Abstract

The aim of the present study was to develop sustainable processes for maximum recovery of energy from domestic food waste (DFW) via their bioconversion to energy carriers i.e. the biofuels bio-ethanol, bio-hydrogen and methane. DFW was produced at the municipality level and were heat-dried and milled upon collection. Drying and milling were applied in order to prevent the biodegradation of the waste and to ensure its stable and unchanging composition during its storage. Two different approaches were tested. In the first approach, the dried DFW was initially subjected to extraction using warm water resulting to a liquid fraction (extract) and a solid residue. The extraction process was optimised and the fractions i.e. the rich in sugars extract and the residual solids were forwarded for ethanol and hydrogen production and subsequently the effluents were used as substrate for methane production via anaerobic digestion (AD). In the second approach, the DFW was suspended in water and directly used as substrate for the production of ethanol or hydrogen via simultaneous saccharification and fermentation and the effluents in both cases were subsequently subjected to AD, aiming at extra energy recovery via methane. The DFW and each fraction were also used as substrate for direct methane production, in order to estimate the overall biodegradability of the waste. Mono-cultures and co-cultures of the C5-yeasts *Pachysolen tanophilus* and *Pichia stipitis* as well as the typical C6-yeast *Saccharomyces cerevisiae* were used for the alcoholic fermentation, supplemented with cellulolytic enzymes, where applicable. For the production of hydrogen, mixed acidogenic consortia were used under mesophilic conditions. The effect of solids loading was studied for both processes, aiming at the optimisation of final yields and substrate bioconversion efficiency. Taking into account the optimum yields of biofuels, the amount of recoverable energy was estimated based for each different approach. Direct AD of either the whole DFW or individual fractions led to lower overall energy recovery, compared to that obtained when fermentation and subsequent AD were applied. Moreover, it was shown that the extraction process and separate bioconversion of the extract and the solid fraction led to higher yields of biofuels and consequently to higher energy recovery.

Keywords: food wastes, biohydrogen, bioethanol, methane
Introduction

In December 2015 the European Commission presented its package on the circular economy, in which the ambition of making a transition from a linear economy to a circular economy was presented [1]. It was noted that such a transition would greatly contribute to boosting the economic development of EU and improve its competitiveness and resource efficiency. In this framework, emphasis was given to the minimization of food wastes, an issue of importance for global food security and good environmental governance, directly linked with environmental, economic and social impacts.

Attempts have been made to quantify global food waste over several decades, motivated partly by the need to highlight the scale of ‘waste’ in relation to global malnutrition. Such assessments are reliant on limited data sets collected across the food supply chain (FSC) at different times and extrapolated to the larger picture. The most often quoted estimate is that ‘as much as half of all food grown is lost or wasted before and after it reaches the consumer.

Food waste (FW) is generated at different stages in the FSC, although it is most clearly defined in the retail and consumer stages, where outputs of the agricultural system are self-evidently ‘food’ for human consumption. In contrast to other commodity flows, FW is a biological material subject to degradation, and different food stuffs have different nutritional values. According to recent data of FAO (Food and Agriculture Organization) in the FSC almost 1.3 billion tons of food are disposed annually worldwide.

Thus, FW is exploitable, constituting an abundant source of renewable biomass, containing large amounts of proteins, carbohydrates, lipids and other nutrients that could be actually recovered and/or converted to other high added value products. Different strategies have so far been proposed for the valorization of different types of FW, depending on their composition and source. Domestic FW (DFW) i.e. FW occurring at the end of the FSC, is considered an ideal substrate for the production of various biofuels via microbiological processes, due to their high content in readily fermentable carbohydrates, such as sugars and starch and the necessary nutrients that can support efficient growth of different types of microorganisms. Another challenge is the heterogeneity that DFW presents, which is highly affected by the source from which the wastes are derived [2]. Nutritional habits and season of collection can also affect the composition of the DFW. In general, fruit and vegetable residues represent a significant portion of the wastes [3].

The aim of the present study was to develop a sustainable process for maximum recovery of energy from DFW via its bioconversion to energy carriers i.e. the biofuels bio-ethanol, bio-hydrogen and methane. DFW was produced at the municipality level and was heat-dried and milled upon collection. Drying and milling were applied in order to prevent the biodegradation of the waste and to ensure its stable and unchanging composition during its storage. For the production of biofuels, two different approaches were tested, in which either the whole DFW or the separated (liquid and solid) fractions obtained after an extraction process, were used. Energy inputs and outputs were estimated based on the operational conditions of the reactors for each different scenario and the final energy balances were assessed. The results of the study indicated that the exploitation of the DFW is quite appealing leading to promising energy recovery in all cases.
2. Materials and Methods

2.1. Raw material

The DFW was collected at municipality level twice a week from 230 houses of the Municipality of Halandri, Greece. Upon collection, DFW was subjected to simultaneous heat-drying at 95-98°C and shredding, resulting to a homogeneous organic product with the following characteristics: Total solids (TS) 91.28±0.75%, volatile solids (VS) 92.34±0.73%, soluble sugars 0.21±0.01 g/g TS, starch 0.16±0.01 g/g TS, total carbohydrates 0.43±0.03 g/g TS, total Kjeldahl nitrogen (TKN) 1.63±0.17 g/100 g TS, proteins 10.17 ± 1.06% (g/g TS). Drying and shredding were applied in order to homogenize the waste and to prevent its biodeterioration, ensuring its stable and unchanging composition during its storage.

