



# **The "Zero Miles Product" Concept Applied to Biofuel Production: A Case Study**

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**Abstract:** To make biofuel production feasible from an economic point of view, several studies have investigated the main associated bottlenecks of the whole production process through approaches such as the "cradle to grave" approach or the Life Cycle Assessment (LCA) analysis, being the main constrains the feedstock collection and transport. Whilst several feedstocks are interesting because of their high sugar content, very few of them are available all year around and moreover do not require high transportation' costs. This work aims to investigate if the "zero miles" concept could bring advantages to biofuel production by decreasing all the associated transport costs on a locally established production platform. In particular, a specific case study applied to the Technical University of Denmark (DTU) campus is used as example to investigate the advantages and feasibility of using the spent coffee grounds generated at the main cafeteria for the production of bioethanol on site, which can be subsequently used to (partially) cover the campus' energy demands.

Keywords: spent coffee ground; ethanol fermentation; biofuel



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# 1. Introduction

As defined by the European Union, "Biofuels are liquid or gaseous transport fuels such as biodiesel and bioethanol which are made from biomass" [1].

Bioethanol is the most common biofuel obtained by a fermentation process and can be run by using a variety of carbon sources. Based on the starting feedstock, biofuels are classified in three categories [2]:

- 1. First-generation biofuels, which are those produced from sources like starch, sugar, animal fats, and vegetable oils.
- 2. Second-generation biofuels, also known as advanced biofuels, produced from feedstocks which refer to non-food biomass (such as by-products and residues from agriculture, forestry, and related industries, as well as the non-fossilized and biodegradable organic fractions of industrial and municipal wastes).
- 3. Third-generation biofuels. These are obtained from aquatic autotrophic organisms (such as algae, which can use light and carbon dioxide to grow).

Biofuels production from lignocellulosic biomasses takes three main steps: biomass pretreatment (to release the fermentable sugar fraction), fermentation, and recovery [3].

During the biomass pretreatment, the feedstock is sequentially both physico-chemically and enzymatically treated. During the physico-chemical treatment, the lignin is broken down in order to increase the enzymes accessibility (enzymatic treatment) to the polysaccharides for the fermentable sugars release [3]. Several reviews can be found reporting the advantages and disadvantages of the physico-chemical treatments currently used [3,4]. Nowadays, the main investigated pretreatment processes are the steam explosion, the acid/alkaline hydrolysis and the autohydrolysis pretreatment [4]. They all present several advantages with respect to the other available options, e.g., a reduced use of chemicals and less waste production compared to ionic liquid and deep eutectic solvents pretreatment [5], higher productivity with respect to biological pretreatment [6], and easier scalability compared to ultrasound pretreatment [7].

Once the fermentable sugar fraction has been released, different microbes can be used to convert those into biofuel through a fermentative process. The most widely known is the ethanol production by the yeast *Saccharomyces cerevisiae* (which will be further analyzed in Section 4). Another fermentation type is the butanol production by *Clostridia* [8]. This anaerobic fermentation is characterized by the production of Acetone –Ethanol–Butanol in molar ratio 3:6:1. This fermentation occurs in two steps: the acidogenic and the solventogenic phases. During the acidogenic phase, there is production of acetic and butyric acids; therefore, there is a decrease in the pH of the medium. In this condition, the microorganism reconverts the acids into solvents (solventogenic phase) [8]. The butanol yield is low, and its recovery is very energy demanding; however, butanol has several advantages with respect to ethanol (e.g., high energy content, high burning efficiency, low hygroscopicity, and low volatility). Thus, several innovative solutions for butanol production are currently being investigated [8].

The last step in the biofuel production is the recovery, which is also considered as highly energy demanding. For this reason, there are dedicated studies in the literature specifically dedicated to its optimization [8,9]. The production of biofuels by microbial fermentation goes back to the second half of the 19th Century. The first studies were focused on the characterization of the fermentation process [10,11], and more recently with the advances and application of genetic and "-omic" sciences, most of the studies have been focused on the development of engineered microorganisms able to increase production yields [12,13].

Nowadays, biofuel production through biotechnological processes has been entirely analyzed with the so-called "cradle-to-grave" approach [14]. This analysis allows to identify the main issues of a given process by considering the whole production chain: from the starting feedstock to the final product use/disposal. Thus, both the environmental benefits as well as the gas emission during the whole process are evaluated. As reported by several of these studies [14–16], one of the main drawbacks in the biorefinery approach is the feedstock's selection and collection: some feedstocks are produced in a particular area, but they are available in low amounts throughout the year [17], while others are available in high amounts, but their production requires broad land use, increasing the collection/transport costs [18]. In order to overcome this problem, some studies proposed the "multi-feedstock" biorefinery concept: a biorefinery able to pretreat and convert different types of feedstocks into fermentable sugars [19]. However, based on the feedstock's characteristics, different pretreatments can be required, and the use of different feedstock(s)/pretreatment(s) may result in the production of different types of undesired fermentation inhibitors [3]. Nevertheless, the collection and transport costs will still remain.

A possible solution in order to reduce or even eliminate these costs could be to implement the "zero miles product" approach into biofuel production. This approach aims to create a link between the final product and the environmental sustainability and thus reducing the carbon footprint of the production process [20]. Nowadays, it is still unclear whether or not the most effective way to reduce the carbon footprint of a process is only "buying local" [21]. Therefore, the focus of the present study is to evaluate if the "zero miles" approach could bring any benefit into the biofuel industry. The most investigated feedstocks to date for biofuel production as well as the state-of-the-art related to the ethanol production are addressed. Finally, a particular case study is presented to further investigate the advantages of using the spent coffee grounds produced locally at the Technical University of Denmark (DTU) campus' cafeteria, for the production of ethanol that can cover the energy needs for the campus operations that rely on fuel. The potential advantages of a scale-up of the process to the entire Copenhagen municipality are also discussed.

