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ORIGINAL ARTICLE

Valorisation of source-separated food waste to bioethanol: pilot-scale demonstration

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Abstract

Food waste is a waste stream that is produced globally in huge amounts and therefore constitutes a major environmental concern. Additionally, the growing consumption of fossil fuels sets the need for alternative energy sources. To this end, in this paper, an holistic approach towards bioethanol production from source-separated food waste was studied as an effective strategy to cope with both issues. Source-separated food waste collected from a Greek Municipality was used as raw material. Two fermentation modes, separate hydrolysis and fermentation and simultaneous saccharification and fermentation, were examined in laboratory and pilot scales with varying solids loadings. For separate hydrolysis and fermentation (SHF) trials, the solids loading increase led to a significant ethanol yield reduction from 79 to 55 g/kg food waste, whereas for simultaneous saccharification and fermentation (SSF), the ethanol yield was increased by 77% (from 62 to 110 g/kg food waste) as the solids loading was increased. This is also related to greater ethanol concentrations, which are beneficial in terms of technoeconomics. The lowest bioethanol production cost, 1.57 €/kg ethanol, was estimated for the scenario of SSF with 20% solids loading while for SHF the lowest production cost was achieved (4.40 €/kg ethanol) when 15% solids loading is applied. In most cases, the energy and enzyme costs presented the most pronounced impact on the total bioethanol cost. In conclusion, it was proved that the food waste valorisation towards bioethanol production is technically feasible on a pilot scale. However, further techno-economic factors of the whole value chain must also be taken into consideration while aiming to assess the viability of the process.

Keywords Enzymatic saccharification · Ethanol yield · Separate hydrolysis and fermentation · Simultaneous saccharification and fermentation · Upscaling

Abbreviations

MTBE	Methyl tert-butyl ether
SG	Glucose yield
SHF	Separate hydrolysis and fermentation
SSF	Simultaneous saccharification and fermentation
UEST	Unit of environmental science and technology
Yeth	Ethanol yield

1 Introduction

Nowadays, the management of food waste is a major environmental concern, given that more than 30% of the food that is destined for humans is either wasted or lost within the value chain. This amounts to more than 1300 million tonnes annually. Due to the exponential increase of the world population, which is directly connected to energy and natural resource consumption, this figure is constantly rising [1]. For the case of Greece, the respective quantity has been assessed to be higher than most European countries and twofold the international average [2]. Food waste constitutes the largest part of municipal solid waste and is rather underutilized. Yet, it holds an increased energy potential but its exploitation is rather challenging given its complex and heterogeneous nature [1].

Thus, it is of high priority to develop environmentally friendly alternatives for the valorisation of food waste, particularly for cases such as Greece. The utilization of this

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waste for biofuel production still remains a challenge. Considering various alternative energy sources, biomass and in particular food waste has been an indispensable part of energy debates within the policy context, strongly promoted by EU, which has been able to transform saving and environmental protection provisions into strategic execution plans for development [3]. The sustainability of schemes treating food waste towards bioethanol production has been an emerging research field for the last decade [4, 5]. There are several reports in literature suggesting different processes, conditions and formulations [6, 7]. Fuel ethanol is also examined as a replacement for conventional octane boosters such methyl tert-butyl ether (MTBE) which are carcinogenic and environmentally hazardous [6].

Food waste must be processed in order to fully convert its structural carbohydrates into ethanol. Starch and cellulose need to be hydrolyzed into glucose, which is afterwards fermented by microorganisms into bioethanol [1]. Separate hydrolysis and fermentation (SHF) is a two-step process including the saccharification of carbohydrates as a first step followed by fermentation. An alternative to SHF is simultaneous saccharification and fermentation (SSF) that incorporates the above-mentioned steps into one. This process presents numerous benefits in relation to other fermentation modes, such as the use of a single reactor for both steps, leading to reduced cost and residence time [8]. However, during SHF, enzymatic formulations and yeast operate under their optimum operational conditions in regard to pH and temperature [9, 10]. During SSF, the operational conditions are not the optimum for the enzymes and, thus, the saccharification rate is lower; the saccharification products are consumed as soon as they are produced given that the fermentation step is on-going. This way, the saccharification products under no circumstance may act inhibitory to fermentation, as is occasionally the case during SHF [11]. Additionally, the presentation of bioethanol in the broth also acts as an antimicrobial agent preventing possible contamination [9].

The heterogeneous composition of food waste and the high moisture content are obstacles to the development of large-scale and high-efficiency processes. Several studies have been published examining the possibility of food waste valorisation by applying the bioethanol route. Yet, the majority is restricted to a lab scale. The maturity status of ethanol production is still at the pilot plant setup or demonstration stage [12]. The performance stability and sensitivity of the process parameters are essential aspects of the upscaling approach. In other words, the same or enhanced yields and rates should be achieved by the application of the same operational parameters based on a sequence of proof-of-concept procedures in the transition from lab to pilot scale [13].

In this context, for bioprocessing to be successful, effective upscaling is required. A suitable and thorough approach must be defined for a certain product, such as bioethanol from food waste, which incorporates a thorough analysis of the operational factors that are correlated to the product's yield. To assure the effectiveness of the operation, a unique, highly scalable strategical approach is performed by keeping constant a specified set of characteristics during the upscaling. However, because various aspects affect dynamics and transport phenomena in a reactor, this is fairly complicated. Furthermore, agitation, energy input, rheological characteristics and mass transfer phenomena caused by aeration and/or mixing, substrate's and product's concentrations, macro- and micro-nutrients and micro-conditions within the bioreactor are all strongly connected to these parameters [14].

The main goal and novelty of this paper was therefore to upscale the whole bioethanol production process from a novel biomass source, food waste, to pilot scale using real source-separated feedstock by means of novel enzymatic preparations. Prior to upscaling of the process, an optimization was performed in a laboratory scale. More specifically, the factors considered were the hydrolysis temperature (35–65 °C), the solids loading (10–20%) and the type of fermentation process (SHF and SSF).