2.2. Process approaches

Biofuels generation was performed using either a two-stage process or a two-stage configuration as illustrated in Figure 1.

![Figure 1](image-url)

**Figure 1.** Schematic illustration of alternative processes for the recovery of energy from DFW.

In the first approach, the dried DFW was initially subjected to extraction using warm water resulting to a liquid fraction (extract) and a solid residue. The rich in sugars extract was forwarded for ethanol and hydrogen production, whereas the solid fraction of the extraction process was also tested as substrate for hydrogen and ethanol production as well as for methane production via anaerobic digestion (AD). In the second approach, the water-suspended DFW was directly used as substrate for the production of ethanol or hydrogen via simultaneous saccharification and fermentation (SSF). The effluents in all cases were subsequently subjected to AD, aiming at extra energy recovery in the form of methane.

2.3. Extraction process

The extraction process was optimized in terms of maximum sugars recovery (% sugars/TS, w/w) and maximum concentration of sugars in the extract, by altering
the extraction period and temperature of water. Thus, different solids loadings i.e. 5, 10, 15 and 20 % wTS/v (mass/volume ratio of solids DFW (g TS) to water (mL)) were mixed for 30 or 60 min at room temperature (25°C) or upon heating at 30°C and the liquid fraction (extract) was characterized in terms of soluble sugars concentration.

2.4. Biofuels production

2.4.1 Bioethanol production

2.4.1.1. Microorganisms and media

All alcoholic fermentation tests were performed using the yeasts *Saccharomyces cereviceae*, CECT 1332, *Pichia stipitis* CECT 1922 and *Pachysolen tannophilus* CECT 1426. *S. cereviceae* and *P. stipitis* were stored at 4°C in slant solid cultures in the following medium (in g.L\(^{-1}\)): yeast extract 3; malt extract 3; myco-peptone 5; d-glucose 10; agar 20, while the composition of the medium for *P. tannophilus* stock cultures was: malt extract 20; myco-peptone 1; d-glucose 20; agar 15. For the startup of each experiment, slant cultures were used for the inoculation of 100 mL fresh liquid medium under sterile conditions. Cultures were incubated at 27°C, under mechanical agitation at 250 rpm for 24 h, in order to obtain cells at the same growth stage for every experiment. The cells contained in equal volumes of *S. cereviceae*, *P. stipitis* and *P. tannophilus* cultures were then harvested via centrifugation and used as inoculum for each experiment.

2.4.1.2. Experimental design for bioethanol production

Alcoholic fermentation was carried out in duplicate, in Erlenmeyer flasks of 100 mL, under microaerobic conditions, using mono-cultures and co-cultures of the yeasts *S. cereviceae*, *P. stipitis* and *P. tannophilus*. The experiments were performed using as substrate either the whole DFW under two different solids loadings i.e. 10 % and 20 % (wTS/v) or using separately the liquid and solid fractions that were obtained from the optimized extraction process. In the latter case, the solids loadings of the residues tested were 10 % and 20% (wTS/v), whereas the extract had a concentration of sugars of 42.84 ± 2.15 g/L, resulting from the extraction of 20% wTS/v DFW at 25°C for 30 min. In all experiments, inoculation was performed with centrifuged cells from pre-cultures of the three strains, in the late exponential phase, while 1 g/L KH\(_2\)PO\(_4\) and 1 g/L MgCl\(_2\)*6H\(_2\)O were also added for nutrients supplementation. In the experiments containing solids (the whole fraction of DFW or the solid fraction of the extraction process), 10 FPU/g TS of Celluclast (Cellulase from *Trichoderma reesei*, ATCC 26921) and Novozyme 188 (Cellobiase from *Aspergillus niger*) at a ratio of (3:1) [4] were also added under sterile conditions before inoculation, in a SSF concept. The flasks were plugged with hydrophobic cotton to allow microaerobic conditions and were incubated at 30°C with mechanical agitation at 250rpm, for 68 h, whereas sampling was performed every 8 h.