# 2. Biofuel Production from Non Lignocellulosic Biomass

Nowadays, second generation biofuels are the ones attracting the most research interest according to scientific literature. As previously stated, they are defined as biofuels produced from feedstocks which refer to non-food biomass. In turn, these can be divided into two subcategories: non-lignocellulosic biomass and lignocellulosic biomass derived feedstocks. The non-lignocellulosic ones are for example vegetable or animal fats and organic matter. In this section, the most representative biofuels obtained from nonlignocellulosic biomass through both thermochemical and fermentative processes will be presented. Biodiesel can be generated from oils or fats by transesterification. Vegetable or animal fats and oils react with short-chain alcohols such as: methanol or ethanol, ethanol is the most used because of its lower cost; however, greater conversion rates can be achieved by using methanol. Although the transesterification reaction can be catalyzed by either acids or bases, the base-catalyzed reaction is more common due to low reaction time and costs [22]. Glycerol is a major by-product of biodiesel production, with around 100 kg of crude glycerol generated per ton of biodiesel [22]. The total European biodiesel production in 2020 has been estimated at about 4.2 billion gallons [23].

Biogas is composed by methane, carbon dioxide, and may contain small amounts of hydrogen sulfide. It is produced by the breakdown of organic matter by microbes such as methanogens and sulfate-reducing bacteria by anaerobic respiration [24]. The main factors affecting the biogas production yields are temperature and pH. As reported by Issah et al. [24], these parameters strictly depend on the microorganism involved. The higher biogas yields are achieved when co-digestion involving the mixture of two or more substrates is applied. The objective of co-digestion is to balance environmental conditions such as pH or alkalinity in a digester and to maintain the optimum carbon to nitrogen ratio [24]. The biomethane plants in Europe have increased by 51% in two years, from 483 in 2018 to 729 in 2020 and there are currently 18 countries producing biomethane in Europe. Germany has the highest share of biomethane plants (232), followed by France (131) and the UK (80). By 2050, 1170 TWh of biogas are expected to be generated [25].

Biohydrogen may be produced by steam reforming of methane (biogas) produced by anaerobic digestion of organic waste. The natural gas and steam react producing hydrogen and carbon dioxide [26]. Biohydrogen can also be produced by fermentation; however, the process renders low yields; thus, there is a need to identify new strains for the process [26]. Moreover, still several hurdles need to be fixed in relation to this particular type of biofuel, such as the storage and its transportation [26].

# 3. Lignocellulosic Feedstock for Biofuel Production

Lignocellulosic biomass refers to a broad range of feedstocks which, after a more or less harsh pretreatment step, can be used as carbon source for different fermentation processes. In the following sections. a new classification as "traditional" and "alternative" lignocellulosic feedstocks is introduced, as well as an analysis regarding to the transport costs and Greenhouse Gas (GHG) emissions of this kind of feedstock. Moreover, a literature study related to bioethanol (the most investigated biofuel production in lignocellulosic biorefinery plant) is carried out.

# 3.1. "Traditional" Second Generation Feedstocks

Traditional second generation feedstocks include short rotation coppice (SRC), lignocellulosic crop, and agricultural residues [3].

Short rotation coppice refers to the growth of particular species (such as willow and poplar) that can grow on marginal land and can be used as feedstock for biofuel production. These species have a sugar content of about 60% [27] and they typically require five-year cycles [28]. Considering that Europe possesses over 10 million hectares of marginal and abandoned lands [29] and that the biomass yield from poplar ranges between 68 and 148 t/ha [30], this results in about  $10 \times 10^5$  Mt of poplar that could be grown and therefore used for biofuel production using all the European estimated marginal land

(Table 1). However, as early mentioned, this could be only obtained after about five-year growth cycles.

Table 1. Availability of "traditional" second generation feedstocks used as sugars' source for biofuel production.

Feedstock		Sugar Contant (%)	Amailahility (Mt/Vaar)	Defe	
Group	Specie	Sugar Content (76)	Availability (with lear)	Keference	
Short Rotation Coppice	Poplar	60	$10  imes 10^{5  1}$	[27–29]	
Lignocollulosis operav	Giant reedgrass	65	$16 imes 10^{42}$	[28,31,32]	
Lightcentulosic energy	Miscanthus	70	$10 imes 10^{42}$	[28,33]	
ciops	Switchgrass	56	$8  imes 10^{42}$	[28,32,34]	
Agricultural residues	Corncob, corn stover, vineyard pruning residue, rice straw	30–50	140	[33–35]	

<sup>1</sup> After 5-year growth cycles, using all the marginal land estimated in Europe. <sup>2</sup> After 1 year, using all the marginal land estimated in Europe.

Lignocellulosic energy crops refer to the growth of crop species such as Giant reedgrass (*Arundo donax*), Miscanthus (*Miscanthus giganteus*), and Switchgrass (*Panicum virgatum*). Compared to SRC, lignocellulosic energy crops require few growth cycles [28].

Giant reedgrass (*Arundo donax*) has a sugar content of about 65% and its growth has minimal requirements on soil tillage, fertilizer, and pesticides [31]. This species offers additionally protection against soil erosion, is well adapted to saline soils and saline water, and is resistant to biotic and abiotic stresses. Moreover, Giant reedgrass can be cultivated for 20–25 years without replanting [28]. Biomass production from this crop is about 16.3 t/ha [32], thus considering the 10 million hectares of marginal and abandoned land in Europe, this could render about  $16 \times 10^4$  Mt/year of Giant reedgrass as feedstock for biofuel production.

Miscanthus (*Miscanthus giganteus*) is a type of grass with narrow leaves, which grows well in temperate climates requiring limited fertilizer, and its sugar content is about 70% [33]. Its biomass yield is about 10.5 t/ha [28]; thus, about  $10 \times 10^4$  Mt/year of Miscanthus as feedstock for biofuel production could be produced using all the marginal European land.

Switchgrass (*Panicum virgatum*) is a perennial warm season bunchgrass native to North America which can grow across a wide geographic range. Its sugar content is about 56% [34], and its biomass yield is 7.9 t/ha [32]. This means that  $8 \times 10^4$  Mt/year of switchgrass could be produced in Europe as feedstock for biofuel production.

