Ecological Modelling 315 (2015) 46–56

Contents lists available at ScienceDirect

Ecological Modelling

journal homepage: www.elsevier.com/locate/ecolmodel

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

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ARTICLE INFO

Article history:
Available online 22 September 2014

Keywords:
Cellulase enzyme
Embodied energy analysis
Emergy accounting
Emission inventory
Monte Carlo simulation
Second generation ethanol

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.

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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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http://dx.doi.org/10.1016/j.ecolmodel.2014.09.005
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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

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