2.4.2 Hydrogen production

2.4.2.1 Seed culture

Sludge from the anaerobic digester of Metamorphosis, Athens wastewater treatment plant, operating at steady state at an HRT of 15 d, was used as inoculum. The sludge characteristics were: pH=7.78 ± 0.10, Total Suspended Solids (TSS) = 39.65 ± 0.02 g/L and Volatile Suspended Solids (VSS) = 18.32 ± 0.52 g/L. The sludge was gassed with a mixture of N\(_2\)/CO\(_2\) (80/20) in order to secure anaerobic conditions.
and then was boiled at a constant temperature of 100°C for 15 min to eliminate methanogens and preserve selectively the hydrogen producing clostridia [5].

2.4.2.2 Biohydrogen production potential (BHP) experiments

Batch hydrogen production experiments were carried out in duplicate at 35°C, in 160 mL serum bottles. The BHP experiments, were performed using either the whole DFW under different solids loadings (1, 2 and 5 % (wTS/v) or using separately the liquid and solid fractions (loading 1 % (wTS/v)) obtained after the optimized extraction process (20% (wTS/v), 25°C, 30 min). For the experiments with the DFW or the solid residues, 10 mL of mixed anaerobic culture were seeded with 40 mL of a nutrient medium (g/L): NaH₂PO₄⋅2H₂O 8.98; Na₂HPO₄⋅2H₂O 5.2; yeast extract 0.625, 10 mL/L of the inoculum of trace elements and appropriate amounts of solids samples were added. All experiments were performed either without or with the addition of enzymes, in SSF mode (40 FPU /gTS of Celluclast 1.5 L and Novozyme 188, at a ratio of 3:1). For the experiments with the liquid fraction obtained after the extraction process, 20 mL or 10 mL of extract were used at a final volume of 50 mL culture, corresponding to final sugars concentrations of 17.14 ± 0.86 g/L and 8.56 ± 0.43 g/L, respectively. The seed cultures were 10 mL in both cases, whereas, for the supplementation with nutrients, the following basal nutrient medium was also used (g/L): NaH₂PO₄⋅2H₂O 17.84; Na₂HPO₄⋅2H₂O 10.4; yeast extract 1.3 and the solution of the trace elements. The initial pH was set to 6, with 6N NaOH or 6N HCl solutions and the cultures were incubated at 35°C at 100 rpm. Blank experiments were also carried out, in order to determine the background hydrogen productivity of the inoculum. The content of the vials was gassed with a mixture of N₂/CO₂ (80/20), in order to ensure anaerobic conditions. The vials were sealed with butyl rubber stoppers and aluminum crimps, and hydrogen production was monitored over time.

2.4.3 Methane production

2.4.3.1 Seed culture

Sludge from the anaerobic digester of Metamorphosis, Athens wastewater treatment plant, operating at steady state at an HRT of 15 d, was used as inoculum. The sludge characteristics were: pH=7.41 ± 0.15, TSS = 39.53 ± 0.12 g/L and VSS = 18.62 ± 0.52 g/L. The sludge was gassed with a mixture of N₂/CO₂ (80/20) in order to secure anaerobic conditions.

2.4.3.2 Biochemical methane potential (BMP) experiments

BMP experiments were carried out in duplicate at 35°C in serum bottles of 160 mL, at 100 mL working volume. The experiments were performed according to the modified protocol of Owen and Chynoweth [6]. BMP tests were performed on the whole DFW, on the separate liquid and solid fractions obtained after the optimized extraction process (20% (wTS/v), 25°C, 30 min) and on the liquid effluents of the hydrogen and ethanol production experiments. For the experiments with the DFW and the solid residues after extraction, 20 mL of mixed anaerobic culture, 80 mL of water and appropriate amounts of samples were added, in order to acquire the desirable VS content of 2 g VS / L, while for the experiments with the liquid extract and effluents, 20 mL of mixed anaerobic culture was seeded with water and appropriate volumes of liquids, so that their final Chemical Oxygen Demand (COD) concentration was 2 g/L. For all experiments, the microbial culture was supplemented with 10 mL/L of the solution of the trace elements. Control experiments for checking the methanogenic biomass activity were carried out using glucose. Blank experiments were also carried out in order to determine the background gas productivity of the inoculum. The
content of the vials was gassed with a mixture of N₂/CO₂ (80/20) in order to secure anaerobic conditions. The vials were sealed with butyl rubber stoppers and aluminum crimps and methane production was monitored as a function of time according to Owen and Chynoweth [6].

2.4.4 Analytical methods

2.4.4.1. Analysis of chemical composition

The measurements of TS, VS, TSS, VSS and COD were carried out according to Standard Methods [7]. For the quantification of the carbohydrates, a colored sugar derivative was produced through the addition of L-tryptophan and sulphuric and boric acids and subsequently measured colorimetrically at 520 nm [8]. Total Kjeldahl Nitrogen (TKN) was carried out according to Standard Methods [7]. Crude protein content was estimated by multiplying TKN by a factor of 6.25 [9]. The oil and grease content of the waste was determined according to the Soxhlet extraction method [7].