2 Materials and methods

The source-separated food waste used in this paper was obtained from the Municipality of Vari-Voula-Vouliagmeni in Greece. It was transported directly to NTUA, School of Chemical Engineering, UEST laboratory (Unit of Environmental Science and Technology), where it was dehydrated and ground using a GAIA dryer (model GC-100). The feedstock was then fully characterized.

All of the chemicals utilized were of analytical grade. Novozymes (Denmark) generously contributed non-commercial enzymatic formulations (NS22109: amylolytic and NS22177: cellulolytic) to our work. NS22109 amylase activity was tested using the method provided by Xiao et al. [6] and was 2420 U/mL. The total cellulase activity (FPU) of NS22177 was determined using the standard IUPAC method outlined by Ghose [7] against filter paper and found to be 227 FPU/mL. *Saccharomyces cerevisiae* was employed as the fermentation yeast.

The NREL laboratory analytical methodologies were used to characterize structural carbohydrates and acid-soluble lignin and acid-insoluble residue in biomass from raw and treated materials [8]. Analysis of ethanol and glucose was carried out by an HPLC apparatus (high-performance liquid chromatography) with a HyperREZ XP Carbohydrate H+ Counter-ion (8 µm) column. The analysis was executed

at a 0.6 mL/min flow rate and at 70°C in acidified (0.005 M sulphuric acid) ultrapure water as the mobile phase. The specific heat capacity of source-separated food waste was measured according to ASTM D2766–95. All analyses were performed in duplicate.

2.1 Lab-scale experiments

2.1.1 Optimisation experiments

The temperature of enzymatic saccharification was set as the optimisation parameter for the lab-scale experiments. The latter was performed in 250-mL Erlenmeyer flasks. A 10% solids loading was applied and feedstock "I" was used. The optimum operating conditions for the enzymes were disclosed by Novozymes; 65 °C for NS22109 and 50°C for NS22177 and pH 4.5–5.5 for both enzymatic formulations. The mixture's initial pH was 4.8 and was not regulated given that it was within the pH range that the enzymes needed to function properly. According to our previous work [15, 16], the optimum results for source-separated food waste saccharification were obtained after 2 h of hydrolysis at 65 °C by 40 μL NS22109/g starch and after 5 h of hydrolysis at 50 °C by 175 μL NS22177/g cellulose. In this experimental plan, the hydrolysis of starch was performed using 40 μL NS22109 per g of starch in an Unitronic-Orbital, PSelecta water bath for 2 h for all samples. Temperatures of 35, 50 and 65 °C were used. Furthermore, the hydrolysis of cellulose was conducted with 175 μL NS22177 per g of cellulose for 5 h. Temperatures of 35 and 50 °C were used. The samples were analysed at the end of each experiment.

Further optimisation experiments were performed in a lab scale regarding applying both SHF and SSF under different solids loadings (10, 15 and 20%).

2.1.2 Separate hydrolysis and fermentation (SHF)

A two-step enzymatic saccharification was conducted. The first step included the addition of 40 μL NS22109/g starch at 65 °C and lasted 2 h, while at the second step, 175 μL NS22177/g cellulose was added at 50 °C for 5h. After 7h, the glucose generated was bioconverted to bioethanol by *Saccharomyces cerevisiae* (2% w/w) in the autoclavable containers at 35 °C for 18 h. After an 18-h fermentation process, the samples were centrifuged (3200**g* for 8 min) and the resulting solid and liquid phases were analysed.

2.1.3 Simultaneous saccharification and fermentation (SSF)

Erlenmeyer flasks with a capacity of 250 mL were used. The mixture's pH was around 4.8 at the beginning, and it was not changed because it was in the ideal pH range for enzyme activity. The mixture was supplemented with 40 μL NS22109/g starch, 175 μL NS22177/g cellulose and 2% w/w *Saccharomyces cerevisiae*. In the water bath, the bottles were incubated for 18 h at 35 °C (Unitronic-Orbital, PSelecta). The solid and liquid phases were separated and analysed after an 18-h fermentation period using centrifugation (3200*g for 8 min).

All of the experiments were carried out three times, with the average results reported.

2.2 Pilot trials

The upscaling of bioethanol production from food waste was achieved using a bioconversion pilot-scale unit (Fig. 1) developed at UEST. Two stainless steel reactors, of 200L capacity each, that continually stirred are included in the pilot plant and can run independently. For the case of SHF, the enzymatic saccharification occurred in the first reactor, whereas fermentation was carried out in the second. The entire procedure for SSF was conducted in a single reactor. The temperature in the reactors is controlled by the water circulation through the vessel's double walls. The recovery of produced ethanol was achieved through vacuum distillation at 70 °C. Distillation takes place in a coil-type heat exchanger that heats a water reflux system to cool the ethanol vapours. The prototype plant's operation is totally automated via a PLC.

For the SHF pilot trials, 40 μL/g starch NS22109 for 18 h at 65 °C, 175 μL/g cellulose NS22177 for 18 h at 50 °C and 2% *Saccharomyces cerevisiae* at 35 °C for 24 h were added for increasing solids loadings 10–20%. Similarly, for the SSF trials, 40 μL/g starch NS22109, 175 μL/g cellulose NS22177 and 2% *Saccharomyces cerevisiae* at 35 °C for 18 h were added for increasing solids loadings 10–20%.

3 Results and discussion

3.1 Food waste characterisation

In the following table (Table 1), the physicochemical characteristics of the various biowaste batches collected from the Municipality of Vari-Voula-Vouliagmeni are presented.