Agricultural residues are generated as consequence of crop harvesting. They include arboreal residues, residues of grains, corns, flowers, grass, and straws. These residues are characterized by a higher water content and their sugar composition can range between 30 and 50% [35]. Their availability is seasonal, coinciding with the harvesting periods. Some of the main agricultural residues investigated in the scientific literature as source of fermentable sugars are corncob (which is the central core of an ear of corn), corn stover (which consists of the leaves and stalks of an ear of corn), vineyard pruning residues, and rice straw. The European availability of agricultural residues is about 140 Mt/year [33]. The values reported in Table 1, are a rough estimation obtained by considering the feedstock production spread out in all the European area. Therefore, high transport costs to collect the feedstock to the biofuel plant can be expected.

# 3.2. "Alternative" Second Generation Feedstocks

Alternative second generation feedstocks include all feedstocks coming from agroindustrial processes such as brewers' spent grains (BSGs), potato peel, coffee silverskin (CS), and spent coffee grounds (SCG).

Brewers' spent grains (BSGs) are the residues from beer production, and they represent about 20% of the process. Their sugar content is about 35% and the European availability of this waste is about 6 Mt/year [5].

Potato peels are one of the main by-products of the potato processing: around 3%. The sugar content of this waste is around 38% and the estimated European availability is 0.45 Mt/year [5].

Coffee silverskin (CS) is the only by-product generated during the coffee beans roasting. It represents about 4.2% of coffee beans. Carbohydrates content of CS is about 30% and the CS production in Europe is about 0.2 Mt/year [5].

Spent coffee grounds (SCG) is the residue generated after instant coffee preparation. The sugar content of SCG ranges between 10 and 20% and the European production is about 2.5 Mt/year [5].

Municipal solid wastes (MSW) are currently investigated as possible source of fermentable sugars. This waste consists of organic materials, paper, plastic, glass, and metals collected by municipal authorities. As reported by Nair et al. [36], 1.42 kg/capita/day of MSW are expected to be produced by 2025. Considering the current population numbers,  $4 \times 10^5$  Mt/year of MSW is estimated to be produced in Europe. Organic waste accounts for about 60% of the MSW [36], thus  $2 \times 10^5$  Mt/year of urban organic waste are expected to be available as feedstock for biofuel production in Europe.

Currently, several studies are focused on a new and peculiar waste which can be used as feedstock: the so-called "green waste" [37,38]. Green waste by definition is any organic waste that can be composted, and it is most usually composed of leftovers from gardening activities (e.g., grass clippings and leaves). This kind of waste does not include wastes such as dried leaves, pine straw, and all agricultural residues generated as consequence of crop harvesting (which have been already discussed in the previous section). Due to the specific nature of this kind of waste, it is difficult to make an estimation related to its availability, however, promising results have been reported in literature regarding its use [37,38]. Compared to the "traditional" second generation feedstock reported in the previous section, the "alternative" one includes several advantages: (i) continuous production all the year round (not seasonal); (ii) low lignin content (thus, low energy pretreatments are required); and (iii) local production (thus, low transport costs are expected).

# 3.3. Cost and GHG Emission Related to the Feedstock Collection and Transport

There are several examples of studies addressing the cost and the GHG emission related to the feedstock collection and transport [39–41]. In this section, data available for one example of feedstock corresponding to each of the groups previously discussed have been gathered and compared. Table 2 summarizes the related costs for the use of pine, switchgrass, corn stover, BSGs, and urban wastes, respectively.

Feedstock		Costs (€/t)					
Group	Specie	Nutrient Replacement (N-P-K)	Collection/Transport to Local Storage	Local Storage	Transport <sup>3</sup>	Total	Ref.
Short Rotation Coppice	Pine <sup>1</sup>	16.4	10.6	6.7	8.5	42.2	[39]
Lignocellulosic energy crop	Switchgrass <sup>2</sup>	37.3	16.8	2.7	8.5	65.4	[39]
Agricultural residues	Corn stover	16.4	26.1	2.8	7.0	51.2	[40]
Industrial	Brewers' spent grains	-	-	-	3.6	3.6	[41]
processes	Urban wastes	-	-	-	3–50	3–50	[42]

**Table 2.** Collection and transport cost for the main feedstock used in biofuel production.

<sup>1</sup> Whole tree woodchip. <sup>2</sup> Square bale. <sup>3</sup> Truck transport within 50 km.

Xiaoming et al. [39] performed a logistic analysis related to the use of pine and switchgrass as feedstock for biofuel production. They analyzed the influence of the deliver format (e.g., square and round bale for switchgrass, clean and whole tree woodchip for pine, etc.) on the total cost. The square bale and the whole tree woodchip reported the lowest cost (Table 2).

A study by Morey et al. [40] analyzed the supply logistic system for corn stover as feedstock for biofuel production. Each step from the field production to the transport to the production plant was investigated and the main results are also reported in Table 2.

Unfortunately, there are still very few studies regarding the logistic analysis of feedstock coming from industrial processes. However, no nutrient replacement, transport to local storage and local storage costs are expected, and due the nature of their generation, this feedstock does not have a nutrient cost associated either. As the amount of this feedstock is lower than the traditional lignocellulosic biomass, it can be directly transported to the production plant while generated; hence, local storage is not needed. This direct use is also necessary because of their physicochemical composition: if stored for too long it can be easily degraded in a short time. Hamed et al. [41] calculated about  $3.6 \notin /t$  of transport cost for BSGs. As reported by Xiaoming et al. [39], the transport cost can be affected by several variables such as the transport mode (truck, rail, and barge), the biomass format (woodchip, bale, and pellet) and the transportation distance. In Table 2 all the transport costs (except the one related to the urban waste) have been normalized taking into accounts transport by truck within 50 km. The transport cost related to the urban waste can remarkably change depending also to other factors like the country and disposal methods, thus a broad range value has to be considered [42].

The main cost for SRC and lignocellulosic energy crop is caused by the nutrient replacement. For agricultural residues, the collection and transport to the local storage represents the main cost. As discussed before, both nutrient replacement and collection and transport to the local storage costs, are not even present (equal zero) if wastes come from industrial processes. Thus, if wastes are used as feedstock for biofuel production, the cost item related to the feedstock transport might be significantly minimized.

GHG emissions are generated during the feedstock's supply to the biofuel plant. These emissions have been calculated for different feedstocks in several studies [39–44] in order to estimate the GHG emission net efficiency related to the biofuel use. Table 3 reports the GHG emission related to the collection and transport cost for pine, switchgrass, corn stover, BSGs and urban wastes, respectively.

