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LIFE CYCLE ASSESSMENT AND TECHNO-ECONOMICAL ANALYSIS OF ON-SITE ENZYME PRODUCTION IN 2ND GENERATION BIOETHANOL

Report from an f3 project

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Authors:

Johanna Olofsson
Lund University, Environmental and Energy Systems Studies

Zsolt Barta
Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Science

Pål Börjesson
Lund University, Environmental and Energy Systems Studies

Ola Wallberg
Lund University, Department of Chemical Engineering

THE SWEDISH KNOWLEDGE CENTRE
FOR RENEWABLE TRANSPORTATION FUELS

PREFACE

This report is the result of a collaborative project within the Swedish Knowledge Centre for Renewable Transportation Fuels (f3). f3 is a networking organization, which focuses on development of environmentally, economically and socially sustainable renewable fuels, and

- Provides a broad, scientifically based and trustworthy source of knowledge for industry, governments and public authorities,
- Carries through system oriented research related to the entire renewable fuels value chain,
- Acts as national platform stimulating interaction nationally and internationally.

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SUMMARY

Production of ethanol from lignocellulosic materials is a very complex process, which consists of various interdependent steps, such as pretreatment of the raw material, enzymatic hydrolysis of the polysaccharides into sugar monomers, fermentation of the sugars to ethanol, and purification of ethanol. Life cycle assessment (LCA) is a potential tool for comparing and analyzing environmental performance of different pathways for lignocellulosic ethanol as well as finding hot spots for future improvements. Several previous LCA's have identified the production of cellulase enzymes as a process that have a large impact on overall results, especially regarding energy consumption and greenhouse gas (GHG) emissions. Due to the new and quickly developing technology of cellulase production, partly not up-to-date and uncertain input data have been used in previous studies. Furthermore, energy use and environmental impacts could potentially be reduced by integrating the production with the ethanol conversion process, e.g. by making use of excess heat and electricity from biomass, and by avoiding upgrading and refining processes for cellulases which are necessary when enzymes are to be stored and transported. Some studies have taken steps towards investigating potential benefits from co-locating and partly integrating enzyme production with ethanol conversion but the full potential of an integrated process approach has not yet been assessed.

The aim of the present study was to investigate GHG performance, primary energy use and ethanol production cost from two different process designs regarding cellulase enzymes for lignocellulosic ethanol production: (i) integrated in ethanol plant versus (ii) purchased from a centralized facility. On-site cellulase production in a full-scale bioethanol plant was modelled together with the whole ethanol production process, and the economic impact of the enzyme fermentation step on the ethanol production cost was assessed.

The results show that primary energy efficiency is somewhat higher in the cases with integrated enzyme production, but no major differences are identified. Regarding GHG emissions, results show that by using part of the lignocellulosic feedstock for enzyme production by the microorganism, emissions from bioethanol in a well-to-wheel perspective can be reduced significantly, compared to a scenario using purchased enzymes from a centralized facility. Information regarding purchased enzymes is scarce and data is connected to large uncertainties. The sensitivity analysis shows that assumptions regarding purchased enzymes, such as dosage and type of energy utilized in production, largely affect the comparison with an integrated enzyme production approach.

The feasibility of including enzyme production in the lignocellulosic ethanol process highly depends on the full-scale price of commercial cellulase enzyme preparation, which is still very uncertain. At the premises of the study, one scenario proved to be more economical feasible than that with purchased enzymes, which implies that on-site enzyme production can be an alternative also considering the process economics.

SAMMANFATTNING

Produktion av etanol från lignocellulosa är en mycket komplex process, som består av olika samverkande åtgärder, såsom förbehandling av råvaran, enzymatisk hydrolys av polysackarider till sockermonomerer, jäsning av socker till etanol, och rening av etanol. Livscykelanalys (LCA) är ett verktyg för att jämföra och analysera miljöpåverkan från olika processalternativ för etanolproduktion från lignocellulosa. Tidigare studier har visat att enzymproduktionen har en stor inverkan på de totala växthusgasutsläppen från etanolproduktion ur ett livscykelperspektiv. På grund av den snabba utvecklingen av tekniken för cellulasproduktion har dock delvis inaktuella och osäkra indata använts i tidigare studier. Dessutom skulle primärenergianvändning och miljöpåverkan eventuellt kunna minskas genom att integrera enzymproduktionen med etanolproduktionsprocessen, t.ex. genom att använda intern överskottsvärme och överskottsel där detta är fördelaktigt, och genom att undvika uppgradering och förädling av cellulaser som krävs då dessa ska transporteras och lagras. Vissa studier har undersökt potentiella fördelar med att samlokalisera och delvis integrera enzymproduktionen i etanolproduktionen, men den fulla potentialen av en integrerad process har inte tidigare utvärderats.

Syftet med denna studie var att undersöka växthusgasutsläpp, primärenergianvändning och produktionskostnad för etanol från två olika processalternativ när det gäller tillverkning av cellulasenzymer för lignocellulosaetanol: (i) integrerad i etanolproduktionsprocessen kontra (ii) köpt från en central anläggning. Integrerad cellulasproduktion i en fullskalig bioetanolanläggning modellerades tillsammans med hela etanolframställningsprocessen. De ekonomiska konsekvenserna av enzymproduktionen på etanolproduktionskostnaderna bedömdes och miljöprestandan utvärderades.

Resultaten visar att primärenergiutbytet är något högre i fallen med integrerad enzymproduktion, men inga stora skillnader kan identifieras. Växthusgasberäkningarna visar att utsläppen från bioetanol ur ett livscykelperspektiv kan minskas väsentligt genom att en del av lignocellulosaråvaran används för enzymproduktion med hjälp av mikroorganismer, jämfört med att använda inköpta enzymer. Informationen om inköpta enzymer är dock bristfällig och indata är kopplade till stora osäkerheter. Därför har antaganden om exempelvis enzymernas dosering och de energikällor som används i produktionsprocessen stor påverkan på jämförelsen med en integrerad enzym- och etanolproduktion. Resultat från den ekonomiska analysen indikerar att det även ur kostnadssynpunkt kan vara fördelaktigt med integrerad enzymproduktion, men även här finns stora osäkerheter i indata när det gäller kostnader för inköpta enzymer.

CONTENTS

1 INTRODUCTION

Production of ethanol from lignocellulosic materials is a very complex process, which consists of various interdependent steps, such as pretreatment of the raw material, enzymatic hydrolysis of the polysaccharides into sugar monomers, fermentation of the sugars to ethanol, and purification of ethanol. Since the process has not yet been demonstrated on a commercial scale, only a limited number of studies are available on its techno-economic aspects, and large variations in the estimated overall ethanol production costs (from about 0.93 to 5.49 SEK/L ethanol) can be seen, due to differences in the process design and in the assumptions used in the studies [1-10].

According to recent techno-economic evaluations, the main contributors to the overall costs of producing ethanol from biomass are the raw material (30-40 %) and the capital investment (30-45 %), followed by the cellulase enzymes (10-20 %) [6,7,11-13]. The cost of cellulases not only represents a significant part in the overall production costs, but is also one of the most uncertain parameters in the evaluations [3]. Most authors assume that cellulases are purchased from enzyme manufacturers, and calculate with an estimated future enzyme price, which varies from about 0.2 to 0.7 SEK/L ethanol in the investigations reviewed [1,6,7,11-15]. However, some other studies presume that on-site or near-site production on cheap lignocellulosic raw materials will be desirable to meet the targeted enzyme costs of <0.5 SEK/L [8,16-20]. In any case, improvement of cellulolytic microorganisms, enhancement of the hydrolytic capacity of cellulases, and optimization of the technology of enzyme production are essential today in order to further reduce the enzyme costs for the biomass-to-bioethanol process.

Spruce is the most abundant wood in Sweden, and it was shown to be a suitable raw material for bioethanol production in several studies [21-24]. Hypercellulolytic mutants of *Trichoderma reesei*, the most widely used fungus for cellulase production, were reported to grow well and secrete high amounts of cellulolytic enzymes on steam-pretreated spruce [25,26]. The most economical way of employing the enzymes produced would be the direct use of whole crude fermentation broths, containing fungal cells and substrate residues, in order to avoid expensive cell removal, enzyme concentration and purification steps. Previous investigations showed that due to the effect of mycelium-bound enzymes, application of the whole broth of *T. reesei* could not only lead to cost reduction, but also to improved saccharification and enhanced ethanol yields [27-30]. These suggest that on-site enzyme production with *T. reesei* could be a possible alternative to purchasing cellulases for a bioethanol plant using spruce as raw material.

Life cycle assessment (LCA) has become a widespread tool for analyzing the environmental performance of a product by mapping the resource use and emissions related to its life cycle. Thus, LCA is a potential tool for comparing and analyzing different pathways for lignocellulosic ethanol as well as finding hot spots for future improvements. Several previous LCA's have identified the production of cellulase enzymes as a process that have a large impact on the overall environmental impact, especially regarding energy use and greenhouse gas (GHG) emissions [31-36]. Due to the new and quickly developing technology of cellulase production, partly not up-to-date and uncertain input data have been used in previous studies. Furthermore, energy use and environmental impacts could potentially be reduced by integrating the production with the ethanol conversion process, e.g. by making use of excess heat and electricity from biomass where this is beneficial, and by avoiding upgrading and refining processes that are necessary for storage and transport of cellulases [36]. Some studies have taken steps towards investigating potential benefits from co-locating and partly

integrating enzyme production with ethanol conversion [34-36] but the full potential of an integrated process approach has not yet been assessed.