It is evident that the composition of the biowaste samples presented fluctuations and that the total hydrocarbon content ranges from 26 to 45% implying a promising bioethanol potential. The fluctuations in Table 1 are prominent in accordance with well-established literature [17]. These might be linked to a variety of management approaches, as well as nutritional, socioeconomic and geographical differences.

Most parameter values fall within the range reported in literature, e.g., 88.0±1.04% volatile to total solids

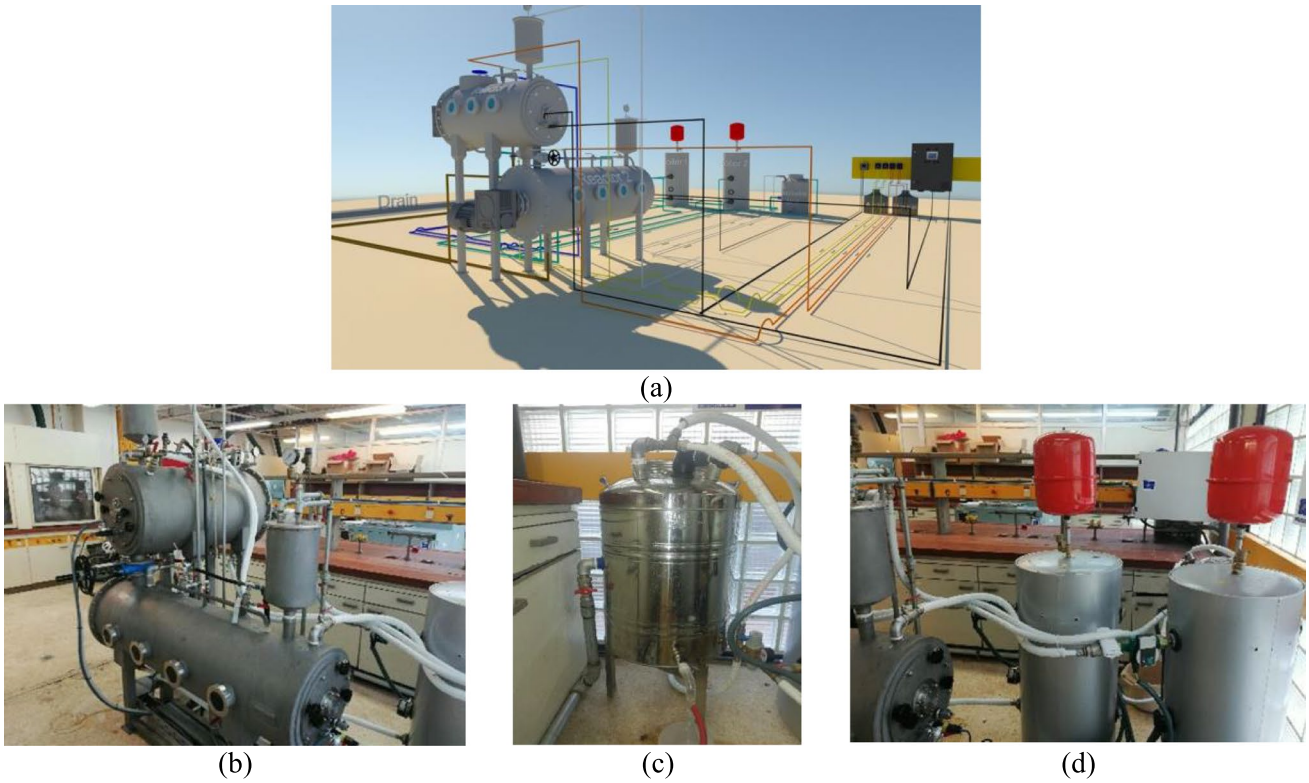

Fig. 1 **a** Representation of the full-scale plant, **b** bioconversion pilot plant, **c** distillation unit, **d** boilers

Table 1 Composition of different batches of source-separated food waste utilized in this study

	"I" (% w/w)	"II" (% w/w)	"III" (% w/w)	"IV" (% w/w)	"V" (% w/w)
Total solids	95.7 ± 0.01	95.7 ± 0.00	94.1 ± 0.01	96.3 ± 0.01	95.9 ± 0.00
Residual moisture	4.27 ± 0.05	4.27 ± 0.04	5.89 ± 0.02	3.69 ± 0.03	4.05 ± 0.03
Fats & oils	9.90 ± 0.11	11.03 ± 0.14	12.99 ± 0.14	11.82 ± 0.14	12.54 ± 0.14
Water-soluble solids	26.9 ± 0.03	42.4 ± 0.01	40.5 ± 0.15	39.7 ± 0.05	36.9 ± 0.09
Volatile solids	89.4 ± 0.01	88.3 ± 0.01	88.4 ± 0.01	87.2 ± 0.01	86.8 ± 0.02
Ash	10.6 ± 0.03	11.7 ± 0.04	11.6 ± 0.04	12.8 ± 0.1	13.3 ± 0.13
Cellulose	20.3 ± 0.1	13.6 ± 0.12	18.7 ± 0.13	23.8 ± 0.14	19.7 ± 0.1
Hemicellulose	16.9 ± 0.04	33.8 ± 0.03	3.94 ± 0.04	4.41 ± 0.26	6.75 ± 0.18
Starch	3.40 ± 0.15	3.55 ± 0.12	4.49 ± 0.36	3.78 ± 0.25	2.86 ± 0.28
Acid-soluble lignin	1.10 ± 0.07	1.23 ± 0.05	1.38 ± 0.08	1.2 ± 0.02	1.35 ± 0.14
Acid-insoluble residue	20.6 ± 0.2	9.2 ± 0.11	11.0 ± 0.14	10.8 ± 0.11	11.8 ± 0.09
Free glucose	3.1 ± 0.4	1.6 ± 0.3	2.6 ± 0.1	0.8 ± 0.1	2.4 ± 0.2

ratio (51.6% in UK - 94.9% in Turkey [17]), 11.7±1.23% fats and oils (5.6% in Italy - 24.7% in Turkey [17]), 19.2±3.67% cellulose (15.2 ± 14.6% [17]). On the other hand, the mean value of hemicellulose was 13.2±12.7%, higher than literature (7.4 ± 4.6% [17]), while starch presents an average value of 3.62±0.59%, significantly lower than the reported values (20.2 ± 13.9% [17]). These deviations could be attributed to the seasonality of dietary habits of the residents of Vari-Voula-Vouliagmeni municipality, taking into account that the sampling took place within a short period of the year (2 months).