Feedstock GHG Emission (kg<sub>CO2e</sub>/t) Nutrient Collection/Transport Ref. Local Transport<sup>3</sup> Total Group Specie Replacement (N-P-K) to Local Storage Storage Short Rotation Pine<sup>1</sup> 15.1 9.9 11.2 4.841.0 [39] Coppice Lignocellulosic Switchgrass<sup>2</sup> 131.7 14.01.0 4.8151.5 [39] energy crop Agricultural 31.0 Corn stover 13.12.2 4.851.1[40] residues Brewers'  $115^{4}$ Industrial 115 [43] spent grains processes  $227^{5}$ 227 Urban wastes [44]

Table 3. Greenhouse Gas (GHG) emission due to collection and transport for the main feedstock used in biofuel production.

<sup>1</sup> Whole tree woodchip. <sup>2</sup> Square bale. <sup>3</sup> Truck transport within 50 km. <sup>4</sup> Wet BSG. <sup>5</sup> Depending on waste's kind.

As reported in Table 3 the main contribution to the GHG emission is represented by the nutrient replacement for SRC, lignocellulosic energy crop and agricultural residues. Wastes coming from industrial processes have not this entry value but due to their composition, the transport emission to the biofuel plant can be high. However, still few studies can be found in literature regarding this aspect.

The total GHG emission for the transport of industrial wastes is comparable with the value related to the transport of traditional feedstock; thus, the use of industrial wastes for biofuel production should be further investigated.

#### 3.4. Lignocellulosic Feedstock Pretreatment

Several reviews are currently available regarding feedstock pretreatment for fermentable sugars production [3]; therefore, our study will not touch upon this matter extensively. Because of the aforementioned advantages related to the use of industrial wastes as feedstock for fermentable sugars production, only the latest results regarding the pretreatment of this kind of wastes is further investigated. In particular, our analysis focuses on those pretreatments currently most advantageous, such as steam explosion, acid/alkaline hydrolysis and autohydrolysis.

Steam explosion is the benchmark pretreatment process for fermentable sugars production. This is a hydrothermal pretreatment in which the feedstock is rapidly heated by introducing high-pressure saturated steam in a reactor at temperatures typically between 160 and 260 °C (0.69–4.83 MPa) and kept for a short period of time that can range from seconds to several minutes. After this period, the pressure is instantaneously relieved causing the mechanical disruption of the lignocellulosic matrix [3]. Up to a 75% of sugars yield are obtained when BSGs is treated at 200 °C for 10 min [45]. This pretreatment is characterized by high productivity and does not require additional chemicals. However, fermentation inhibitors can be produced depending on both the operating conditions and the type of feedstock [5,6].

Acid/alkaline pretreatment involves the use of acid or base to remove the external layer of lignin from the feedstock [3]. A yield of sugar up to a 32% is obtained when coffee silverskin is treated with 2% NaOH at 1:10 (solid to liquid ratio) and 120 °C for 30 min [46]. However, chemical use and their follow up disposal have to be taken into consideration. Moreover, inhibitors are produced too [3].

Autohydrolysis is a hydrothermal pretreatment in which the feedstock is dissolved in water at high temperature. A sugar recovery efficiency of 55% is reached when potato peels are treated at 140 °C and 56 min [47]. This pretreatment is characterized by no chemical use, high productivity, high scalability, and low inhibitors formation [3].

Because of all the advantages mentioned above, from an economic and environmental point of view, the use of "alternative" feedstock and the autohydrolysis pretreatment could make feasible the scale up of the biofuel production to industrial level.

#### 4. Ethanol Production

Considering the importance of bioethanol being produced across the world to satisfy the energy demand, it is extremely important to understand the overall process design of bioethanol production. The annual world production of ethanol is over 100 billion liters, which around 70% is produced by fermentation [48]. This fermentation process is performed in different kinds and by diverse microbes (mainly yeasts due to their high ethanol yields and high tolerance limits). The product is finally recovered by different methods, being highly linked to the type of fermentation used during the process [48].

# 4.1. Types of Fermentation

## 4.1.1. Batch, Fed-Batch, and Continuous Fermentation

In batch fermentation, the process is highly controlled but provides lower ethanol yields. The microorganisms have to function at high substrate and product concentrations at the beginning and the end of the fermentation process, respectively. Therefore, other fermentation techniques are required in the commercial market [49]. In fed-batch fermentation, improved yields are obtained compared to the batch mode. However, the feed rate is limited, and the cell mass density is not increased in excess [49]. Finally, continuous fermentation provides maximum ethanol productivity and is easy to control, although higher risk of contamination is faced during the operating process [50]. A high cell concentration

of microorganisms is achieved in the continuous fermenter, which gives higher production and short processing time [50].

#### 4.1.2. Separate Hydrolysis and Fermentation (SHF)

It is a conventional bioethanol production method in which enzymatic hydrolysis and fermentation are performed separately, what makes the overall process more time consuming and expensive. On the other hand, both separate steps are carried out at their optimal reaction conditions. The main limitation of the SHF method is the cellulase activity inhibition by the sugars released in the hydrolysis phase [49].

#### 4.1.3. Simultaneous Saccharification and Fermentation (SSF)

In this method, the biomass saccharification is combined with simultaneous fermentation of sugars in a single step. SSF is considered a better method than SHF, reducing both residence times and costs of the process. Other advantage is the reduction of inhibitory compounds from the enzymatic hydrolysis, which improves the overall performance of the process [50]. On the other hand, the pH and temperature required for the enzymatic hydrolysis are normally different from the optimal fermentation process, forcing to find a compromise in order to make the process work properly [51].

#### 4.1.4. Solid State Fermentation (SSF)

This is a heterogeneous process which combines solid, liquid, and gaseous phases. It demands lower energy requirements while it is associated to higher product yields and less wastewater [52]. SSF processes are performed on a solid substrate with a moisture content (mostly agro-industrial wastes such rice straw, sugarcane bagasse, or corn cobs that also makes the process eco-friendly). The solid matrix can be either used by the microbes or serves as a support for their growth. A major factor to be considered is the size of the matrix's particles, as small ones have better exposed surface and therefore, better accessibility of the microbes for their nutrition [52]. The best considered microorganism for this method is the filamentous fungi, which due to their morphology, can easily grow and cover the particle surfaces as well as the intraparticle channels [53]. The SSF method shows several other advantages such as easy gaseous transportation, pH control, or smaller fermenters needed. On the other hand, some limitations have also been observed, like difficult downstreaming properties, heat dissipation during the process, and sustainability issues [52,53].