The aim of the present study was to investigate GHG performance, primary energy use and ethanol production cost from two different process designs regarding cellulase enzymes for lignocellulosic ethanol production: (i) integrated in ethanol plant versus (ii) purchased from a centralized facility.

On-site cellulase production in a full-scale bioethanol plant was modelled together with the whole ethanol production process, and the economic impact of the enzyme fermentation step on the ethanol production cost was assessed. Cellulases were assumed to be produced using a mutant of *T. reesei*, employing the whole crude fermentation broth of the fungus in the ethanol production step. The effect of varying the carbon source of enzyme fermentation, at constant protein and mycelium yields, was investigated through the whole process. Pretreated liquid fraction and pretreated liquid fraction supplemented with molasses were evaluated as feed for enzyme production. As reference case, ethanol production without integrated enzyme production was modelled, and cellulase enzymes were assumed to be purchased from an external plant.

2 MATERIAL AND METHODS

This chapter introduces the methods and data applied for this study. Sections 2.1 to 2.6 present the assumptions and data used in the techno-economic assessment, followed by a presentation and background for LCA in section 2.6 and 0.

2.1 RAW MATERIAL

The dry spruce chips contain 37.9 % glucan, 9.9 % mannan, 1.8 % galactan, 4.3 % xylan, 1.3 % arabinan and 28.0 % lignin. These values were derived from compositional analyses performed in EU-project NILE (contract no. 019882) according to the standardized method of National Renewable Energy Laboratory (NREL, Golden, CO) [37]. The remaining part is made up of acetyl groups, extractives and other compounds, which were estimated from a previous study [6]. The dry matter (DM) content was assumed to be 50 %. Theoretically, 356 L of ethanol could be produced from the hexose sugars per dry tonne of raw material.

2.2 OVERALL PROCESS DESCRIPTION

The proposed ethanol plant is assumed to be located in Sweden and process 200 000 dry tonne spruce chips annually. It is run by 28 employees and is assumed to be in operation 8000 h per year.

The process scheme is shown in Figure 1. Each step, except cellulase enzyme fermentation (CEF), has been described in detail elsewhere [6], and will only be discussed here briefly, focusing mainly on the minor modifications. Further description of enzyme production configurations in the different cases studied, can be found in sections 2.3 and 2.4.

Live steam was assumed to be available at 20 and 4 bar, and secondary steam is used to replace live steam whenever possible.

The conversion of carbohydrates is carried out in steam pretreatment and in simultaneous saccharification and fermentation (SSF) (Figure 1). Process data for steam pretreatment (210°C, 2 % SO_2) and SSF were based on results recently obtained from experimental work performed at the Department of Chemical Engineering, Lund University, Sweden.

Water needed to adjust the dry matter in the SSF step to 10 % water-insoluble solids (WIS) is added before pressing the pretreated slurry. The diluting stream consists of fresh water and part of the evaporation condensate. It also contains ammonia to neutralise the slurry. The pressed liquid supplemented with molasses containing 50 % sucrose is used in yeast cultivation (YC) without adding extra fresh water, hence the inhibitor concentrations in YC and SSF are approximately the same. Yeast seed train consisting of three stages provides SSF with 7.5 % inoculum. Only the first and second stages are designed to be sterile, i.e. those vessels are pressure-rated for steam sterilization. In SSF, the concentration of ordinary baker's yeast and the enzyme dosage are 3 g DM/L and 10 FPU (filter paper unit)/g WIS, respectively. The SSF takes place in twelve agitated non-sterile fermentors with a total volume of 920 m^3 each. An SSF cycle including filling, fermentation, draining, and cleaning lasts for 60 h. The number of the YC fermentors was calculated from the cycle time, which was assumed to be 15 h for all YC stages.

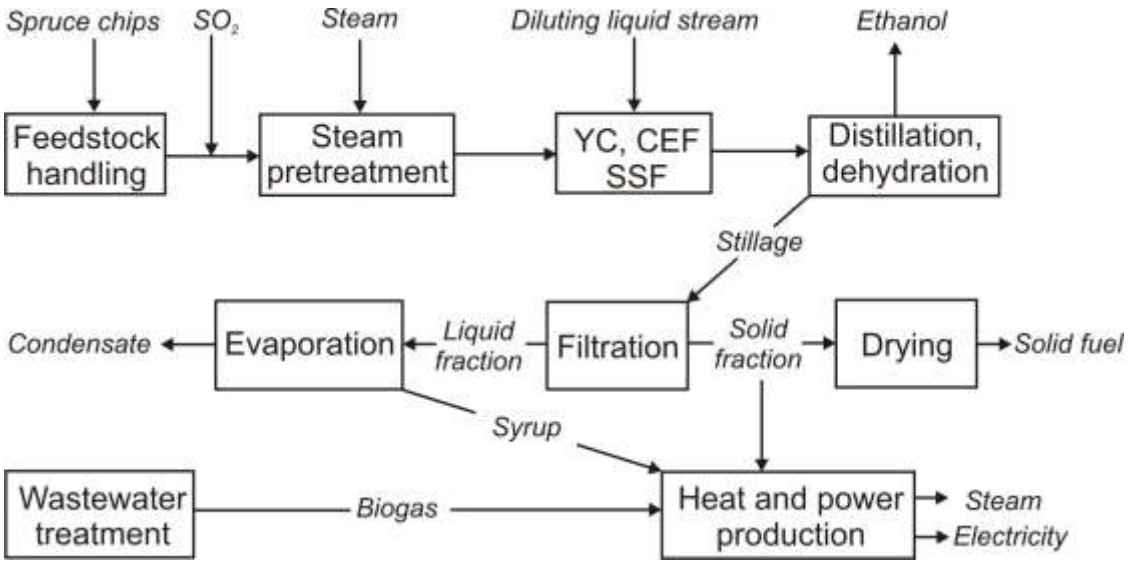

Figure 1. Overall process scheme for the proposed ethanol plant. In the reference case there was no enzyme production, the enzymes were purchased. CEF: cellulase enzyme fermentation, YC: yeast cultivation, SSF: simultaneous saccharification and fermentation.

According to the model calculations the ethanol content of the SSF broth is 3.8 wt-%, which corresponds to a concentration of 40.4 g/L in the liquid phase. Distillation and molecular sieve adsorption are used to produce pure (99.8 wt-%) ethanol. The distillation step consists of two stripper columns and a rectifier, which are heat integrated by operating at different pressures. The remaining water in the overhead vapour leaving the rectifier is removed in the dehydration columns that are regenerated with pure ethanol vapour. The regenerate is returned to the rectifier.

The stillage of the stripper columns is separated in a filter press resulting in a solid fraction with a WIS content of 40 %. The liquid fraction of the stillage is concentrated to 60 % DM in an evaporation system which contains five effects in a forward-feed arrangement, i.e. only the first effect is heated by live steam, the subsequent ones utilize the vapour from the previous effect, operating at higher pressure. Boiling point elevation was accounted for [38], and overall heat transfer coefficients were estimated to vary between 500 and 2000 $W/m^2°C$, depending on the temperature and concentration of the liquid. Based on the work of Olsson et al. [39] , it was assumed that by applying a stripper column after evaporation, recycling of part of the evaporation condensate to dilute the whole slurry was possible. The rest of the condensate is sent to the wastewater treatment facility, where together with the condensed flash streams mainly originating from the pretreatment, it is treated by anaerobic digestion followed by an aerobic step [6]. It was assumed that 50 % of the chemical oxygen demand (COD) was converted with a yield of 0.35 m^3 methane/kg COD consumed.

Steam and electricity are generated by burning the biogas, the concentrated liquid fraction and part of the solid fraction of the stillage in the combined heat and power plant (CHP). The generated steam is allowed to expand to 4 bar through the turbine system, however, part of the steam is withdrawn at 20 bar for pretreatment and drying. The heat from flue gas condensation could be utilized by integrating a district heating system with the heat and power producing facility, however, this was not included in the model. The excess solid residue, i.e. the solid fraction not

required for steam generation, is dried in a superheated steam dryer to 88 % DM. The secondary steam generated by drying is utilized in the process.

2.3 REFERENCE CASE

In the reference case, no enzyme production was modelled. Instead enzymes were assumed to be purchased from an external plant and added directly to the SSF.

Information on industrial large-scale production of cellulases cocktails is scarce and aggregated. In general, the main steps of enzyme production are i) production by microorganisms using inputs of carbohydrates, protein, mineral salts and vitamins, followed by ii) recovery of enzyme liquor, and iii) formulation of enzyme product [40]. For the purpose of this study, the main important differences between enzyme production in integrated scenario and at a central facility, are the treatment steps applied to refine and stabilize the enzymes intended for use elsewhere (see e.g. [36]). However, since no cost breakdown is available for the purchased enzymes and data in general is available in an aggregated form, purchased enzymes are analysed in less detail.