3.2 Lab-scale experiments

In all lab-scale experiments, feedstock "I" was utilized at 10% solids loading.

3.2.1 Impact of temperature on the enzymatic saccharification of starch and cellulose

In Fig. 2, the results of the optimization experiments are presented. More specifically, the glucose concentration of the saccharified samples with the amylolytic formulation (40 μL NS22109 per g of starch for 2 h) at 35, 50 and 65 °C or the cellulolytic formulation (175 μL NS22177 per g of cellulose for 5 h) at 35 and 50 °C is given.

From this figure (Fig. 2), it is clear that the impact of temperature within the range of 35–65 °C was insignificant on glucose production. From the characterisation of the solid residue, it was calculated that nearly 100% of starch was hydrolysed for all cases when amylase was applied. The measured glucose concentrations could be attributed by 50–55% to saccharification of starch and by 45–50% to free glucose content of feedstock "I" (Table 1). For the case of cellulase, lower cellulose degradation efficiencies were obtained (25–31%) from the analysis of solid residue. Nevertheless, the glucose concentrations measured indicated that cellulose was not completely saccharified to its monomers as was also observed by Barampouti et al. [18]. Thus, given the energy needs that are necessary to maintain the temperature at higher levels, 35 °C seems favorable for the enzymatic saccharification for both starch and cellulose, implying that it would be interesting to test both fermentation modes (SHF, SSF).

3.2.2 Impact of fermentation mode on ethanol yield

Regarding the fermentation mode, in Fig. 3, the ethanol yields of the SHF and SSF experiments with increasing solids loadings (10, 15 and 20%) are presented. Ethanol yield is expressed as ethanol production per kg of dry food waste processed.

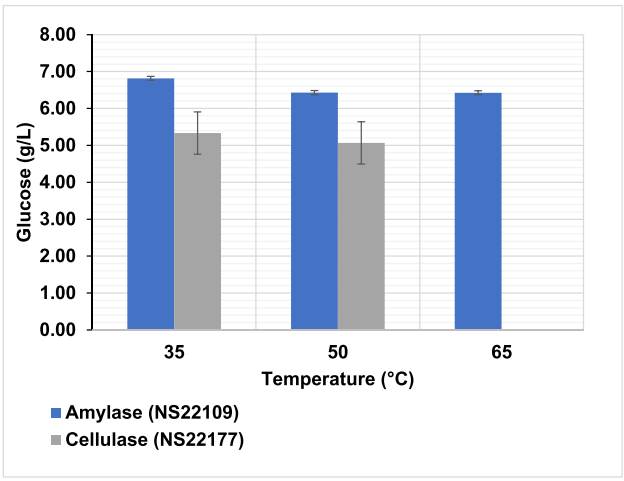

Fig. 2 Glucose concentration after the enzymatic saccharification of source-separated food waste (10% solids loading, feedstock "I") with 40 μL NS22109 (amylase) per g of starch for 2 h or 175 μL NS22177 (cellulase) per g of cellulose for 5 h

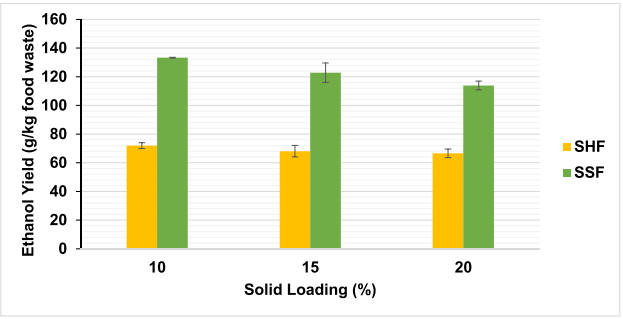

Fig. 3 Ethanol yields of SHF experiments (40 μL NS22109 per g of starch for 2 h, 175 μL NS22177 per g of cellulose for 5 h, 2% *S. cerevisiae* for 18 h) and SSF experiments (40 μL NS22109 per g of starch, 175 μL NS22177 per g of cellulose, 2% *S. cerevisiae* for 18 h) with increasing solids loading (10–20%)

It is evident from Fig. 3 that the higher the solids loading, the lower the yield for both cases. However, the ethanol yields during SSF were higher (up to 30%) than the respective of SHF. Higher yields of SSF compared with SHF were also observed in the work of Rana et al. [9] who compared the SSF and SHF modes of wet-exploded loblolly pine and corn stover. Yet, in the case of Rana et al. [9], higher ethanol yields were observed when more elevated solids loadings were applied during SSF. In addition, Wang et al. [19] reported higher ethanol yields (as high 230g/kg kitchen garbage) while applying SSF on kitchen garbage with 11.5% solids loading. However, in this study, the feedstock was very rich in carbohydrates (46% starch, 2% cellulose and 22% free glucose). Koike et al. [20] also observed ethanol yields ranging from 200 to 220 g/kg kitchen garbage (35.9% total sugars) for SSF ethanol production with 10% solids loading, managing to ferment successfully 76.7–84.3% of available carbohydrates. Lower ethanol yields (80–110 g/kg food waste) were obtained in the study of Alamanou et al. [21] who applied SSF on household food waste (18.3% cellulose, 4.39% free glucose) with 20% solids loading. Wyman et al. [22] also observed that the SSF mode presents more elevated ethanol yields with respect to SHF. This was attributed to the low sugar concentrations that alleviate their inhibitory action on enzymes.