#### 4.1.5. Simultaneous Saccharification and Co-Fermentation (SSCF)

This process is analogous to SSF except for the fermentation of hexose and pentose sugars covered in a single step. It is a cascade process involving microbial assimilation of sugars released from the pretreatment process and following hydrolysis of lignocellulosic material [54]. SSCF process can reduce the total cost of the production as pentose sugars are also used during the process and the inhibitory effects of xylose are also reduced [55]. Moreover, one of the main advantages of this method is the simultaneous fermentation of the released glucose which maintains the glucose concentration within the medium [55].

## 4.1.6. Simultaneous Saccharification, Filtration and Fermentation (SSFF)

In this method, pretreated lignocellulosic material is enzymatically hydrolyzed in a reactor, while the suspension is continuously pumped through a cross-flow membrane. The retentate goes back to the hydrolysis vessel, while a clear sugar-rich filtrate continuously perfuses through the fermentation vessel before it is pumped back to the hydrolysis vessel. SSFF includes both the advantages of SSF and SHF. The microorganisms and the enzymes are used at their different optimal conditions in each reactor, and the glucose released during the hydrolysis, and filtered afterwards, is assimilated by the microorganisms to form ethanol. This method also allows the fermenting cultures to be reused for several cultivations. On the other hand, important considerations are, e.g., the capacity and life

span of the filter module or the long time fermentation performance of the fermentation unit [56].

# 4.1.7. Direct Microbial Conversion/Consolidated Bioprocessing (CBP)

This recent developed method is considered the most hopeful fermentation approach for bioethanol production using cellulosic materials. Although it is yet in a premature stage, many studies are being performed in order to increase the knowledge about this process and making it commercially viable [57]. In CBP, all the different steps (enzyme production, saccharification, hydrolysis, fermentation) are carried out simultaneously [57]. Therefore, a great number of microorganisms (bacteria, yeasts, fungi), genetically modified or not, have been also tested for this purpose [58]. The main limitations faced with this method are the higher enzyme production cost and low production efficiency. Actually, novel enzymes development, with the help of genetic approaches, is taking place in order to find those with high capability to hydrolyze a wide range of feedstocks reducing the cost of production [58].

# 4.2. Microorganisms Used

A great number of microorganisms have been tested for industrial ethanol production among the years, being the yeast Saccharomyces cerevisiae the best known industrial specie for fermentation (Table 4). S. cerevisiae ferments only the hexose sugars present in the hydrolyzate, but not the pentose sugars [59]. Other yeast species, such as *Pichia stipitis* or *Candida shehatae* are capable to ferment both C5 and C6 sugars, although their ethanol yield is around five-fold lower than S. cerevisiae using glucose [60]. Another yeast, *Brettanomyces claussenii* has been found able to use cellobiose in SSF processes of cellulose to ethanol [61]. Moreover, other less known yeasts (non-conventional yeasts) have been also studied as alternative organisms in industrial ethanol fermentation, due to its tolerance to a variety of stresses faced during the fermentation process. Some species that can naturally cope with those unfavourable conditions are *Zygosaccharomyces rouxii*, *Kluyveromyces marxianus*, *Dekkera bruxellensis*, or *Pichia kudriavzevii* [62].

Other traditional microorganisms used for ethanol fermentation include the bacteria *Zymomonas mobilis* or the fungus *Mucor indicus* (Table 4). Compared with *S. cerevisiae*, *Z. mobilis* has been reported to exhibit excellent ethanol productivity, high ethanol tolerance and efficient sugar uptake [63]. Sufficient nutrient level seems necessary for satisfactory glucose and xylose co-fermentation in *Z. mobilis*. Sugar consumption rates significantly decreased in lignocellulosic hydrolysates, which resulted in the decrease of ethanol productivity. On the other hand, high cell density fermentation with cell recycling, greatly shortened fermentation time and improved ethanol productivity [64]. The filamentous fungi *M. indicus*, has a great potential for lignocellulose bioconversion, and it is broadly used to manufacture fermented products such as beer, fermented rice, or soybean seeds [65]. *M. indicus*, which transforms into yeast-like form under anaerobic conditions [66], is able to ferment both glucose and xylose and with great resistance to inhibitors like furfural, hydroxymethylfurfural and acetic acid [66]. Many studies compare its fermentation efficiency to *S. cerevisiae*, being close to 92% of the theoretical value [67].

To overcome any limitation found in the host microbe, cellular and metabolic engineering methods can successfully be applied to improve the performance of those microorganisms and make them more suitable for industrial uses (Table 4). The most common strategy is to investigate the efficiency of microbial metabolism for improved enzyme production, which has been applied in biotechnological studies enabling improvements in yield and reaction titer [68]. The metabolic engineering of microorganisms provides new microbial cell factories for production of biochemicals and bioproducts. Microorganisms have been engineered to produce specific enzymes required to breakdown bonds in biomass polymers and release simple sugars, which can be used to produce ethanol among other compounds [69].