For the purchased enzymes, data are based on the commercially available cellulase enzyme cocktail Cellic CTec3 from Novozymes A/S. As there is no official data for Cellic CTec3 dosage when applied in lignocellulosic ethanol production, assumptions regarding specific activity and dosage have been made. Assuming 213 FPU ml^{-1} for the cocktail [41, see also 42] and a density of 1.1 g ml^{-1} (valid for CTec2, the predecessor product) [43], enzyme dose is calculated to 30.4 g enzyme cocktail kg^{-1} DM.

2.4 DESCRIPTION OF ENZYME FERMENTATION

For modelling of integrated enzyme production, process data for CEF were obtained from the literature [44,45]. However, some key-assumptions were also made. The applied *Trichoderma* strain was assumed to be able to produce cellulase enzymes in the presence of monosaccharides, i.e. it was not catabolite-repressed (e.g. *T. reesei* RUT C30). The mycelium, soluble protein and activity yields were 0.27 g, 0.26 g, 185 FPU per g carbohydrate in anhydro equivalent [45], respectively, which resulted in a specific activity of 710 FPU/g protein. After complete hydrolysis of polysaccharides, all the monosaccharides are consumed entirely in CEF, while other compounds are not involved in any reaction. Based on the work of Szengyel et al. [46], it was assumed that inhibition due to compounds present in the pretreated material, such as furan derivatives and organic acids, did not occur.

The 5 % inoculum is received from the second stage of a two-stage seed train. Both stages operate with 5 % inoculum at a cycle time of 30 h. The first stage receives inoculum from a stock culture, while the second is inoculated with the broth of the first. Concerning the composition, the seed stages are assumed to be run on the same feed as the production stage, where 120 h cycle time is presumed. As this time is double of the cycle time of SSF, the number of vessels is 24 in the enzyme production stage. Considering the ratio of the cycle times of seed and production stages, the number of seed vessels is 6 in both stages. In all scenarios these numbers were kept constant, hence the total vessel volume varied in the production stage. The fermentors of the seed train are pressure-rated, and can be sterilized at 120°C, however, it was assumed at the production stage, that sterilization was not necessary. Cleaning-in-place is sufficient, since the evaporation condensate and the pretreated material were considered to be sterile and the fresh water added

before pressing the pretreated slurry is sterile-filtered beforehand. Furthermore, the nutrients (soy-meal 0.5 %, $(NH_4)_2SO_4$ 0.15 %, KH_2PO_4 0.07 %, $FeSO_4 \cdot 7H_2O$ 0.001 %) and the molasses were assumed not to cause any contamination, so the seed vessels can be sterilized empty. At all stages, 30°C and pH 5 are kept. The feed is cooled down in a heat exchanger and the heat released during fermentation is removed by cooling water that circulates in jackets at the first stage and in coils at later stages. The cooling jacket is favourable in terms of cleaning, however, it is not sufficient at larger volume. The pH is controlled using ammonia. Aeration of 0.5 VVM was assumed to ensure sufficient agitation. The whole broth containing mycelia and enzymes is added to SSF. This can be done, since SSF is carried out at 37°C, and above 35°C the growth of mycelia is completely inhibited [45].

2.5 ENZYME FERMENTATION CONFIGURATIONS

Two configurations, denoted with A-B, were investigated in the model of enzyme fermentation (Figure 2). They differed in the carbon source: in configuration A part of the liquid fraction of the diluted slurry was used, while in configuration B the liquid fraction was supplemented with molasses to increase the sugar content. In the scenarios denoted with "+", the specific activity of the soluble proteins was enhanced 1.5-fold, resulting in an increase of 50 % in the productivity in terms of enzyme activity, while protein and mycelium yields remained the same.

The liquid fraction also contained water-insoluble particles, as a WIS retention of 99 % was assumed in the filtration of the slurry. In the CEF feed the total carbohydrate content expressed in monomer equivalent (ME) and WIS concentration, in parentheses, were the following: A: 4.6 % (0.5 %), A+: 4.7 % (0.7 %), B: 10 % (0.8 %), B+: 10 % (0.9 %). In scenario B, molasses served as a complex nutrient source, hence nutrient supplementation was omitted.

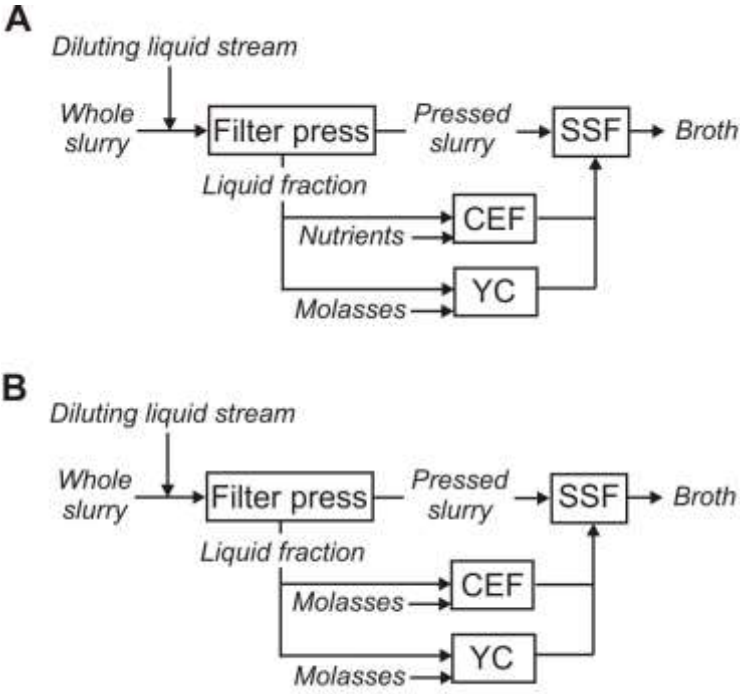

Figure 2. Lay-out of cellulase enzyme fermentation (CEF), yeast cultivation (YC) and simultaneous saccharification and fermentation (SSF).

2.6 ANALYSIS METHODS

Mass and energy balances were solved using the commercial flow sheeting program Aspen Plus V8.0 (Aspen Technology, Inc., Cambridge, MA). Physical property data for biomass components such as polysaccharides and lignin were derived from the NREL database [47]. Fixed capital investment (FCI) costs were estimated either with Aspen Process Economic Analyzer V8.0 (Aspen Technology, Inc.) or from vendor quotation. The construction material was assumed to be 304 stainless steel for all process vessels. To obtain the annual FCI, an annuity factor of 0.11 was used, corresponding to a depreciation period of 15 years and an interest rate of 7 %. Working capital investment (WCI) was calculated according to the recommendations in literature [48]. Annual WCI is the product of WCI and interest rate.

All costs are presented in Swedish kronor (SEK, 1 US$ ≈ 8.3 SEK, 1 € ≈ 9.2 SEK). In the reference case the purchase price of enzyme is 33 SEK per million FPU, which was obtained by updating the estimate of a previous study [49]. Purchase prices of raw material, nutrients, chemicals, and utilities, costs of labour, insurance, maintenance, and selling prices of co-products are listed in Table 1.

In the economic analysis minimum ethanol selling price (MESP) and annual cash flows are calculated. The former refers to the ethanol price at the break-even point, that is at this price the annual cost and the annual income are equal. The annual cash flows show the difference between the annual cost and annual income, and in this case the annual income is calculated at an ethanol selling price of 5.5 SEK/L.

Table 1. Purchase prices of raw material, nutrients, chemicals, utilities, costs of labour, insurance, maintenance, and selling prices of co-products [6,13].

Type	Input or product	Purchase or selling prices	
Raw material	Spruce	560	SEK/dry tonne
Chemicals	SO_2	1.5	SEK/kg
	H_2SO_4	0.5	SEK/kg
	NH_3 (25 %)	2	SEK/kg
	H_3PO_4 (50 %)	5	SEK/kg
	Defoamer	20	SEK/kg
	$(NH_4)_2HPO_4$	1.5	SEK/kg
	$MgSO_4 \cdot 7\ H_2O$	4.4	SEK/kg
	Molasses	1	SEK/kg
	Soy-meal	1.5	SEK/kg
	$(NH_4)_2SO_4$	0.9	SEK/kg
	KH_2PO_4	1	SEK/kg
	$FeSO_4 \cdot 7H_2O$	1	SEK/kg
Utilities	Electricity	450	SEK/MWh
	Cooling water	0.1	SEK/m^3
	Process water	1.4	SEK/m^3
Other costs	Labour	600 000	SEK/employee/year
	Insurance	1	% of annual fixed capital
	Maintenance	2	% of annual fixed capital
Co-products	Pellets	1150	SEK/dry tonne
	Electricity, spot price	350	SEK/MWh
	Electricity certificate	200	SEK/MWh
	CO_2	30	SEK/tonne

2.7 LIFE CYCLE ASSESSMENT

Life cycle assessment (LCA) results for biofuels have proven largely affected by methodological choices such as that of allocation procedure and handling of different co-products [50-53]. This study applies two methodological approaches; one following the standardized methodology of ISO 14040 and 14044 [54, 55], and the other following the methodology presented in the EU renewable energy directive (RED) [56]. These are referred to as the ISO and RED method, respectively. Where the ISO method offers a frame and structure for the LCA with recommendations regarding methodological considerations, the RED method goes further into stating how the calculation of environmental impact in terms of GHG emissions from biofuel systems is to be conducted [56]. While the main focus of this study is to evaluate a comparison of scenarios with on-site or purchased and centralized enzyme production, the inclusion of two methodological approaches allows for more reliable results regarding the importance of enzyme production to the life cycle of lignocellulosic ethanol.