The respective ethanol concentrations for both modes are depicted in Table 2.

The increase of ethanol concentration while increasing the solids loadings presented in Table 2 was anticipated since higher solids loadings imply higher carbohydrates loading, thus higher glucose production and thus higher ethanol production unless inhibition phenomena arose. The effect of food waste loading during SSF on the bioethanol

Table 2 Ethanol concentration of SHF experiments (40 µL NS22109 per g of starch for 2 h, 175 µL NS22177 per g of cellulose for 5 h, 2% *S. cerevisiae* for 18 h) and SSF experiments (40 µL NS22109 per g of starch, 175 µL NS22177 per g of cellulose, 2% *S. cerevisiae* for 18 h) with increasing solids loading (10–20%)

Solids loading (%)	Ethanol concentration (g/L)	
	SHF	SSF
10	7.20±1.51	13.3±1.00
15	10.2±3.28	18.4±2.66
20	13.3±2.42	22.8±4.49

concentration was also studied by Hong et al. [23] who observed that the bioethanol concentration increased with an increase in solids loading, while the respective yield decreased slightly. In the work of Rana et al. [9] as well, in all experiments, the ethanol titers were more elevated for SSF in comparison with SHF under identical operational conditions. Furthermore, when using empty fruit bunch as a substrate for bioethanol [24], SSF also presented superiority over SHF since ethanol was produced rapidly, its concentration was higher and ethanol yield was also higher. Wyman et al. [22] reported that SSF may lead to increased ethanol concentration and yield with reduced CAPEX and energy needs given the lower temperatures applied. Thus, a comparison between SHF and SSF resulted that SHF was less efficient than SSF in a lab scale, even though optimal temperatures were applied during the enzymatic saccharification.

Hence, although it seems that the SSF fermentation mode may be considered a sustainable choice for the valorisation of source-separated food waste, it was decided that all three solids loadings should be examined in a pilot scale for both fermentation modes in order to assess the impact of upscaling in the pilot plant's performance.

3.3 Pilot trials

For the pilot trials of SHF, feedstocks "II", "III" and "IV" were used for the trials of 10, 15 and 20% solids loadings respectively. Figure 4 presents the glucose and ethanol production during the three phases of SHF (enzymatic hydrolysis with amylolytic enzyme - phase 1; enzymatic hydrolysis with cellulolytic enzyme - phase 2; fermentation - phase 3) of source-separated food waste with 40 µL/g starch NS22109 for 18 h at 65°C, 175 µL/g cellulose NS22177 for 18 h at 50 °C and 2% *Saccharomyces cerevisiae* at 35 °C for 24 h for increasing solids loadings 10–20%.

In phase 1, the increase of solids loading from 15 to 20% did not induce an increase in glucose concentration as was anticipated, implying possible inhibition due to mass transfer phenomena, as was also reported by Rana et al. [9]. This inhibition is provoked by the viscous nature of the feedstock mixture [9, 25]. It is evident that during the first 2 h of hydrolysis, 56–69% of the final glucose concentration was obtained. The addition of cellulolytic enzyme and the initiation of phase 2 further triggered glucose production. Similarly, almost 100% of glucose production was obtained after the first 5 h of saccharification with the cellulolytic enzyme. The additional 13 h contributed slightly to the glucose production. Moreover, when *Saccharomyces cerevisiae* was added to the mixture, glucose was quickly consumed. This was more obvious for the low solids loadings (10 and 15%) while more time was necessary for 20% solids loading. As far as ethanol is concerned, its concentration started to increase once *Saccharomyces cerevisiae* was added. The initial production rate was almost identical for 10 and 15% solids loading while a slower rate was observed for 20% solids loading.

Similarly, for the pilot trials of SSF, feedstocks "III", "V" and "II" were used for the trials of 10, 15 and 20% solids loadings respectively. Figure 5 presents the glucose and ethanol production during SSF of source-separated food waste with 40 µL/g starch NS22109, 175 µL/g cellulose NS22177 and 2% *Saccharomyces cerevisiae* at 35 °C for 18 h for increasing solids loadings 10–20%.

From this figure (Fig. 5), it is observed that in all cases after 2 h the maximum glucose was produced from the breakdown of polysaccharides; for the next 5 h (from 2 to 7 h of processing), the glucose production was either reduced or its consumption by the yeast towards ethanol production was increased. Maximum glucose concentration (32.1 g/L) was achieved for solids loading 20%. Similarly, the maximum ethanol concentration was observed at around 6 h for all solids loadings. The highest ethanol concentration (22 g/L) was observed, as anticipated for 20% solids loading. After 6 h of processing, the ethanol concentration remains almost stable or decreases slightly.

In Fig. 6, the ethanol yields for all pilot trials performed under both SHF (with 40 µL/g starch NS22109 for 18 h at 65 °C, 175 µL/g cellulose NS22177 for 18 h at 50 °C and 2% *Saccharomyces cerevisiae* at 35°C for 24 h with increasing solids loadings 10–20%) and SSF (with 40 µL NS22109 (amylase) per g of starch, 175 µL NS22177 (cellulase) per g of cellulose and 2% *Saccharomyces cerevisiae* for 18 h with increasing solids loading (10–20%)) of source-separated food waste are presented.

From Fig. 6, it is obvious that for SHF trials, ethanol yield slightly increased (18%) from 10 to 15% solids loading whereas a more pronounced effect was observed (41% decrease) at a further 5% increase of solids loading. A similar trend was not observed in lab-scale experiments (Fig. 3) where ethanol yield was almost irrespective to solids loading. Nevertheless, starch degradation was almost complete for all solids loadings whereas cellulose degradation ranged from 32.4 to 66.0%.