2.4.4.2. Analytical methods for bioethanol, BHP and BMP experiments

The filter paper activity (FPU) of cellulase (Celluclast 1.5L) was measured with the improved method developed by Ghose [10]. For ethanol analysis, an HPLC-R1 with an Aminex HPX-87H column (Biorad) and a Cation H micro-guard cartridge (biorad Laboratories) at 60°C using H₂SO₄ 0.006 N, as an eluent, at a flow rate of 0.7 mL/min, was used. The produced gas composition in hydrogen and methane was quantified with a gas chromatograph (SRI 8610c MG#1), equipped with a thermal conductivity detector and a packed column. The carrier gas was nitrogen for hydrogen measurements and helium for methane. The injector, column and detector temperatures were set at 90°C, 35°C and 100°C, respectively. The volume of the produced gas was measured by the method of displacement of acidified water. For the quantification of the Volatile fatty acids (VFAs) i.e acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and hexanoate, acidified samples with 3μL 20% H₂SO₄ were analyzed by a Varian CP-3800 GC equipped with a flame ionization detector. The measurement of the pH was done using a HANNA (pH 211) pH-meter with a HANNA electrode (HI 1230).

2.4.5 Statistical analysis

The statistical analysis of the obtained data was conducted with the use of the SPSS Inc.17 software package. After checking for homogeneity of the variance (Levene's test of equality of error variances), the significant differences for each treatment were assessed non-parametrically, using the Mann Whitney u test (p < 0.05, ANOVA).

3. Results and discussion

3.1 Optimization of extraction method

In order to obtain maximum recovery of sugars from the DFW via extraction, different handlings were followed in terms of solids loading, temperature of water and time of extraction process. In Figure 2, the concentration of sugars in the extract as well as the yield of sugars obtained from DFW are presented for the different handlings.
The statistical analysis of the values obtained for each solids loading revealed no significant differences in neither sugars’ concentration nor yields. As such, the mean yield of sugars was estimated to be 21.93 ± 1.46 %, indicating thus that the whole amount of the soluble sugars contained in the DFW (0.21±0.01g sugars/g DFW) can actually be recovered even using the smaller temperature and extraction time and the highest solids loading. The maximum concentration of sugars achieved was 43.27±1.95 g/L for 20% TS w/v used, 35°C and 30 min of extraction time, whereas the maximum mean value for all handling at the same solids loading was a value that was 42.76±0.45 g/L. According to those findings the extraction protocol that was selected for all following experiments was 20% TS w/v, 25°C and 30 min of extraction time.

### 3.2 Valorization of DFW through different approaches

#### 3.2.1 Ethanol production

DFW contains soluble sugars that can be directly fermented from different yeast strains towards ethanol, as well as complex polysaccharides such as starch, cellulose and hemicellulose, which need to be hydrolyzed in order to be fermented by yeasts. In the present study, the efficiency of ethanol production from DFW was tested via two different approaches i.e. the direct fermentation of the whole DFW biomass and the separate fermentation of the soluble sugars upon recovery and the remaining solids, which contain the complex polysaccharides only. In order to exploit the complex polysaccharides of the waste, the culturing media were supplemented with cellulytic enzymes when DFW and solids were used as substrate.

In Figure 3 the maximum ethanol concentrations ($C_{E_{OH}}$) obtained via mono- and co-cultures of *S. cereviceae* (S.c), *P. stipitis* (P.s) and *P. tannophilus* (P.t), from the whole DFW and the remaining solids after extraction of the sugars and the extract are shown. It can be observed that for the fermentation of 10% TS w/v of the whole DFW, the use of different microorganisms did not have any significant effect on the maximum $C_{E_{OH}}$ achieved. Indeed, in all cases the $C_{E_{OH}}$ was similar, i.e. ranging from 12.10±0.58g/L to 13.04±0.44g/L. However, when the solids loading was doubled, the use of co-cultures exhibited a tendency for higher $C_{E_{OH}}$ production, reaching...
24.71±0.78 g/L and 24.83±1.05 g/L for the S.c-P.t and S.c-P.s co-cultures respectively. Results were similar when the solid residue was used as substrate, with the highest $C_{E_{OH}}$, 25.7±0.6 g/L, observed for the S.c-P.s co-culture. For the extract, the highest and the lowest $C_{E_{OH}}$ observed were 10.5±0.6 g/L 7.83±0.45 g/L, for the S.c-P.s co-cultures and the P.t. monoculture, respectively.