The RED method is designated to calculations of GHG emissions. For the purpose of this study, the RED system boundaries and assumptions are also applied to calculate primary energy efficiency in the ethanol systems.

Both calculation methods call for sensitivity analyses in which the sensitivity of results to changes in different parameters is tested. Focus is directed towards the comparison of integrated versus purchased enzymes, and related input data and assumptions are the main objects for scrutiny. As for the different calculation approaches applied, the choice of the two methods can be regarded as a sensitivity analysis in itself. The importance of choices regarding system expansion and crediting potential co-products in the ISO method is discussed only briefly, again motivated by the main focus on enzyme production.

2.7.1 Multi-functionality and allocation

Multi-functionality in a studied system can be handled in different ways. According to the ISO order of priority, expansion of the system to include co-products is preferred prior to allocation based on physical or economical relationships [55]. For the ISO method, we apply substitution which is a form of system expansion where co-products are assumed to provide an added function in the expanded system, thus substituting corresponding products. The avoided impacts from substituted products are credited to the main product. In the RED method, environmental impacts are allocated to co-products based on lower heating value (LHV) [56]. Electricity is regarded as a co-product if generated from by-products or waste at the plant, and in other scenarios it is assumed to substitute grid electricity [56].

2.7.2 System description and functional unit

According to RED methodology, wastes and agricultural crop residues used as feedstock in biofuel production should not be burdened by GHG emissions from activities prior to its collection [56]. The directive also states that neither CO_2 uptake during cultivation nor CO_2 emissions from combustion of biofuels are to be included. For the ISO method, we assume that emissions from combustion are cancelled out by CO_2 uptake during biomass growth, and that no impact from forestry is allocated to forest residues. However, the collection of residues may affect the soil carbon content, as a result of removing biomass from the forest (see e.g. [57]). This aspect is not

included in the calculations but is brought up in the discussion. The reason for this is that the primary focus of this study is on integrated versus centralized enzyme production, and the potential improvements in energy efficiency and GHG performance. Removal of forest residues may also affect nutrient balances negatively, although ash recovery can help reduce the issues [58]. The importance of including other parameters than enzymes production in a complete analysis is illustrated further in the sensitivity analysis of LCA results.

The studied systems deliver electricity and lignin solid fuel as co-products. For the ISO method we assume an expanded system where electricity is delivered to grid, replacing Swedish electricity mix. Lignin solid fuel is assumed to replace wood-based pellets in the base case, based on an assumption of competing interests for forest residues for biofuel and biobased heat [59]. The avoided impacts are credited to ethanol. For the RED method, co-products (including electricity) are handled by allocating GHG emissions and energy use based on LHVs. Figure 3 shows the studied systems according to ISO and RED methods.

According to RED methodology, the functional unit (FU) to which environmental impact is related is 1 MJ of fuel using the LHV [56]. For the purpose of comparison, the FU is 1 MJ bioethanol (LHV) in both ISO and RED calculations. GHG performance is calculated as global warming potential (GWP) with a 100 year time frame. Emissions of CO_2, CH_4 and N_2O are taken into account, where 1 g of CH_4 and N_2O is regarded as 34 and 298 g CO_2-equivalents, respectively [60].

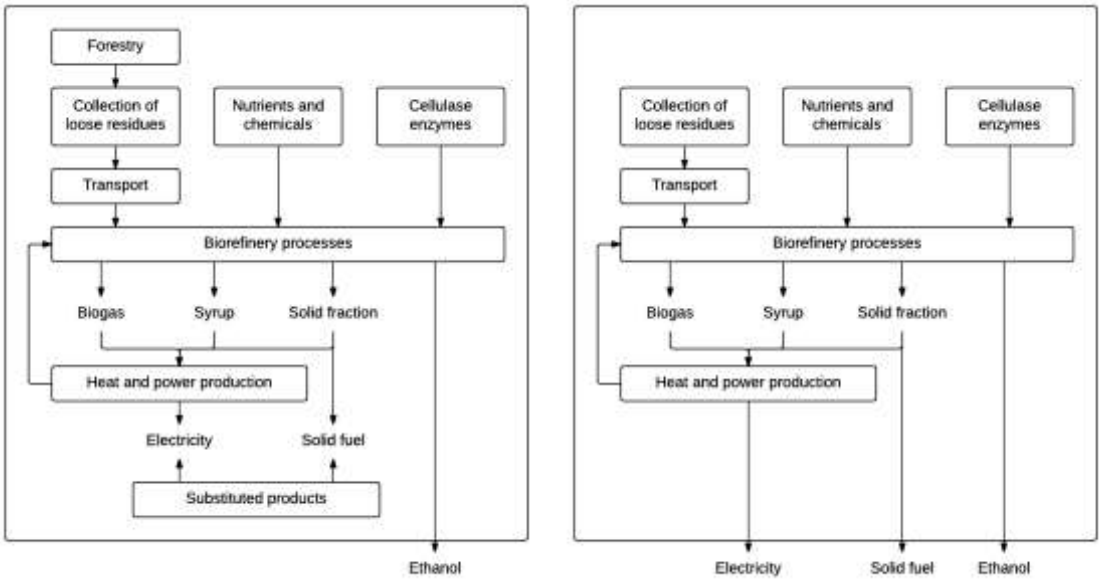

Figure 3. Studied system according to ISO (left) and RED (right) methods. Developed from [31] and [49].

2.8 INVENTORY

2.8.1 Collection and transportation of feedstock

Both methods assume collection, forwarding, loading, unloading, comminution and transport of feedstock as loose residues according to Lindholm et al. [61, 57]. We assume collection of feedstock as loose residues in Northern Sweden where transportation distance is 138 km. Potential changes in soil organic carbon due to removal of residues [57] are not accounted for but included in the sensitivity analysis.

Assuming 19.2 MJ kg^{-1} DM for forest residues, GHG emissions related to harvest and transport activities are 65 g CO_2-eq. kg^{-1} DM and 42 g CO_2-eq. kg^{-1} DM collected, respectively [57]. Energy input is 0.25 MJ kg^{-1} DM for harvest and 0.25 MJ kg^{-1} DM for transport [61].

2.8.2 Enzyme and nutrients

Table 2 shows all nutrient and enzyme inputs to the studied system in each case. Scenarios A and A+, and B and B+, assume different carbon sources for in-house enzyme production, and scenarios A+ and B+ assume an increased enzyme activity of 50 %. Thus the input of nutrients and chemicals differ between all cases.

The case of purchased enzymes from centralized production uses assumptions based on a commercially available cellulase enzyme cocktail, with data referring to the Cellic CTec3 product from Novozymes A/S.

Updated carbon footprint data for Cellic CTec3 was provided by Novozymes A/S [62]. The data refers to aggregated GHG emissions from production at the company site in North Carolina, United States, which amounts to 5.5 kg CO_2-equivalents per kg product. Previously released data from Novozymes also contains information on the total input of fossil primary energy to the production process. As presented in [31], fossil primary energy input is 100 MJ per kg formulated product based on aggregated data from 2012, where the corresponding data for GHG emissions is 8 kg CO_2-equivalents per kg product. For the purpose of this study, assumptions regarding primary energy input to cellulase production are necessary:

- The total primary energy input is assumed to correspond to the reported input of fossil primary energy. The source of electricity in production is natural gas [62], while the source for heat is unknown.
- The primary energy input to enzyme production is assumed to be 69 MJ per kg enzyme product. This assumption is based on the update of carbon footprint from 8 to 5.5 kg CO_2-equivalents per kg product, a reduction by 31 %, and the previously reported data on fossil primary energy input of 100 MJ per kg enzyme product. Thus the update of (fossil) primary energy input is assumed to follow the reduction of carbon footprint.

Note that data for external enzyme production is available only on an aggregated form, showing the total input of primary energy and total emissions of GHG. Alternative input data and assumptions are tested in the sensitivity analysis.

For calculations of primary energy input, the energy for producing all chemicals and nutrients in Table 2 is included. Regarding energy content of the products, only the energy content of molasses (13.6 MJ kg^{-1} DM based on Aspen modelling) and enzymes (assuming 10 % protein concentration and 11.2 MJ kg^{-1} protein, based on Aspen modelling) is regarded.

Table 2. Input data for chemicals, nutrients and enzymes.