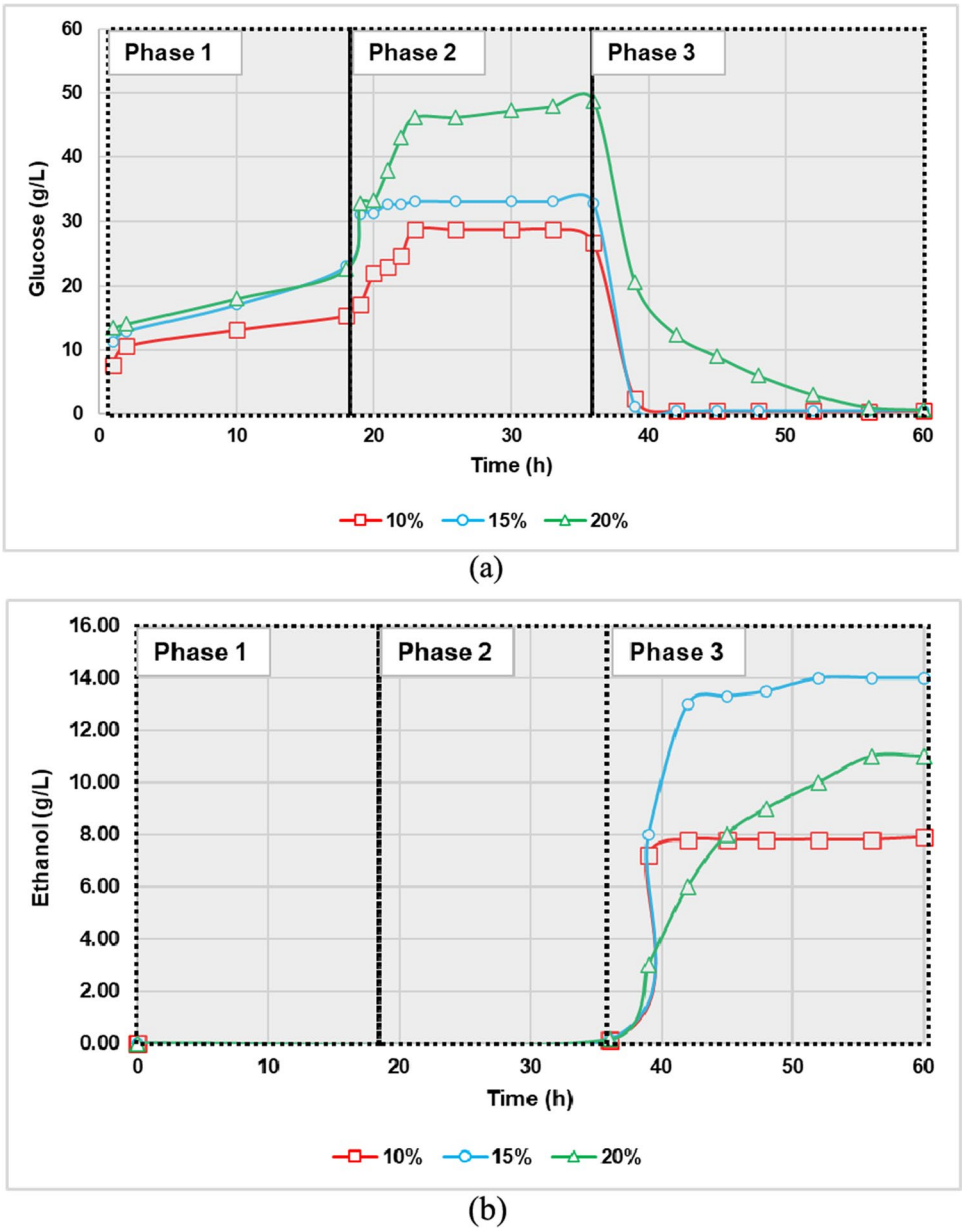

Fig. 4 Glucose (**a**) and ethanol (**b**) production during the three phases of SHF (enzymatic hydrolysis with amylolytic enzyme - phase 1; enzymatic hydrolysis with cellulolytic enzyme - phase 2; fermentation - phase 3) of source-separated food waste with 40 µL/g starch NS22109 for 18 h at 65 °C, 175 µL/g cellulose NS22177 for 18 h at 50 °C and 2% *Saccharomyces cerevisiae* at 35 °C for 24 h for increasing solids loadings 10–20%

For SSF pilot trials, an increase in ethanol yield (77%) was observed as the solids loading was increased. This is also related to greater ethanol concentrations, which are beneficial in terms of technoeconomics. The maximum ethanol concentrations of 13.2 g/Land 22.0 g/L were observed after 7 h of SSF with 15 and 20% solids loadings respectively. The degradation of starch was over 90% for all cases while cellulose degradation was nearly 62%. Additionally, 84% of bioethanol was distilled and recovered.

Similarly, in the study of Rana et al. [9] who compared the SSF and SHF modes of wet-exploded loblolly pine and corn stover, both higher ethanol yields and higher ethanol concentrations were also observed by applying more elevated solids loadings during SSF that could lead to a reduction in the cost of production. On the other hand, Kim et al. [25] reported a 28% ethanol yield increase (0.31 to 0.43 g/g) when SHF instead of SSF was applied on cafeteria food waste.