![Figure 3](image-url)

Figure 3. Maximum ethanol concentration achieved by alcoholic fermentation of (a) DFW (10 and 20% wTS/v), (b), remaining solids after the extraction of sugars (10 and 20% wTS/v) and (c), extract, using mono-and co-cultures of S. cereviceae (S.c), P. stipitis (P.s) and P. tannophilus (P.t).

Table 1: Estimated ethanol yields (g-ethanol/g initial carbohydrates) $Y_{E_{OH/Carb}}$ achieved by alcoholic fermentation of DFW (10 and 20% wTS/v), remaining solids after extraction of sugars (10 and 20% TS w/v) and extract, using mono-and co-cultures of S. cereviceae (S.c), P. stipitis (P.s) and P. tannophilus (P.t).

<table>
<thead>
<tr>
<th>Substrate/microorganism</th>
<th>DFW 10%</th>
<th>DFW 20%</th>
<th>solids 10%</th>
<th>solids 20%</th>
<th>extract 10%</th>
<th>extract 20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.c</td>
<td>0.30 ± 0.01</td>
<td>0.26 ± 0.02</td>
<td>0.46 ± 0.02</td>
<td>0.44 ± 0.01</td>
<td>0.22 ± 0.01</td>
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<tr>
<td>P.s</td>
<td>0.29 ± 0.03</td>
<td>0.25 ± 0.01</td>
<td>0.48 ± 0.01</td>
<td>0.45 ± 0.02</td>
<td>0.19 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>P.t</td>
<td>0.28 ± 0.01</td>
<td>0.26 ± 0.00</td>
<td>0.44 ± 0.04</td>
<td>0.42 ± 0.01</td>
<td>0.18 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>S.c-P.s</td>
<td>0.30 ± 0.00</td>
<td>0.27 ± 0.01</td>
<td>0.51 ± 0.03</td>
<td>0.46 ± 0.01</td>
<td>0.22 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>S.c-P.t</td>
<td>0.30 ± 0.01</td>
<td>0.27 ± 0.02</td>
<td>0.52 ± 0.02</td>
<td>0.44 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>
Taking into account that the initial concentrations of total carbohydrates were estimated to be 43g/L and 86g/L approximately, for the 10% TS w/v and the 20% TS w/v of the whole DFW; 28g/L and 55g/L approximately, for 10% TS w/v and the 20% TS w/v of the remaining solids, and 42g/L for the extract, the respective yields of ethanol from the initial carbohydrates ($Y_{\text{ETOH carb}}$) can be estimated and are presented in Table 1. As shown, regardless the type of microorganism used, the highest $Y_{\text{ETOH carb}}$ were observed in the case that the solids were used as substrate and the lowest when the extract was used as substrate. Moreover, in all cases $Y_{\text{ETOH carb}}$ tends to be higher for the lower solids loadings. These observations could be attributed to possible substrate inhibition. Indeed, during the fermentation of the solids residues of the DFW the concentration of sugars ($C_{\text{sug}}$) in the fermentation media is controlled by the hydrolysis step of carbohydrates which is low compared to the accumulation capacity of the microorganisms [11], and consequently the $C_{\text{sug}}$ is expected to be low and having thus no inhibiting effect on the alcoholic fermentation. On the contrary when extract or even DFW is used as substrate, yeasts are directly subjected to an environment of quite high $C_{\text{sug}}$ that could have an inhibitory effect on ethanol production [12].

![Figure 4](image_url)

**Figure 4.** Ethanol yields expressed as g EtOH/g initial DFW used, during alcoholic fermentation of (a) DFW (10 and 20% wTS/v), (b) remaining solids after extraction of sugars (10 and 20% TS w/v), (c) extract and (d) extract and 10% TS w/v solids (sum of yields), using mono-and co-cultures of *S. cereviceae* (*S.c*), *P. stiptis* (*P.s*) and *P. tannophilus* (*P.t*).

In Figure 4, the respective ethanol yields expressed as g EtOH/g initial DFW ($Y_{\text{ETOH/DFW}}$) used, are presented. As shown, the higher solids loading resulted in lower yields for both DFW and residues for all microorganisms used, an observation that is
in agreement with the previous assumptions. It has to be mentioned that the results concerning ethanol yields from solid residues are normalized, taking into account the loss of biomass (20% approximately) due to the removal of sugars via extraction. As expected, the yields from DFW are higher than the respective ones from solids and extract. However, the comparison of achieved yields via direct fermentation of DFW and via separate fermentation of soluble sugars and the remaining solids reveals that in the second case the overall yields of ethanol per mass unit of initial DFW is considerably higher. Indeed as shown in Figure 4.d the $Y_{ETOH/DFW}$ achieved from the co-cultures of $S.c$-$P.s$ and $S.c$-$P.t.$ via separate fermentation of extraction fractions both reached the value of 0.16 g EtOH/g DFW, whereas the respective values for direct fermentation of the DFW were 0.12±0.00 g EtOH/g DFW and 0.13±0.00 g EtOH/g DFW, respectively.