Input	kg CO_2-eq. kg^{-1}	MJ primary energy kg^{-1} (for production)	Source
SO_2 (as S)	0.84	7.8	[63]
Ammonia (as N)	3.23	11.1	[64]
Phosphoric acid (as P)	0.95	5.52	[63-64]
Antifoam	1.33	24.4	Mean value based on [65] and [66]
$(NH_4)2HPO_4$	0.87	8.19	Based on data for diammonium phosphate [63-64]
$MgSO_4$	0.308	3.65	[63]
Molasses	0.142	0.57	[67, 31]
Soybean oil meal	0.8	5.95	[67]
$(NH_4)_2SO_4$	2.76	7.78	[63]
KH_2PO4	1.39	7.96	[34]
$FeSO_4*7H_2O$	0.284	1.13	Data for $FeSO_4$ [63]
Enzymes	5.5	69[1]	[62], estimation based on [31] and [62]

[1] Carbon footprint was re-evaluated by Novozymes A/S from 8 to 5.5 kg CO_2-eq. kg^{-1} enzyme cocktail, a reduction by 31 %. Fossil energy use was 100 MJ kg^{-1} cocktail, from which the estimate here is reduced by 31 %.

2.8.3 Other input data

Table 3 presents electricity and fuel data used in calculations of GWP and primary energy input.

In all calculations, the Reference case assumes natural gas based electricity for the external production of cellulase enzymes as this is included in aggregated input data. Alternative assumptions and data are tested in the sensitivity analysis. Case A, which is the only case where grid electricity is used as input to the bioethanol production, assumes Swedish electricity mix as the proposed ethanol plant is assumed to be located in Sweden. For the same reason, exported electricity is assumed to substitute Swedish electricity mix in all ISO calculations. These assumptions are tested in the sensitivity analysis, where grid electricity is assumed to correspond to natural gas based electricity instead.

For base case calculations with the ISO method, exported lignin pellets are assumed to replace wood residues as heating fuel (using the data for wood residues presented above). In the sensitivity analysis, lignin pellets are assumed to substitute hard coal for heating instead.

Table 3. Additional input data for LCA.

		g CO_2-eq. MJ^{-1}	Primary energy factor	Source
Base case	Swedish electricity mix	10.1	2.1	[68]
Sensitivity analysis	Natural gas based electricity	124	1.9	[68-69]
	Hard coal	106	1.15	[68]

3 RESULTS AND DISCUSSION

3.1 MASS AND ENERGY BALANCES

The sulphur dioxide has the same mass flow in all the scenarios, since the raw material input is identical (Table 4). The ammonia demand is greater in the CEF scenarios than in the Reference case, as extra ammonia is required in the CEF, which is directly proportional to the total mass flow fed to the CEF. The phosphoric acid is added in the WWT, and its mass flow is equal in all the scenarios. The addition of antifoam in the ethanol fermentation is based on experimental data, and is directly proportional to the produced ethanol. The antifoam requirement of the CEF is assumed to be equal to that of ethanol fermentation. The mass flows of $(NH_4)_2HPO_4$ and $MgSO_4$ depends on the mass flow fed to the SSF, and therefore they are constant. The molasses addition does not differ significantly in the Reference case and Scenarios A and A+, since in these scenarios the molasses is required only for the yeast fermentation. In Scenarios B and B+ the CEF also utilises molasses, and due to the higher activity yield, in Scenario B+ less molasses is added than in Scenario B. In Scenarios A and A+ the mass flows of soybean oil meal, $(NH_4)_2SO_4$, KH_2PO_4 and $FeSO_4*7H_2O$ are directly proportional to the total mass flow fed to the CEF. The mass flow of enzyme preparation is obtained by assuming a volumetric activity of 213 FPU/ml and a density of 1.1 g/ml.

Among the scenarios electricity is imported only in Scenario A: the total mass flow fed to the CEF and consequently the power consumption of the compressor are the highest in this scenario. The cooling water demand differs because of the heat released in the fermentations. It is the lowest in the Reference scenario due to the lack of the CEF. In Scenarios A and B the cooling water usage is higher than in Scenarios A+ and B+, respectively, since at lower activity yield more carbohydrate is metabolised to reach the given activity level, that is more heat is released. The process water demand varies in a narrow range, and refers to the make-up water used in the CHP, as the diluting water added to the pretreated slurry before filter pressing is recycled from the evaporation. The make-up water is used to generate steam directly injected in the pretreatment. However, vapours of the dryer are also recycled to the pretreatment. The more solid fuel is produced, the more vapours the dryer generates, consequently the less make-up water is required by the CHP for steam generation for the pretreatment.

The enthalpy flow of solid fuel is higher in the case of molasses addition in the CEF (Scenarios B and B+). The highest ethanol production is obtained in the Reference case, and the more carbohydrate is consumed from the liquid fraction of the diluted slurry in the CEF, the less ethanol is produced. The carbon dioxide is evolved in the yeast cultivation, SSF and CEF, and there is higher total mass flow of carbon dioxide in the CEF scenarios than in the Reference case. The electricity export depends on the power consumption of compressor of the CEF, which is lower at lower total mass flow fed to the CEF. The mass flow of cleaned water from the aerobic treatment is lower in the CEF scenarios, as in these cases more water is recycled from the evaporation to dilute the pretreated slurry than in the Reference case.

Table 4. Material and energy flows of the modelled cases.

		Reference	A	A+	B	B+
Spruce dry matter	kg/h	25000	25000	25000	25000	25000
Sulphur dioxide	kg/h	641	641	641	641	641
Ammonia (25 %)	kg/h	641	699	679	668	659
Phosphoric acid (50 %)	kg/h	5.6	5.6	5.6	5.6	5.6
Antifoam	kg/h	13	24	24	24	25
$(NH_4)_2HPO_4$	kg/h	74	74	74	74	74
$MgSO_4$	kg/h	3.7	3.7	3.7	3.7	3.7
Molasses	kg/h	885	886	887	1807	1497
soybean oil meal	kg/h	0	97	63	0	0
$(NH_4)_2SO_4$	kg/h	0	29	19	0	0
KH_2PO_4	kg/h	0	14	9	0	0
$FeSO_4*7H_2O$	kg/h	0	0.19	0.13	0	0
Enzyme preparation	kg/h	761	0	0	0	0
Electricity imported	MW	0	0.2	0	0	0
Cooling water	m3/h	1961	2079	2041	2086	2048
Process water	m3/h	12.5	12.4	12.5	12.2	12.3
Ethanol	L/h	6758	6360	6493	6584	6640
Solid fuel	MW (LHV)	28.0	28.5	28.1	30.2	29.1
Carbon dioxide	kg/h	5731	5906	5847	6085	5967
Electricity exported	MW	2.4	0	0.6	1.1	1.5
Water from aerobic treatment	m3/h	29	27	27	27	27

3.2 ECONOMICS: MINIMUM ETHANOL SELLING PRICE, ANNUAL CASH FLOWS

In regard to MESP, case B+ was the most favourable, furthermore, this was the only scenario with on-site enzyme production, in which the MESP was lower than that in the reference case, with purchased enzymes (Figure 4). In spite of the extra expenses, molasses could improve the process economics considerably, since CEF supplemented with molasses reduced the overall ethanol yield, the most important parameter in the production cost of ethanol [70], to a smaller extent.

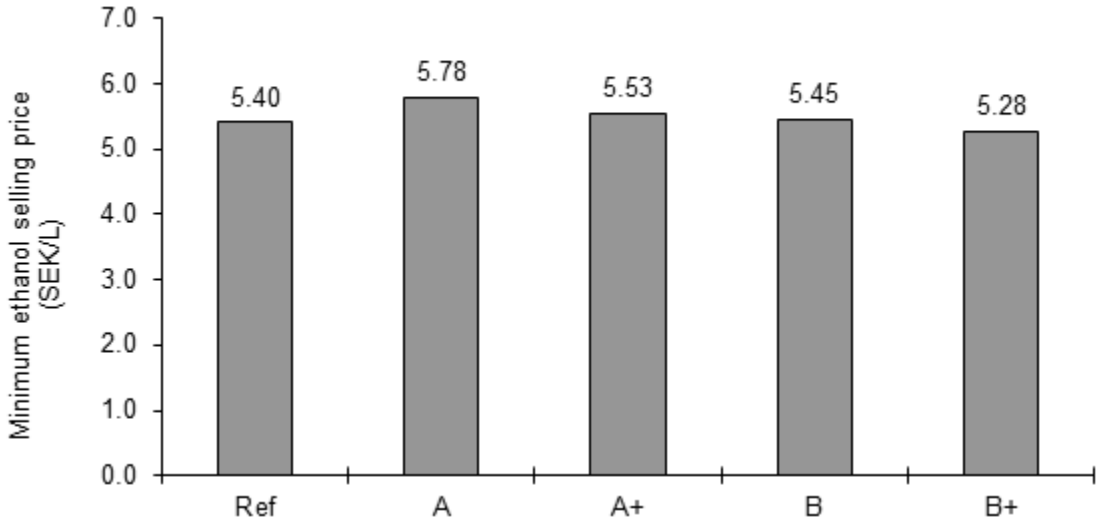

Figure 4. Minimum ethanol selling price. Carbon source A: pretreated liquid fraction, B: pretreated liquid fraction and molasses; +: 1.5-fold specific activity; Ref: reference case with purchased enzyme preparation, SEK: Swedish kronor.

Annual cash flows are presented in Table 5, calculated for a selling price of ethanol of 5.5 SEK/L. The CEF increased the capital costs significantly (11-14 %) compared to the reference case. The second largest cost contributor after the capital cost was the raw material cost, which did not change due to constant annual capacity. Also these costs have been proved to be the main contributors to the production cost of lignocellulosic ethanol in previous, similar studies [3,6,7,13]. Chemical expenses increased by 12-34 % compared to the reference case. The utility costs were the lowest among the cost elements in each scenario, since only process and cooling water had to be purchased, as steam and electricity were generated on-site.