Feedstock	Pretreatment	Microorganism	Method	Ethanol (g/L)	Reference
Corn stover hydrolysate	Acid hydrolysis	S. cerevisiae (recombinant)	SSCF	41.9/48.6/54.0	[70]
Lignin-reduced sugarcane bagasse	Acid hydrolysis	S. cerevisiae (recombinant)	SSCF	14.8	[71]
Wheat bran hemicellulose Detoxified	Liquid hot water + alkaline hydrolysis	D. hansenii (wild type)	SSF	9.5	[72]
hemicellulosic hydrolysate	Acid hydrolysis	P. stipitis (wild type)	SHF	7.1	[73]
Spent coffee grounds hydrolysate	Acid hydrolysis	P. stipitis (wild type)	SHF	9–10	[60]
Rice straw hydrolysate	Alkaline hydrolysis	<i>C. shehatae</i> (wild type)	SSF	15–20	[74]
Rice bran hydrolysate	Alkaline + acid hydrolysis	Z. mobilis (wild type, biofilm)	SHF	13.4	[75]
NMMO-treated wheat straw	Liquid hot water	<i>M. indicus</i> (wild type)	SHF	10.6	[76]
Brewer's spent grains	Acid hydrolysis	E. coli (recombinant, mutant)	SHF	16	[77]
Potato peel waste	Acid hydrolysis	S. cerevisiae (wild type)	SSF	22.54	[78]
Coffee silverskin	Liquid hot water	K. marxianus (mutant)	SHF	9–10	[79]
Urban waste	Acid hydrolysis	S. cerevisiae (wild type)	SHF	N/A <sup>1</sup>	[80]

Table 4. Ethanol production from different feedstocks and microorganisms.

<sup>1</sup> Data not provided in g/L. Max. EtOH yield observed by the authors was  $62.5 \text{ g kg}^{-1}$ .

## 4.3. Ethanol Recovery

One of the major challenges to successfully replace fossil fuels by bioethanol, is the availability of efficient separation and purification methods that represents between 40 and 80% of the ethanol production costs [81]. Distillation have been the preferred method for many years, due to its high alcohol recovery, or energy efficiency, among others. On the other hand, high energy costs and high operation temperatures which affect microorganisms and proteins, are considered disadvantages [82]. As an alternative to produce energy that could be used to power the distillation process, the liquid stillage can optionally be turned into biogas by anaerobic digestion [82]. Serra et al. classified the recovery techniques into conventional systems (distillation system) and non-conventional systems [83]. The non-conventional systems are considered as alternatives for ethanol recovery with energy saving and lower cost. Examples of those are pervaporation separation, vacuum fermentation, adsorption, gas stripping, solvent extraction, etc. [81]. Nevertheless, the integration of these unconventional techniques in the large industrial scale is still limited and not 100% implemented.

#### 5. Case Study: Ethanol Production from Spent Coffee Grounds at DTU Campus

The main issue in the biotechnological utilization of reusable feedstock for biofuel production is the systematic and reliable, large scale collection of the waste product. A solution to this may be to select a specific feedstock, and assess its viability in a smaller, pilot-scale format by selecting a defined local community that produces a sufficiently large quantity, has an established waste collection infrastructure, and could benefit directly from the products from biorefinement. This ensures negligible cost of collection and transport of the feedstock, as well as providing a purpose for the derived products that is rooted in a sustainable and circular economic manner.

In the following sections, ethanol production using spent coffee grounds (SCG) collected at the Technical University of Denmark, Campus Lyngby (DTU), is reviewed. In particular, based on data reported in the literature, an idealistic scenario is explored in which SCG are processed into soluble sugars that can be fermented to produce ethanol. The laboratory yields from a selected study will be scaled proportionally to the collection size from DTU to assess the theoretical maximum yield of ethanol from SCG derived sugars [84].

The aim of the following case study is to investigate if the waste produced in a local community could cover the fuel need of the same community through a biotechnological process rather than to make a detailed economic analysis for a biofuel plant (which is already reported in several papers [85]).

#### 5.1. Assesment of Spent Coffee Ground Waste Production

Each year, DTU Strategic Sourcing purchases roughly 18 tons of coffee beans which is brewed and enjoyed in the departments around campus (data from DTU Procure). Added to that, another 2.5 tons of beans are brewed and sold by private cafeterias at DTU annually (data from Fazer Group). This would amount to an estimated 409 kg of dry coffee beans being converted into SCG per week, excluding weekends. Assuming that the entire quantity is processed into cups of coffee, and that the average extraction yield of solids into the brew is 20%, 327 kg of dry SCG are produced every week in DTU campus [86]. Therefore, DTU could make a suitable case-study for investigating SCG as a feedstock in a pilot-scale biofuel operation.

## 5.2. Assesment of Fuel Consumption

DTU Campus Service, which is responsible for maintaining and developing the facilities at campus, purchases about 4000 L of petroleum-based fuels per year to power their vehicles, costing the department an estimated 5429 euros [87]. Thus, the successful valorization of SCG into biofuels at a local DTU biorefinery could potentially alleviate some of the need for purchasing petroleum-based fuels from external sources.

# 5.3. DTU Pilot Plant

Figure 1 shows a schematic of the proposed pilot plant for ethanol production from SCG pretreatment following the steps reported by Kwon et al. [84]. SCG are subjected to dilute acid hydrolysis, and acid insoluble residues are filtered away. Next, the liquid hydrolysate is neutralized and treated with cellulase enzymes. The resulting sugar rich hydrolysate is fermented to produce ethanol which is distilled and stored. The stillage is anaerobically digested to power the plant.

In particular, 1%  $H_2SO_4$  acid solution is used at 15% (wt/v) of dry SCG, heated to 121 °C for 15 min. A 2% (v/v) cellulase mixture is used for cellulose degradation. Fermentation is carried out with a 10% yeast inoculum, anaerobically at 30 °C and pH 5 [84].

During acid hydrolysis, based on the solid to liquid ratio identified by Kwon et al., a total of 2182 L of 1% H<sub>2</sub>SO<sub>4</sub> solution is expected to be required to process the 327 kg of SCG available at the campus every week. During acid hydrolysis pretreatment, two phases are produced: a liquid phase enriched in sugars and a solid phase identified as acid insoluble lignin (AIL). As reported by Kwon et al., AIL constitutes about 29% of the acid pretreated SCG. Thus, if 327 kg of SCG are pretreated at DTU, 95 kg of dry weight AIL is expected per week [84].

For the enzymatic hydrolysis, a 2% (v/v) cellulase mixture was used by Kwon et al. [84]. In particular, after neutralization, the 2% (v/v) cellulase mixture was added and the liquid was pretreated at 50 °C for 24 h. In the present case study, if 327 kg of SCG are pretreated in 2182 L, about 44 L of enzyme are expected to be required weekly.

With a 10% yeast inoculum, the expected fermentation volume amounts to 2448 L [84].



Figure 1. Schematic of the spent coffee ground plant.