Matsakas et al. [13] using commercial $S. cereviceae$ for ethanol production from household food wastes, reached to 24.75 ± 2.20 g/L and 39.15 ± 0.75g/L $C_{EIOH}$ for solids loadings 35% and 45% (on a dry basis), respectively. Alcoholic fermentation was achieved via SSF using Celluclast 1.5 L and Novozyme 188 for the hydrolysis of complex carbohydrates. According to the study of Jeong et al. [14] 40.59 g/L of ethanol were produced during the fermentation of pre-hydrolyzed food wastes via $S. coreanus$, whereas when $P. stipitis$ was introduced as a co-fermenting microorganism, ethanol production increased up to 48.63 g/L. In other studies, using starchy food wastes for ethanol production and high glucoamylase load for their hydrolysis, extremely high $C_{EIOH}$ are reported i.e 81.5 g/L [15] and 57.5 g/L [16] of ethanol. It can be assumed thus that the addition of amylolytic enzymes during fermentation of the DFW used in the current study could further enhance the production of ethanol, resulting in higher yields.

3.2.2 Biohydrogen production

In order to determine the optimum organic loadings for fermentative hydrogen production, BHP experiments under different solids loadings (1, 2 and 5 % wTS/v) were performed. In addition, a mixture of cellulose- degrading enzymes was added, so as to facilitate the hydrolysis of complex carbohydrates and to increase the quantities of free sugars. In figure 5, the cumulative hydrogen production of DFW under different solids loadings, without or with (SSF concept) cellulolytic enzymes addition, is presented. No methane was detected among the gaseous products ($H_2$ and $CO_2$), confirming the efficiency of heat-sock inoculum pre-treatment (100 °C for 15 min) to suppress the activity of methanogens present in the anaerobic sludge. For the experiments which were performed without addition of enzymes (only fermentation), the cumulative hydrogen production increased proportionally to the TS loading increase, i.e. the hydrogen evolution of the 5% DFW suspensions (wTS/v) was approximately 5-fold higher than the respective of the 1%. Figure 6 shows that the increase of TS loading did not affect the final hydrogen yield of DFW, which was 118.89 ± 7.52 mL $H_2$/g TS, taking into account the values obtained from all loadings. The high hydrogen yield obtained in the present study could be attributed to the high amount of soluble sugars contained in the DFW, since it is well known that the production of hydrogen by mixed cultures could be directly correlated to the soluble sugars content [17].

For the 1 and 2% DFW suspensions (wTS/v), the addition of enzymes had a positive effect on the hydrogen cumulative volumes and yields, with the hydrogen yield rising to 204.41 ± 4.37 L $H_2$/kg TS, taking into account the values obtained from
both loadings. In contrast, the addition of enzymes in the 5% DFW did not enhance the hydrogen yield (116.14 ± 5.26 L H₂/kg TS), which could be attributed to a possible substrate inhibition. The DFW that was used in the present study consisted of ~43% (w/wTS) total carbohydrates, ~21% of which corresponded to sugars. The initial concentration of carbohydrates of 5% DFW suspensions (wTS/v) was ~20 g/L, a high portion of which was solubilized during the experimental period, due to the addition of cellulolytic enzymes, in the SSF concept. The high concentration of sugars which were probably released during fermentation might be inhibitory to the fermentative hydrogen production microorganisms or the hydrolytic enzymes, obtaining thus decreased hydrogen yields. The experimental results obtained are in agreement with the results from other studies using different substrates and substrate concentrations. Lay [18] reported that hydrogen production was significantly inhibited, when microcrystalline cellulose exceeded 25 g/L, when batch fermentative hydrogen production from cellulose was studied at initial cellulose concentration of 12.5 – 50 g/L. In addition, other studies in batch systems reported that an initial substrate concentration of 20 g COD/L for xylose [19], 10.7 g COD/L for glucose [20], 9.8 g COD/L for starch [21], and 6.4 g COD/L for food waste [22] was optimum for hydrogen production.

In Table 2, the pH of the culture at the end of the experiments is presented. It is obvious that only for the experiments with 5% DFW suspensions (wTS/v) at SSF, the pH culture is 4.09 ± 0.01, which is far from the optimum pH range for hydrogen production (pH of 5 to 6) [21]. The drop in the pH observed for the 5% DFW could be a cause for the reduced hydrogen yield observed in this case, in comparison with the 1 and 2% DFW cases.

Table 2: Metabolic products distribution and pH values accompanied by their standard deviations, at the end of BHP experiments.