Table 5. Annual costs, revenues and profit of the proposed ethanol plant in million Swedish kronor (MSEK). Carbon source A: pretreated liquid fraction, B: pretreated liquid fraction and molasses; +: 1.5-fold specific activity.

	Reference	A	A+	B	B+
Annual cost (MSEK)					
Raw material	99.5	99.5	99.5	99.5	99.5
Capital	157	183	180	178	175
Chemicals	28.3	32.4	31.7	38.0	35.4
Enzymes	39.0	-	-	-	-
Utilities	2.3	3.3	2.4	2.5	2.4
Other	21.4	22.2	22.1	22.0	21.9
Total	348	340	335	340	334
Annual income (MSEK)	0	0	0	0	0
Ethanol	297	280	286	290	292
Co-products except electricity	45.1	45.9	45.3	48.5	46.8
Electricity	10.5	0.0	2.6	4.7	6.6
Total	353	326	334	343	346
Annual profit (MSEK)	5.2	-14.5	-1.6	2.9	11.8

Ethanol, the main product, gave 84-86 % of the annual revenues. Co-products in Table 5 refer to solid fuel, i.e. the dried excess solid residue, and the carbon-dioxide produced in CEF, YC and SSF, which was also assumed to be marketable. Solid fuel contributed to 97 % of the co-products income in each scenario. While steam generation met the steam requirement of the process, produced electricity was consumed on-site only partially, except Scenario A, where electricity needed to be purchased. The highest profit was achieved in scenarios B+, where the MESPs were the lowest. Table 5 clearly shows the importance of co-product and electricity revenues, since the income of ethanol does not exceed the expenses.

As the enzyme price is uncertain to a great extent, sensitivity analysis of MESP was performed in the Reference case: MESP was plotted as a function of enzyme price in SEK/MFPU (Figure 5 a) and SEK/kg enzyme preparation (Figure 5 b). Using the equations of the fitted curves (Figure 5 a and b) the MESP of the Reference case can easily be adjusted to other enzyme prices, hence the feasibility of the CEF scenarios can be assessed at any enzyme price.

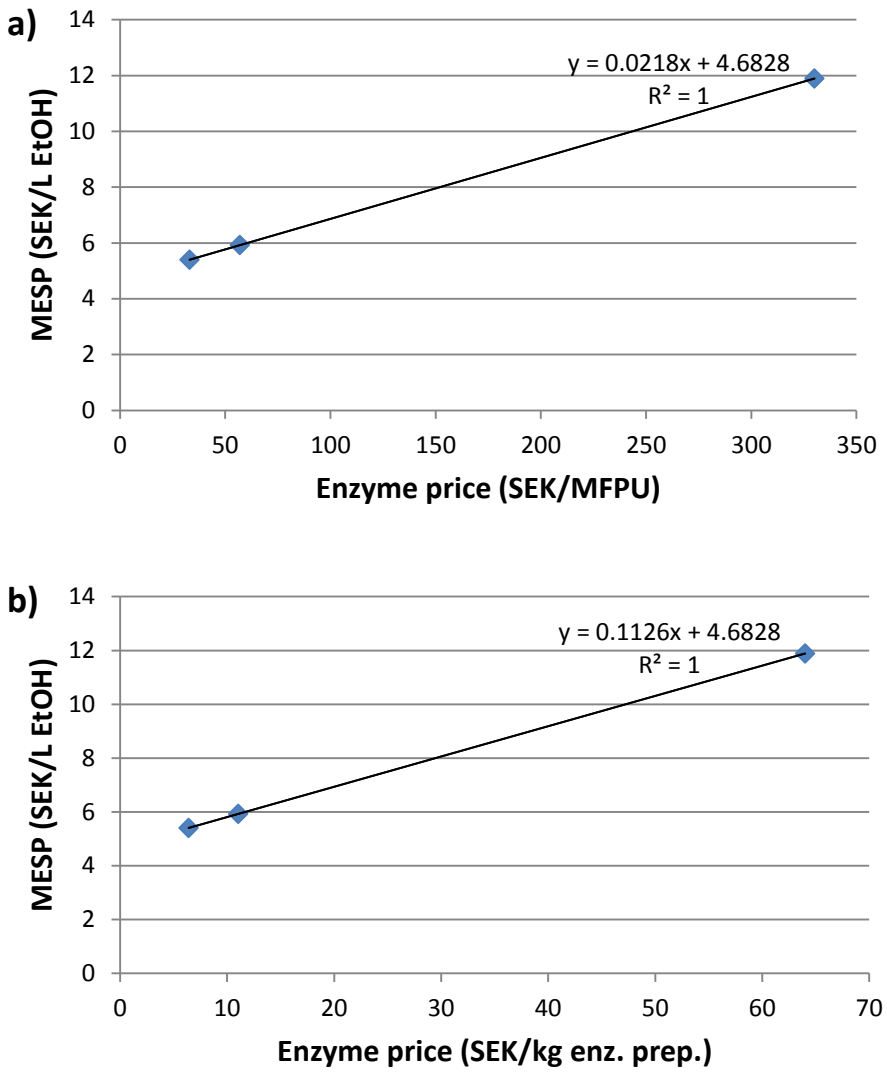

Figure 5. Minimum ethanol selling price as a function of enzyme price in SEK/MFPU (a) and SEK/kg enzyme preparation (b)The enzyme loading of simultaneous saccharification and fermentation was 10 FPU/g water insoluble solid. (M)FPU: (million) filter paper activity unit, SEK: Swedish kronor.

3.3 GREENHOUSE GAS EMISSIONS

With base case assumptions, case B+ showed the lowest emissions of GHG's and consequently the lowest GWP (Figure 6). The Reference case resulted in significantly higher GWP than other cases. Purchased enzymes contributed with 18 to 30 g CO_2-eq. per MJ of ethanol (RED and ISO methods, respectively). Results for GHG emissions are lower using the RED method.

GHG emissions for all cases and both calculation methods, showing relative contribution from different processes, are shown in Figure 6. For illustration purposes, "Chemicals" include SO_2, NH_3, H_3PO_4, antifoam, diammonium phosphate and $MgSO_4$. "Nutrients" include soybean oil meal, diammonium sulphate, KH_2PO_4 and $FeSO_4*7H_2O$ (only occurring in cases A and A+).

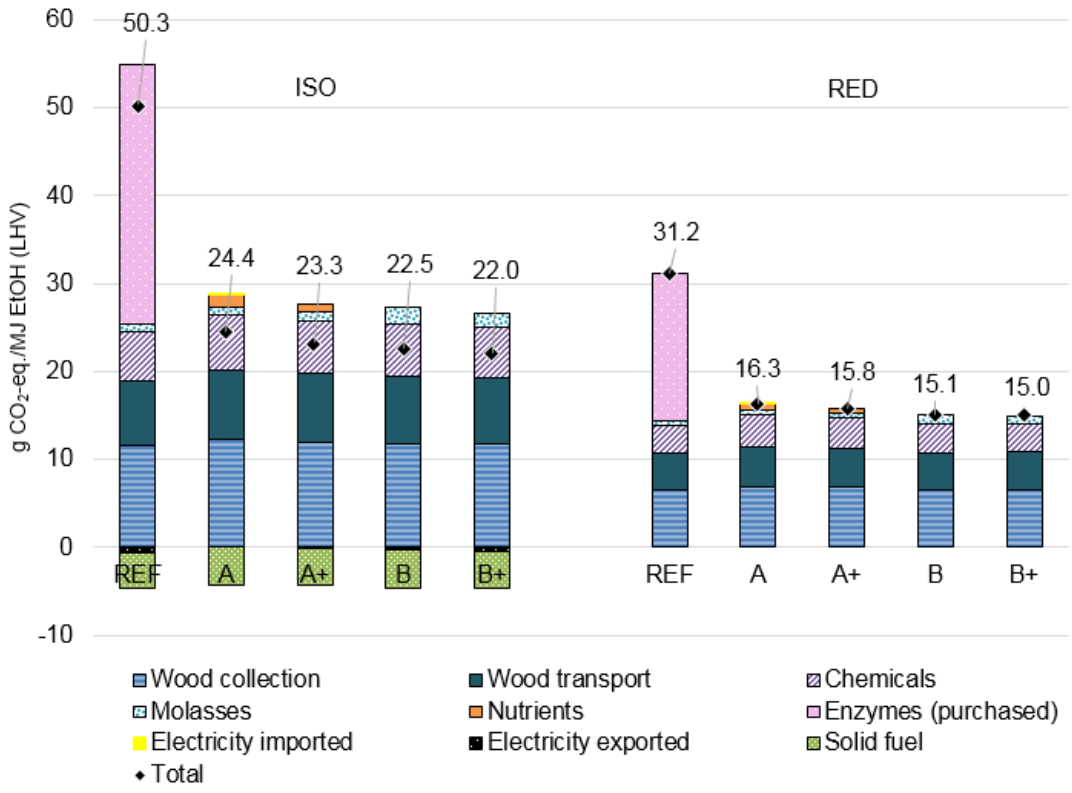

Figure 6. GHG performance (expressed as GWP) for the different ethanol production systems assessed and according to the ISO method (left) and the RED method (right), respectively.