Scaling the laboratory yields from Kwon et al. [84], proportionally to the DTU collection quantity, the theoretical maximum sugar contents from acid- and enzyme hydrolysis, as well as produced ethanol for a week of operation at the SCG plant is presented in Table 5:

**Table 5.** Sugar and ethanol yield (kg/kg dry weight spent coffee grounds (SCG)) from acid- and enzymatic hydrolysis of spent coffee grounds reported in literature and expected in the investigated scenario.

Glucose	Galactose	Mannose	Arabinose	Ethanol	Reference
0.1	0.11	0.27	0.07	0.22	[84] <sup>a</sup>
32.7	37.1	87.3	2.2	73.3	This study <sup>b</sup>

<sup>a</sup> (in kg(kg dry weight SCG)<sup>-1</sup>), adjusted from Kwon et al. [84]. <sup>b</sup> (in kg (327 kg dry weight SCG)<sup>-1</sup>), scaled proportionally from <sup>a</sup>.

The fermentation mash should subsequently be distilled into anhydrous ethanol. With 73.3 kg ethanol produced and the density of ethanol being 0.789 kg/L, the total quantity is 92.8 L per week, assuming that all ethanol is distilled with no loss. This means that the stillage left from distillation is 2355 L, which could be anaerobically digested to power the plant [82].

## 5.4. Financial Analysis

For this analysis, it has been assumed that all the necessary equipment such as vessels, bioreactors, distillers, etc., are already available, thus no up-front investment is reported. The main assumption is that the proposed local community plant should be located in university campuses, scientific incubators or emerging scientific districts were the required equipment is expected to be present. The cost of operation comes primarily from purchasing chemicals and enzymes as well as water and heating of reactors. The water cost for businesses in Denmark is  $4.13 \text{ } \text{€/m}^3$ , and the average heating cost of water is 0.051 €/kWh [88,89]. To estimate the energy requirements for each step, and thus the price, the following formula has been used:

$$E = V \times \rho \times C_p \times \Delta T \tag{1}$$

where *E* is the energy in joule, *V* is the substance volume in  $m^3$ ,  $\rho$  is the substance density,  $c_p$  is the substance heat capacity, and  $\Delta T$  is the temperature change in °C.

To simplify the estimates, water at 20 °C is used as a reference substance for all calculations and vessels are assumed thermally insulated so that heat loss is negligible. As such, the heating requirements for the acid hydrolysis carried out at DTU is expected to be 236.6 kWh. This makes the total cost of water and heating during acid hydrolysis for a week's operation an estimated 8.7 and 12.1 (, respectively. The steam used for heating can be recirculated, and thus the water cost is neglected.

The quantity of sulfuric acid, at a 1% concentration in 2182 L, amounts to about 21.8 L. At a price of approximately 256 $\in$  per metric ton, the cost is 5.6 $\in$  per week [90]. The NaOH for neutralization is assumed to be of the same quantity and price [91].

If during enzymatic hydrolysis, a 2% v/v cellulase mixture is used, the required enzyme amount for the DTU pilot plant is expected to be about 44 L/week. Assuming the claimed enzyme cost of  $0.12 \notin /L$  ethanol produced from Novozymes Cellic CTec<sup>®</sup> (Denmark), and with a weekly ethanol quantity of 92.8 L, the enzyme cost is  $11.1 \notin /Week$  [92,93]. Instead of a heating step, we can estimate the cost of cooling the acid hydrolysate to the desired temperature of 50 °C, calculated using Formula (1), using water as a cooling agent. The kWh of cooling from 121 °C to 50 °C, thus becomes 171 kWh, costing 8.72 $\notin$ . The cost of water in the jacket is neglected here, as it can be recirculated in the same system.

For the fermentation step, it is assumed that inoculums are available due to the hypothesis related to the location of the plant (such as university campus or scientific incubators). As for the enzymatic hydrolysis step, we can assume a cooling cost from 50 °C to 30 °C, which amounts to 64.8 kWh costing 3.3€. The energy required to run the fermentation (agitation, power) is estimated as  $3.5 \text{ kW/m}^3$ , and the fermentation is assumed to run for 24 h. This gives a requirement of 168 kWh costing 8.5€ [94].

Finally, the fermentation mash is distilled to produce concentrated anhydrous ethanol. The stillage is fed through an anaerobic digester to produce biogas, which should cover most of the energy requirements of the distillation, while water can be recycled back into the system [82]. Using the Equation (1) and the same assumptions of heating water only, the energy requirement for heating the mash from 30 to 190 °C is 414.6 kWh, costing around 21€. In this case, no water cost is added.

The total cost of each step is collected in Table 6:

Step	Source of Expense	Costs (€/Week)
Feedstock <sup>1</sup>	Purchase	0
	Water usage	8.7
Acid hydrolysis	Heating	12.1
	$H_2SO_4$	5.6
Neutralization	NaOH	5.6
Enzymatic hydrolycic	Cooling	8.82
Enzymatic nytrorysis	Enzyme	11.1
Formentation	Cooling	3.3
rementation	Operation	8.5
Distillation <sup>2</sup>	Heating	21.1

Table 6. Financial analysis of the Technical University of Denmark (DTU) biofuel plant.

 $\overline{^{1}$  Only collection and manhours required.  $^{2}$  Can possibly be covered by energy from anaerobic digestion of the stillage.

The minimum ethanol selling price (MESP) of second-generation ethanol from sugarcane bagasse is  $1.14 \notin /L$  [95]. Based on this price, and with a total of 92.8 L of ethanol, the total revenue of the plant per week is 105.8 $\notin$ .

With 92.8 L of ethanol produced per week, the productivity of the plant for a year of operation becomes 4642 L, more than enough to supply DTU Campus Service with biofuel for their vehicles. With a revenue of  $105.8 \notin$ /week, the plant's total revenue becomes  $5292 \notin$ /year at the MESP, saving DTU Campus Service  $138 \notin$ /year. If instead, the ethanol was made into 70% ethanol laboratory disinfectant, the yearly revenue could be as much as  $208,715 \notin$ , disregarding cost of denaturation, quality control and packaging [96].

