × 7000 kcal/kg_{EtOH} = 7.82E12 seJ/kg_{EtOH}; scenario SC, G2 not-recycled inputs: 4.52E5 seJ/J_{EtOH} × 4186 J/kcal × 7000 kcal/kg_{EtOH} = 1.32E13 seJ/kg_{EtOH}.
[g] (5.29E19 seJ/year (without Labor & Services)/6.44E14 J_{EtOH}/year) × 4186 J/kcal × 7000 kcal/kg_{EtOH} = 2.41E12 seJ/kg_{EtOH}.
[h] (1.20E22 seJ/3.06E16 J_{EtOH}) × 4186 J/kcal × 7000 kcal/kg_{EtOH} = 1.15E13 seJ/kg_{EtOH}.
[i] 3.71E-4 $kg_{CO2\text{-eq.enzyme}}/L_{ETOH}$ (Table 3) × 1.25 L_{EtOH}/kg_{EtOH} = 4.64E-4 $kg_{CO2\text{-eq.enzyme}}/kg_{EtOH}$.
[j] From 16 to 25 $kg_{CO2\text{-eq.enzyme}}/kg_{enzyme}$ (Kim et al., 2009) × 0.0169 g_{enzyme}/L_{EtOH} × 1.25 L_{EtOH}/kg_{EtOH} × 1 kg/1000 g = from 3.38E-4 to 5.28 E-4 $kg_{CO2\text{-eq.enzyme}}/kg_{EtOH}$.
[k] 8.15E-2 $kgCO_{2\text{-eq.enzyme}}/L_{EtOH}$ (MacLean and Spatari, 2009) × 1.25 L_{EtOH}/kg_{EtOH} = 1.02E-1 $kgCO_{2\text{-eq.enzyme}}/kg_{EtOH}$.
[l] 0.11 $kgCO_{2\text{-eq.enzyme}}/L_{EtOH}$ (Dunn et al., 2012) × 1.25 L_{EtOH}/kg_{EtOH} = 1.38E-1 $kgCO_{2\text{-eq.enzyme}}/kg_{EtOH}$.

6. Global warming and acidification potentials

The global warming potential (GWP) of cellulase enzyme production was estimated as 21.93 $kgCO_{2\text{-eq.}}/kg_{enzyme}$ (3.71E-4 $kgCO_{2\text{-eq.}}/L_{EtOH}$; Table 3), which is equivalent to 0.02% (Table 4) of GWP reported by Agostinho and Ortega (2013) for lignocellulosic ethanol production (1.62 $kgCO_{2\text{-eq.}}/L_{EtOH}$). Thus, similarly to results obtained from emergy and embodied energy assessments, the relative contribution of enzyme on ethanol production can be considered as negligible. This result contradicts the values reported by MacLean and Spatari (2009); (8.15E-2 $kgCO_{2\text{-eq.}}/L_{EtOH}$) and Dunn et al. (2012); (0.11 $kgCO_{2\text{-eq.}}/L_{EtOH}$), in which the enzyme production contribute 5–7% of ethanol's GWP, which could be considered as a moderate contribution.

Regarding the acidification potential (AP), the value obtained for cellulase enzyme production (7.00E-3 $kgSO_{2\text{-eq.}}/kg_{enzyme}$ or 1.18E-7 $kgSO_{2\text{-eq.}}/L_{EtOH}$) represents less than 0.01% of lignocellulosic ethanol's AP (6.78E-3 $kgSO_{2\text{-eq.}}/L_{EtOH}$; Agostinho and Ortega, 2013), and can also be considered a less important item. We were unable to find other studies in the literature about the ethanol's AP for comparison, maybe due to the fact that the GWP has been receiving higher levels of attention due to current concerns about climate change.

The enzyme load of 0.0169 g/L_{EtOH} considered in this study is being reduced over time, i.e., the enzyme activity has been increasing exponentially these recent years. For instance, the Cellic CTec3® of Novozymes company produced in 2012 had 100% higher enzymatic activity than its equivalent enzyme produced in 2009 (Ms. Per H. Nielsen, personal communication). Thus, the influence values presented in Table 4 for all methodologies could be perceived as slightly overestimated, reinforcing the conclusion about the insignificant contribution of cellulases on the impacts herein measured for ethanol production.

7. Conclusions

Considering the methodologies used and the enzyme production system studied in this work, results show that producing 1 kg of cellulase enzyme demands 4.06E14 seJ and 1664 MJ, which results in a global warming potential of 21.93 $kgCO_{2\text{-eq}}$. These

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values correspond respectively to 0.49%, 0.41%, and 0.02% of total emergy, embodied energy, and global warming emission of producing 1 L of lignocellulosic ethanol, therefore deemed insignificant when compared to other system inputs.

Differently from economic approaches in which enzymes contribute from 25 to 50% of lignocellulosic ethanol costs (as reported by Zhuang et al. (2007), Dias et al. (2012), and Hong et al. (2013)), the results of this work highlight their low contribution to environmental issues assessed by biophysical metrics such as emergy accounting, embodied energy, and emission inventory. The impressive difference between economic and biophysical approaches could be explained by the "information" embodied into enzyme production, e.g., all the economic and intellectual investments through years of high quality scientific research until the current enzyme production model was reached. Such hidden costs are disregarded by the biophysical approaches used in this work due to operational difficulties in their quantification. We recognize this theme as very important for future research.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolmodel.2014.09.005.

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