3.4 PRIMARY ENERGY EFFICIENCY

Case B+ showed the lowest primary energy input per MJ of ethanol, and the Reference case showed the highest. Feedstock energy was the main contributor to primary energy input for all cases.

Primary energy balance for all cases and both methods, showing relative contribution from different processes, is shown in Figure 7.

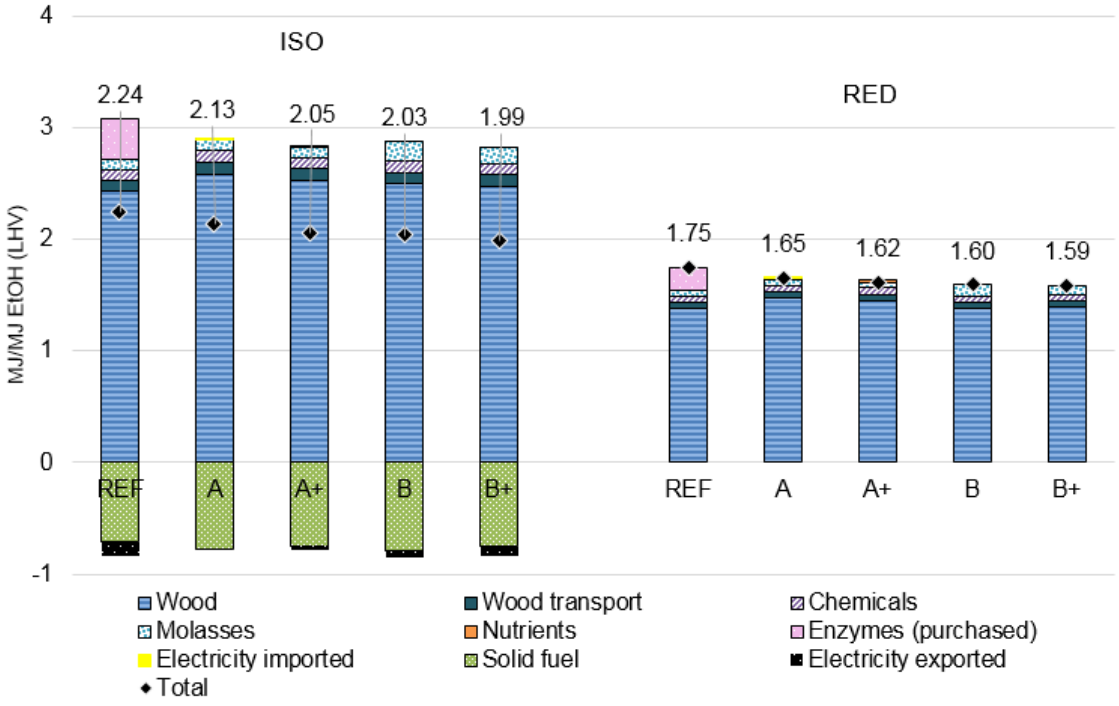

Figure 7. Primary energy balances for the different ethanol production systems assessed and according to the ISO method (left) and the RED method (right), respectively.

The overall primary energy efficiency, expressed as primary energy output over input, differed by 6 percentage units between the lowest and highest case (Table 6). With the ISO calculation method, the primary energy efficiency was 45-50 %, and with the RED calculation method it was 57-63 %.

Table 6. Results for primary energy efficiency (primary energy output as % of primary energy input) for ISO and RED.

Method	**ISO**					**RED**				
Case	REF	A	A+	B	B+	REF	A	A+	B	B+
Primary energy efficency	45	47	49	49	50	57	61	62	63	63

3.5 SENSITIVITY ANALYSIS OF LCA RESULTS

We analyze the robustness of the results in two parts. Firstly the comparison of on-site with purchased enzymes from centralized production is analyzed and discussed in terms of the improvement of centralized production necessary to match the environmental performance of on-site scenarios. Secondly, the sensitivity of results to changes in methodological assumptions such as substituted co-products is tested.

3.5.1 Impact from purchased enzymes

Ethanol from the Reference case with purchased enzymes results in higher GHG emissions and lower primary energy efficiency than the cases with on-site enzyme production. Assuming that all other factors remain unaltered, different reductions of enzyme related impact are necessary to make the Reference case match the environmental and energetic performance of integrated cases. The environmental impact and primary energy use from purchased enzymes could potentially be reduced as result of future improvements, e.g. regarding enzyme activity, energy efficiency or substitution of fossil energy in the enzyme production with renewable alternatives. The size of the potential improvement related to different measures and time horizons is however connected to large uncertainties. A detailed assessment of the impacts from external enzyme production is complicated further by scarce and aggregated data.

In an attempt to relate to and discuss our results, we present the reduction of purchased enzymes' GWP resulting from choosing alternative input data in our calculations.

Enzyme dosage data is adapted from [31], alternative GHG emission values for enzymes from [34] and [35]. Using alternative GHG emissions values for enzyme production requires a different method to decide enzyme dose. For this sensitivity analysis we assume 65 FPU/g whole enzyme preparation containing 10 % enzyme protein according to [49]. In addition to this we test assumptions of 50 % increase in enzyme activity as well as using Swedish electricity mix in enzyme production, illustrating potential future improvements. While the 50 % increase in enzyme activity is based on previous improvements from earlier generations of the Novozymes A/S Cellic CTec product [62], the assumption of a Swedish electricity mix in the production could be seen as an example of a future scenario where the electricity used in production causes lower GHG emissions, e.g. at a Swedish plant or at any plant using a large share of renewable energy sources. Available data does not allow for a precise analysis and thus a rough estimation of electricity use in enzyme production is based on [40]. Electricity is estimated to contribute to roughly 40 % of GHG emissions from enzyme production. Replacing natural gas based electricity [40] with Swedish electricity mix is estimated to reduce GHG emissions by 70 %, thus a reduction of roughly one third of total emissions from enzyme production is accomplished.

The results from the sensitivity analysis are shown in Table 7, presented as percentage reduction of the GHG emissions from purchased enzymes in base cases. Using alternative data and assumptions in calculating GHG emissions from purchased enzymes, the emissions are reduced by 13 to 88 %.

Figure 8 presents the results in Table 7 together with total GWP base case results for all cases (ISO and RED methods). In Figure 8, lines illustrate total GHG emissions from the Reference case using alternative assumptions and data for the purchased enzymes, all other parameters and data identical to base case.

Table 7. Sensitivity analysis of the GHG emissions from purchased enzymes. With alternative data and assumptions, the GWP of purchased enzymes is reduced.

Alternative assumption and data	Reduction of purchased enzymes' GWP compared to base case
Alternative carbon footprint [34]	-13 %
Swedish electricity mix	-30 %
50 % increased enzyme activity	-33 %
Alternative enzyme dose [31]	-73 %
Alternative carbon footprint [35]	-88 %

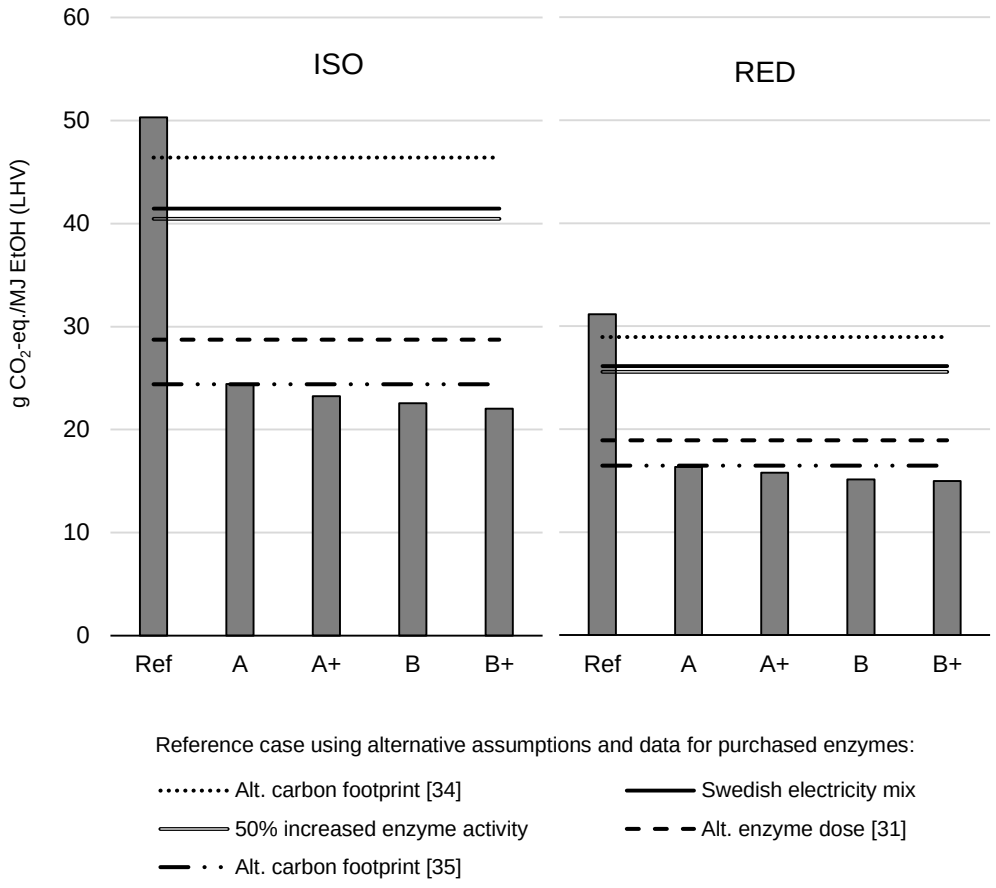

Figure 8. Bars illustrate base case results for GWP using ISO and RED methods. Lines illustrate GWP of the Reference case using alternative assumptions and data regarding purchased enzymes (all other parameters and data identical to base case).