#### 5.5. Copenhagen Municipality SCG Plant

In Denmark, people consume on average 8.6 kg of coffee in their homes every year, of which only 9% is instant coffee [97]. This means that for Copenhagen municipality, which has 602.481 inhabitants, this could be as much as 3.8 million kg of SCG per year. However, collecting SCG from people's homes is not actually feasible due to logistics and transport cost. An alternative option could be to collect the coffee waste produced at businesses, cafés, and institutions. Since there are no official statistics on how much coffee people consume outside their homes, DTU can serve as an estimation: if 20,000 kg is consumed at DTU per year, with 17,000 students and employees, 1.17 kg of coffee is consumed per person per year [98]. Using these figures, a very modest estimation of the total amount of SCG that could be collected in Copenhagen municipality, based on its number of inhabitants, is 708 tons per year or 11.3 tons per work week. Using the DTU pilot plant as a reference and scaling it proportionally to the SCG collection volume in Copenhagen municipality, Table 7 summarizes the cost of the industrial scale plant.

Table 7. Financial analysis of the industrial plant.

Step	Source of Expense	Costs (€/Week)	
Feedstock	Purchase	0	
	Water usage	298.8	
Acid hydrolysis	Heating	434.1	
	$H_2SO_4$	193.7	
Neutralization	NaOH	193.7	
En grupo ati a braduolarcia	Cooling	301.8	
Enzymatic hydrolysis	Enzyme	386.1	
E and a fatter	Cooling	114.3	
Fermentation	Operation	310.0	
Distillation <sup>1</sup>	Heating	731	

<sup>1</sup> Only collection and manhours required.

With 3218 L of ethanol produced per week, the productivity of the plant for a year of operation becomes 160,888 L. With a revenue of  $3668 \notin$  week and a cost of  $2964 \notin$  week, the plant's total profit becomes  $704 \notin$  week, and thus  $35,210 \notin$  year at the MESP. If instead, the ethanol was made as a 70% liquid ethanol for hand disinfectant, the yearly profit could be as much as 2 million  $\notin$ , disregarding cost of denaturation, quality control, and packaging [99].

## 6. Discussion of Pilot- and Industrial Plant

SCG serves as an ideal feedstock for a zero-miles, second-generation biofuel operation. With a continuous supply of SCG in society throughout the year, refineries could potentially be developed in every city or region. These refineries could utilize existing waste management infrastructure to collect feedstock from institutions, local businesses and possibly consumers, processing it into valuable products and introducing said products into society, all the while reducing transportation cost in a financial and environmental sense. Since the feedstock is essentially "free", all costs related to transport come from paying local waste management contractors, which in turn saves money for those that provide the feedstock by reducing their waste streams. Turning SCG away from traditional waste management strategies into biotechnological refinement delays the need for measures like incineration and landfilling, which fulfills the promises of the circular economy [100]. In this sense, the continued research into utilization of SCG directly targets several of the sustainable development goals (SDG), with special emphasis on SDG12, by assisting the reduction of food waste streams, transitioning energy consumption away from fossil fuels, as well as supporting technological advancements in sustainable industry practices [100].

At DTU, the total collecting capacity is roughly 20 tons annually, providing enough feedstock to establish a proof-of-concept pilot plant that is founded in the principles

of the circular economy. The procedures for scaling up from the laboratory to a pilotscale operation require extensive testing and reiteration, a vast amount of resources and expensive equipment. However, in reality, the ethanol produced from a SCG pilot plant can hardly compete with the MESP, there may be another, more important takeaway: it could potentially assist in accelerating the transition of SCG utilization from strictly laboratory settings into industrial scale operation. Furthermore, a DTU-based SCG pilot plant would still serve as a research facility, where the process could be optimized gradually. Thus, the pilot plant provides educational value by enabling students to get hands-on experience with scaling up an industrial process or testing novel production strains they develop in the laboratory. In this sense, the products derived from the pilot plant become by-products to the educational value that it provides, serving more as a small contribution to DTU in the form of biofuel or other products of value for the institution. Moreover, in addition to the educational value, the proposed local approach could contribute to raise the awareness and mind-shift towards the green solutions and transition to the circular instead of linear economy as well as to represent a tangible contribution towards the transition to "zero waste" concept on global scale.

In both reported studies, profitable estimates have been described. However, in both cases, deployment costs have not been taken into accounts, as it was assumed that the required equipment was available in the selected community such as university campuses and scientific incubators. For the pilot- and industrial plants to be truly competitive even with deployment costs, the MESP should be markedly higher. This is a common challenge with advanced biofuels since there is an uneven cost competition with conventional biofuels [101]. This is in great part due to the high capital cost of deployment and high cost of conversion technologies for second-generation operations as compared to conventional operations, while still being in direct competition with lower cost biofuels in blend obligations for commercial fuel blends such as E10. One possible solution could be the implementation of tax exemptions on advanced biofuels, which could allow for a rapid expansion of the industry and thus serve to boost the necessary technologies [102]. Another option is the sequential valorization of the feedstock, by first extracting one of the 40 documented high value bioactive compounds such as chlorogenic acids (33 euro/kg) present in SCG to increase the overall feasibility of the plant.

# 7. Conclusions

As reported in the literature, one of the main bottlenecks in the biofuel production is the selection of a feedstock which simultaneously responds to the following needs: high availability all year around, low transport cost, and low GHG emissions associated with its transport. In the present paper, an analysis regarding alternative feedstocks (i.e., brewers spent grains, spent coffee grounds, and urban waste) as possible alternative source of fermentable sugars has been carried out in term of European availability and transport cost. Moreover, a case study at DTU campus has been investigated in order to identify the possible advantages of the SGG use for ethanol production in a defined local community. On one hand, the proposed local biorefinery can actually decrease the transport cost and reduce the traditional incineration and landfilling practice, while on the on the other hand, there are still high costs unaccounted for in the biofuel plants such as the deployment and equipment costs. One possible solution to create better profit margins could be represented by tax exemption on advanced biofuels in general, in which SCG could possibly compete on price with reduced GHG emission prospects. Further studies related to the main wastes produced in local communities (in particular, university campus and scientific incubators) are required. These could help both: the lab scale research about real and available wastes as well as the scale up of biorefineries using local wastes, thus boosting the circular economy approach.

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