<table>
<thead>
<tr>
<th>Solids loading (% wTS/v)</th>
<th>Enzymes</th>
<th>pH</th>
<th>Acetic acid (mg/gTS)</th>
<th>Propionic acid (mg/gTS)</th>
<th>Butyric acid (mg/gTS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>no</td>
<td>5.63 ± 0.01</td>
<td>176.45 ± 2.38</td>
<td>49.50 ± 0.82</td>
<td>49.50 ± 2.97</td>
</tr>
<tr>
<td></td>
<td>10 FPU/gTS</td>
<td>5.30 ± 0.00</td>
<td>255.35 ± 22.98</td>
<td>45.86 ± 8.43</td>
<td>136.88 ± 5.10</td>
</tr>
<tr>
<td>2</td>
<td>no</td>
<td>5.11 ± 0.02</td>
<td>134.68 ± 2.80</td>
<td>20.97 ± 0.96</td>
<td>76.21 ± 2.60</td>
</tr>
<tr>
<td></td>
<td>10 FPU/gTS</td>
<td>5.05 ± 0.01</td>
<td>169.57 ± 10.14</td>
<td>13.65 ± 0.96</td>
<td>157.18 ± 10.32</td>
</tr>
<tr>
<td>5</td>
<td>no</td>
<td>4.87 ± 0.00</td>
<td>60.77 ± 0.13</td>
<td>2.65 ± 0.02</td>
<td>96.55 ± 3.93</td>
</tr>
<tr>
<td></td>
<td>10 FPU/gTS</td>
<td>4.09 ± 0.01</td>
<td>42.32 ± 8.67</td>
<td>6.65 ± 0.01</td>
<td>92.69 ± 23.96</td>
</tr>
</tbody>
</table>

The involved metabolic pathways in hydrogen production can either be promoted or suppressed, depending on the prevailing operating conditions, which govern the simultaneous production of specific VFAs including acetate, propionate, butyrate and reduced products, such as ethanol and lactate [23]. Among the liquid metabolites produced at the end of the fermentation, acetic acid, n-butyric and propionic acid were detected (Table 2). Acetate and butyrate were the dominant metabolic products, with the concentration of acetate being higher at the BHP experiments with the lower TS loadings. The yield of acetic acid decreased with the increase of TS loading to 5% w/v, with the butyrate being the dominant metabolic product. Butyric acid concentration increased with the enzymes addition at the TS
loading of 1 and 2 % w/v, being 136.88 ± 5.10 and 157.18 ± 10.32 mg/gTS respectively. As expected, hydrogen production yields were strongly related to butyrate production; with the higher hydrogen yield matched with the highest yields of butyrate. At the solids loading of 5% w/v, the metabolic products distribution was similar, either with or without enzymes addition. Under the latter conditions, the pH culture was low (4.87 and 4.09). Under low pH values, the generation of more reduced end products such as lactate and ethanol, which are known as metabolites generated in zero-hydrogen balance pathways, is favored [24]. This is consistent with the decreased acetate and butyrate production which was observed under these conditions.

Figure 5. Cumulative hydrogen production of DFW over time under different solids loadings (1, 2 and 5 % wTS/v), either without or with (SSF) enzymes addition (10 FPU Celluclast 1.5 L/g TS and Novozyme 188 at a ratio 3:1).

Figure 6. Hydrogen yields of DFW under different solids loadings (1, 2 and 5 % wTS/v), either without or with (SSF) enzymes addition.

BHP experiments with the separate fractions (extract and solid fraction) obtained after extraction procedure were also performed and are presented in figure 7. The experiments with the solid fractions were conducted either without or with
enzymes addition, in the SSF concept. For the extract, two different dilutions i.e. 1/2 and 1/4 were tested, resulting to 17g/L and 8.5 g/L initial concentration of sugars ($C_{sug}$) respectively, in order to assess any inhibitory effect due to initial sugars concentration. Hydrogen yields, pH and metabolic products distribution at the end of the experiments are also presented in table 3.

Table 3: Metabolic products distribution and pH values accompanied by their standard deviations, at the end of BHP experiments (the values correspond to the TS of DFW)

<table>
<thead>
<tr>
<th>parameters</th>
<th>Solids (1% wTS/v)</th>
<th>Extract $C_{sug}$ = 17g/L</th>
<th>Extract $C_{sug}$ = 8.5g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No enzymes</td>
<td>10 FPU/gTS</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>5.65 ± 0.05</td>
<td>4.96 ± 0.23</td>
<td>4.97 ± 0.01</td>
</tr>
<tr>
<td>yield, LH$_2$/kg TS</td>
<td>64.18± 3.18</td>
<td>160.59± 7.94</td>
<td>47.63± 2.64</td>
</tr>
<tr>
<td>Acetic acid (g/kgTS)</td>
<td>126.37±3.10</td>
<td>182.91±4.16</td>
<td>36.53±3.88</td>
</tr>
<tr>
<td>Propionic acid (g/kgTS)</td>
<td>8.11±1.25</td>
<td>26.18±3.20</td>
<td>3.83±0.25</td>
</tr>
<tr>
<td>Butyric acid (g/kgTS)</td>
<td>14.31±1.82</td>
<td>68.76±4.24</td>
<td>38.53±2.16</td>
</tr>
</tbody>
</table>