Considering the impact of upscaling (Fig. 3 and Fig. 6), it is evident that for SHF and low and medium solids loadings (10 and 15%), upscaling had a positive impact on ethanol yield whereas for SSF upscaling induced a rather negative impact. Lower ethanol concentrations were also reported by Zou et al. [26] when they examined the upscaling of ethanol production from *Helianthus tuberosus* L. This fact was attributed to the worse agitation in the large-scale system and the uneven distribution of the medium within the bioreactor. Lower yields were also reported by Camus et al. [27] during

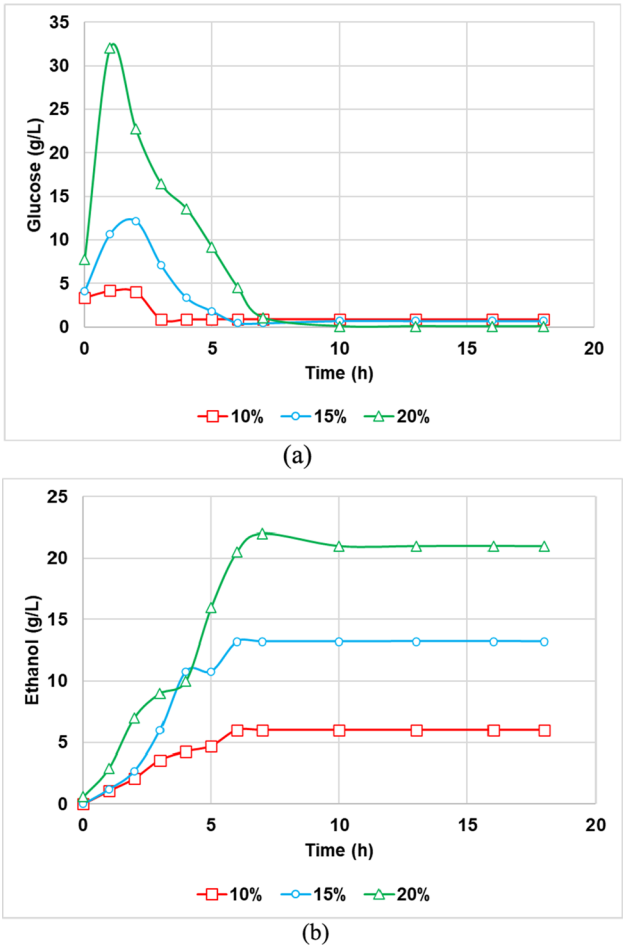

Fig. 5 Glucose (**a**) and ethanol (**b**) production during simultaneous saccharification and fermentation of source-separated food waste with 40 µL/g starch NS22109, 175 µL/g cellulose NS22177 and 2% *Saccharomyces cerevisiae* for 18 h for increasing solids loadings 10–20%

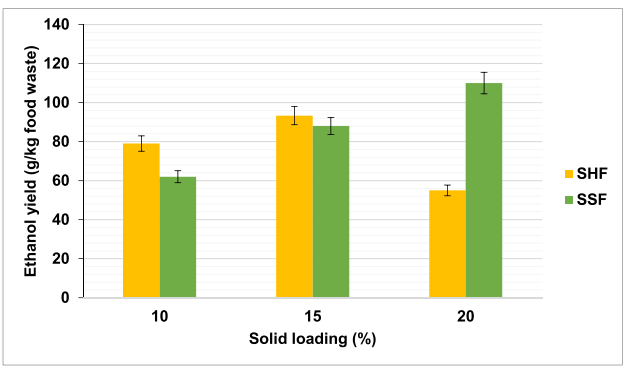

Fig. 6 Ethanol yields for all pilot trials performed under SHF (with 40 µL/g starch NS22109 for 18 h at 65 °C, 175 µL/g cellulose NS22177 for 18 h at 50 °C and 2% *Saccharomyces cerevisiae* at 35°C for 24 h with increasing solids loadings 10–20%) and SSF (with 40 µL NS22109 (amylase) per g of starch, 175 µL NS22177 (cellulase) per g of cellulose and 2% *Saccharomyces cerevisiae* for 18 h with increasing solids loading (10–20%)) of source-separated food waste

the scaling-up of ethanol production from a macroalgae species. On the other hand, an increase in both bioethanol concentration and yield was reported by Das et al. [28] when upscaling SSF of thatch grass. Yet, in this case, the upscaling was performed from 100 mL to 1 L working volume.

Therefore, the results obtained in this study indicate that a fairly elevated bioethanol yield could be achieved by an upscaled enzymatic hydrolysis and fermentation system of source-separated food waste by application of both fermentation modes. Thus, it is considered feasible to use food waste as a suitable feedstock to perform bioethanol fermentation using enzyme mixtures and *Saccharomyces cerevisiae* for industrial production. In order to define the most promising scenario, apart from the parameter of ethanol yield, economic considerations should also be made.

3.4 Economic considerations

In an effort to give precedence to a scenario for bioethanol production, its production cost was chosen as an optimization parameter. This cost could be considered as the sum of 4 factors: energy, water, enzymatic formulations and yeast. The total cost includes a part that is related to insurance, maintenance, labor etc. and is fixed and another part that is associated with consumables which is variable [18, 29]. The energy needs of each scenario were assessed according to Barampouti et al. [18]. In Table 3, the numerical parameters that were used for the estimation of the bioethanol production cost are presented.

Based on the results of the pilot trials, the mass balances equations were set up and the cost of bioethanol production for each case was calculated. In Fig. 7, the total bioethanol production cost and its fractionation for each case are presented.