In order for the Reference case to match the GHG emissions of integrated cases, the impact from purchased enzymes must be reduced by 88 to 96 % according to the ISO method, and by 88 to 97 % according to the RED method (assuming all other factors are constant).

With the GHG emissions value 16 kg CO_2-eq. kg^{-1} enzyme protein from [34], the GWP of the reference case is still higher than in all other cases. Assuming 2.3 kg CO_2-eq. kg^{-1} enzyme protein as in [35], the GWP of the reference case is reduced significantly, and is roughly equal to the GWP of case A using both calculation methods. In [35], co-location of enzyme and ethanol production is assumed, using part of the hydrolysate from ethanol conversion from switchgrass to grow *T. reesei* for cellulases production.

Applying the enzyme dose from [31] to the Reference case entails a 73 % reduction of the base case enzyme GHG emissions. Total GWP of the Reference case is still higher than all other cases, but the gap between them is reduced significantly. The dose in [31] was based on a Novozymes A/S dosage data sheet for cellulose conversion in corn stover.

Assumptions of 50 % increased enzyme activity and a Swedish electricity mix both lower the GHG emissions from enzymes by roughly 30 %. If these improvements are assumed simultaneously, the GWP from enzymes would be reduced by approximately half, leading to roughly 30 % reduction of total GWP in the reference case. Total GHG emissions are still higher in the Reference case than in cases with integrated enzyme and ethanol production.

Different available input data regarding the GHG emissions from external enzyme production [34, 35] indicate the significant uncertainties in estimating emissions. Dosage of purchased enzymes in lignocellulosic ethanol production is one significant uncertainty in assessing the total GHG emissions, as illustrated by adopting dosage data from [31]. The base case illustrates current status of enzyme production, using GHG emissions data based on actual plant performance, and enzyme activity of purchased cellulase cocktail as reported in literature. Data from [35] illustrates a future and partly integrated scenario where enzyme activity is higher and GHG emissions are lower, explaining the difference in results. On the other hand, enzyme dose as calculated in [31] does not make any assumptions of future improvements, and nevertheless results in a significant reduction of the Reference case's total GWP compared to base case.

3.5.2 Methodological choices

In order to test the sensitivity of the results to changes in assumptions and methodological choices, a set of sensitivity analyses were performed. In the different cases, sensitivity of GWP results related to inclusion of changes in soil organic carbon (SOC) due to the recovery of logging residues, dosage of purchased enzymes and substituted products were investigated (Table 8). The sensitivity of primary energy balances was tested using alternative assumptions for purchased enzyme dosage and activity, and solid fuel substituting coal (Table 9).

Table 8. Sensitivity analysis of the GHG performance (expressed as GWP) of the various ethanol production systems including changed assumptions and methodological choices.

g CO_2-eq. MJ^{-1} ethanol (LHV)	ISO					RED				
	REF	A	A+	B	B+	REF	A	A+	B	B+
Baseras	50	24	23	23	22	31	16	16	15	15
SOC 2-3 rotations	78	53	52	51	50	47	33	32	31	31
SOC 1 rotation	110	89	86	84	83	66	53	52	50	50
SOC 20 years	700	710	700	670	670	500	510	500	480	480
Ref. enzyme activity +50 %	41					26				
Solid fuel substitutes coal	-21	-64	-62	-68	-64					
Electricity substitutes natural gas based electricity[1]	43	24	21	19	18					

[1] Imported electricity in case A is also assumed to be natural gas based in this scenario.

The ISO GWP results are sensitive to assumptions regarding substituted products. Assuming that exported electricity replaces natural gas based electricity instead of Swedish electricity mix, decreases the total GHG emissions by 0-18 % in the different cases. However, it affects all cases except for case A where no electricity is exported, and the mutual relation between the Reference case and other cases, increases by 8 percentage units at most. If solid fuel is assumed to replace coal for heating, all cases result in negative GWP values. Again, all cases are affected and the GHG emissions remain higher in the Reference case than in all other cases.

Including potential effects on SOC according to [57] significantly increases GHG emissions in all cases. Based on the assumption that forest residues left to decompose increase the soil carbon storage of the forest, removing residues increases GHG emissions to the atmosphere. Because the degradation of biomass is time dependent results are also affected by the time horizon chosen, where shorter time spans increase the impact from SOC changes. Cases with integrated enzyme production use more feedstock per MJ ethanol produced, and are thus affected to a larger extent than the Reference case.

Assuming a long-term scenario of two to three rotation periods for changes in SOC, GHG emissions increase by approximately 54-126 % using the ISO method and by 50-104 % using the RED method. The Reference case still results in higher GWP than cases with integrated enzyme production. Assuming one rotation period the results increase further, still with the Reference case causing the highest emissions. With a time horizon of only 20 years, all GWP results exceed the RED fossil fuel reference of 83.8 g CO_2-eq. MJ^{-1}. Using this assumption, the Reference case has a lower GWP than case A but higher than case B, with either calculation method.

Table 9. Sensitivity analysis of the primary energy balances of the various ethanol production systems including changed assumptions and methodological choices.

MJ primary energy MJ^{-1} ethanol	ISO					RED				
	REF	A	A+	B	B+	REF	A	A+	B	B+
Base case	2.2	2.1	2.1	2.0	2.0	1.8	1.7	1.6	1.6	1.6
Ref. enzyme activity +50 %	2.1					1.7				
Enzyme alt. dosage (Karlsson et al. 2014)	2.0					1.6				
Solid fuel substitutes coal	2.1	2.0	2.0	1.9	1.9					

Because the main contribution to primary energy input comes with the feedstock, tested parameters have limited impact on results (5-12 % change from base case). However, with alternative assumptions regarding enzyme activity and enzyme dosage, the primary energy balance of the Reference case approximately levels with that of case B+. It should be noted that there was little information on the input data for primary energy input for purchased enzymes.

The focus of this study is on the comparison of different routes for cellulase enzyme production. Thus, the results should be used in this evaluation and not regarding optimized process design for lignocellulosic ethanol nor the environmental impact from ethanol produced in such an optimized process. Many factors must be considered to evaluate overall sustainability of a biorefinery design. For instance, burning of lignin solid fuel in-house could facilitate ash recovery, which may be crucial to ensure the sustainability of forest residue recovery. The necessity of including other factors in complete assessments and design of an environmentally sustainable and commercially viable biorefinery is therefore significant.

Assumptions regarding system expansion and crediting co-products by replacing alternative products are crucial to the end result for GWP of ethanol. However, in most cases they do not significantly affect the comparison of an integrated production approach versus centralized enzyme production performed in the present study. Inclusion of effects on soil organic carbon should be investigated further, as the end results for GWP show significant sensitivity to related assumptions.

4 CONCLUSIONS

In this project the environmental impact and the process economics of lignocellulosic ethanol production from spruce was evaluated, comparing scenarios where the hydrolytic enzymes are produced onsite to cases where enzymes are purchased from centralized facilities. The main question was if on-site and integrated enzyme production can help reduce the environmental impact of lignocellulosic ethanol production and improve the economics.

The results show little significant difference in the primary energy efficiency of integrated cases and using purchased enzymes. The GHG calculations show, however, that emissions from on-site enzyme production are lower than those from production at a centralized site, regardless of calculation method applied. These results are sensitive to assumptions regarding the purchased enzymes, e.g. dosage needed and energy sources utilized in production. The impact of purchased enzymes on GHG performance of bioethanol can be lowered by, for instance, replacing fossil energy sources with lower impact sources, or by lowering the enzyme dose needed. Drawbacks for the purchased enzymes are the refining and stabilization processes not needed in an integrated process. However, it is possible that separated processes could provide other benefits not investigated here, where focus is on the potential of an integrated process. Though our results indicate potential to lower GHG emissions of bioethanol with integrated enzyme and ethanol production, the comparison with purchased enzymes will be affected by future improvements and developments in the centralized enzyme production processes.

The feasibility of including enzyme production in the lignocellulosic ethanol process highly depends on the full-scale price of commercial cellulase enzyme preparation, which is still very uncertain. At the premises of the study, one scenario proved to be more feasible than that with purchased enzymes, which implies that on-site enzyme production can be an alternative considering the process economics. To achieve further cost reduction, the enzyme demand of SSF has to be decreased, whereas the activity yield and productivity, the two most important parameters of enzyme fermentation in terms of economics, have to be increased.

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