From Figs. 6 and 7, it is obvious that the scenario of SSF with 20% solids loading did not result in the highest ethanol yield, yet it presents the lowest bioethanol production cost. Similarly, for SHF, the lowest production cost is achieved when 15% solids loading is applied although this scenario did not perform as well, in terms of ethanol yield, as 10% solids loading. Furthermore, From Fig. 7, it is evident that the energy cost contributes most (49–81%) in the overall ethanol production cost for all cases examined as anticipated, except for SSF and 20% solids loading where the

Table 3 Adopted numerical parameters for the estimation of the ethanol production cost

Unit	Value
$c_{p,\text{food waste}}$	0.52 cal/g/°C
Water	0.83 €/m^3
Enzymes	2.00 €/L
Yeast	1.20 €/kg
Energy	0.0647 €/kWh

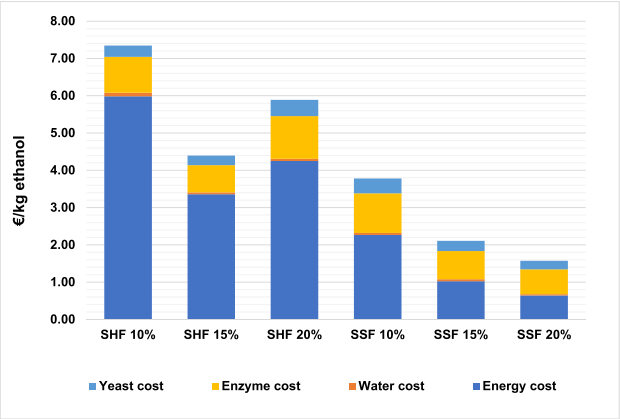

Fig. 7 The total bioethanol production cost and its fractionation for each case

parameter of enzyme cost has the most pronounced impact (43%). In the rest of the cases, the enzyme cost is the second most important parameter contributing 13–36% to the total production cost. The contribution of the cost of yeast is also not negligible (4–15%). On the other hand, water consumption in all cases contributes just slightly (1–3%).

In an effort to determine which factor has the greatest influence on the bioethanol production cost, a sensitivity analysis was performed considering the prices of the following factors: (1) energy, (2) enzymes, (3) yeast and (4) water. To this end, these values were changed by ± 20%. The best scenario from each fermentation mode was examined (15% solids loading for SHF and 20% solids loading for SSF) and the results are presented in Fig. 8.

The non-linear correlation of bioethanol with the four factors is obvious. The minimal unit ethanol cost is observed when the cost of energy is reduced by 20% for SHF and 15% solids loading, since the energy cost has the most pronounced impact on the total bioethanol cost. For SSF, the impact of energy and enzyme cost is similar. Thus, in view of reducing total bioethanol cost, the orientation of the research should focus on minimizing both the energy and enzyme consumption.

4 Conclusive remarks

The application of two fermentation modes SSF and SHF for bioethanol production from source-separated biowaste was investigated at a pilot scale. The performance of enzymes proved to be efficient at 35 °C, lower than their optimum temperature, but ideal for *Saccharomyces cerevisiae* activity. As a result, SSF could be a sustainable fermentation mode for producing bioethanol. The findings of this work could promote ethanol production from food waste at industrial scale. They may also serve as a beneficial guide for future studies aiming at elucidating the upscaling process for bioethanol production. Key stakeholders and interested parties are offered an alternative technology for efficiently treating food waste to reduce the environmental impact and to increase the economic benefit from their production line. Conclusively, an effective waste management system is required to make food waste–derived bioethanol viable. assistance.

However, some limitations and challenges should be noted. For instance, it is difficult to achieve consistency for food waste composition, as it varies and depends on resources such as diet, culture and community. Furthermore, the collection of food waste might be difficult because they are sometimes dispersed over a vast geographic territory. The recovery and purification of bioethanol is another intriguing research area. In addition, to improve profitability, it is recommended to potentially replace the distillation system with membrane distillation, which is a technology with low energy consumption and a potentially effective approach to reduce utility cost. As a result, hotspots for the bioethanol production management scheme must be identified.

Future studies should also consider investigating the sustainability features of the developed platform using

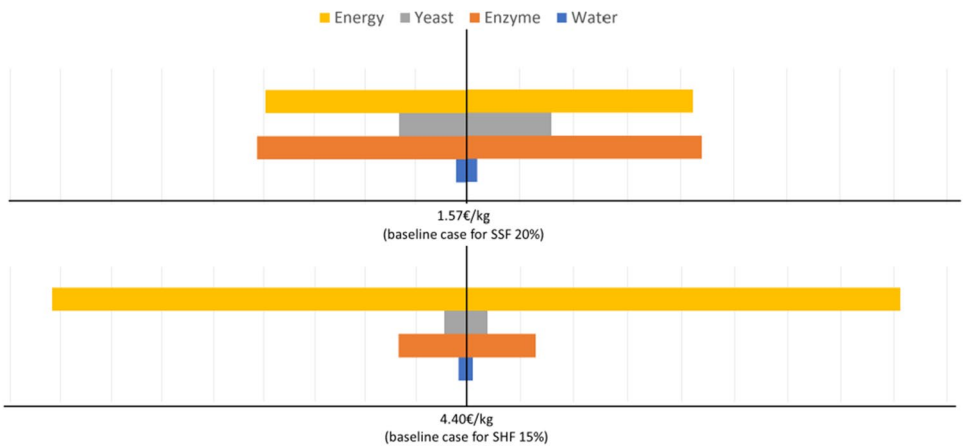

Fig. 8 The results from the sensitivity analysis of 15% solids loading for SHF and 20% solids loading for SSF

advanced sustainability assessment tools such as LCA, exergy and their combinations, including exergoenvironmental analysis. Rosen et al. [30] have elaborated on the significance of the sustainability of biofuel production processes. Similarly, Soltanian et al. [31] and Aghbashlo et al. [32] have elaborated on the significance of these methods in the context of renewable energy. Overall, a thorough analysis can offer more detailed information on the environmental consequences of the bioenergy production plants, thereby diagnosing the breakthrough points for additional environmental improvements.

Author contribution K. Passadis: investigation, data curation
D. Christianides: investigation
D. Malamis: project administration, funding acquisition, resources
E. M. Barampouti: conceptualization; writing—review and editing; writing—original draft
S. Mai: conceptualization; writing—review and editing; supervision; writing—original draft

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Declarations

Competing interests The authors declare no competing interests.

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