It is obvious that the addition of enzymes, strongly enhanced the hydrogen yield from the solid fraction obtained after the extraction process (from 64.18 ± 3.18 mLH$_2$/g TS to 160.59± 7.94 mLH$_2$/g TS under SSF), indicating that a high portion of hydrogen is produced due to the hydrolysis of structural carbohydrates which was carried out with SSF. The fact that the hydrogen yield of the solid fraction without addition of enzymes was much lower than the respective of DFW at the same solids loading could be attributed to the removal of the soluble sugars during extraction. In addition, the experiments with the extract under two different dilutions showed that the different initial concentrations of sugars had no effect on the maximum hydrogen yield, indicating that there is no substrate inhibition, at the conditions tested.

The experiments showed that the separation of liquid and solid fraction had no influence on the hydrogen yield of DFW at low solid loadings (1 and 2 % wTS/v), i.e.
118.89 ± 7.52 mL H₂/g TS and 204.41 ± 4.37 mL H₂/g TS (under SSF) are produced when no separation is performed, while 112.92 mL H₂/g TS and 209.33 mL H₂/g TS (under SSF) are produced when an extraction process is performed.

3.2.3 Anaerobic digestion of DFW and effluents for methane production

Biochemical methane potential experiments were performed for DFW, the separated fractions obtained after the extraction process as well as from the effluents of the hydrogen and ethanol production experiments. In figure 8, the cumulative methane production in the above experiments is presented, while in table 4 the final methane yields are summarized.

The biogas and methane production rates in all BMP experiments were initially high (0-15 d), while the rates decreased later. The calculated yield in methane from DFW after subtracting the methane produced from blank experiments, was almost 429.44 ± 2.66 mL CH₄/g TS, or 465.06 ± 2.88 mL CH₄/g VS, indicating its high biodegradability. This value is similar with the respective of other studies, using DFW as substrate [25]. As shown in table 4, it is obvious that the extraction process and the separation of liquid and solid fractions did not influence the methane yield of DFW since 441.07 mL CH₄/g TS are produced (329.72 ± 0.94 mL CH₄/g TS from the solid and 111.35 ± 7.59 mL CH₄/g TS from the liquid fraction, respectively).

Table 4: Methane yields in the BMP experiments from DFW, from the separate solid and liquid fractions obtained after the DFW extraction process, as well as from the effluents of ethanol and hydrogen fermentation process, using the same substrates.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Methane yield</th>
<th>Methane yield (L CH₄/kg TS DFW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFW</td>
<td>429.44 ± 2.66</td>
<td>429.44 ± 2.66</td>
</tr>
<tr>
<td>Solid fraction</td>
<td>433.52 ± 1.23</td>
<td>329.72 ± 0.94</td>
</tr>
<tr>
<td>Extract</td>
<td>22.27 ± 1.52</td>
<td>111.35 ± 7.59</td>
</tr>
<tr>
<td>Effluents from DFW fermentation</td>
<td>4.20 ± 0.36</td>
<td>419.07 ± 35.42</td>
</tr>
<tr>
<td>Effluent from solid fraction fermentation</td>
<td>4.32 ± 0.35</td>
<td>315.14 ± 25.48</td>
</tr>
<tr>
<td>Effluent from extract fermentation</td>
<td>9.61 ± 0.45</td>
<td>120.17 ± 5.65</td>
</tr>
</tbody>
</table>

1 expressed in L CH₄/kg TS DFW, 2 L CH₄/kg TS solids, 3 L CH₄/L extract, 4 L CH₄/L effluent

By comparing the methane yield of the liquid effluents coming from the fermentations (ethanol and hydrogen production experiments) it can be concluded that the extraction process had no effect on the yield of methane, since 419.07 ± 35.42 mL CH₄/g TS are produced when the DFW was used as substrate during the fermentations, while 315.14 ± 25.48 mL CH₄/g TS and 120.17 ± 5.65 mL CH₄/g TS are produced, when the solid and the liquid fraction were separately used as substrates, respectively.

Comparing the different approaches, it can be seen that the methane yield of DFW in a single stage process (AD) was similar to the respective during a two-stage process (hydrogen -AD or ethanol -AD). This fact is consistent with Monlau et al. [26] who found that a two-stage H₂/CH₄ process had no influence on the methane yield obtained in comparison with the single-stage CH₄ process (152±4 mL CH₄ /gVS).
3.2 Estimation of energy recovery via different approaches

Taking into account the maximum yield of each biofuel generated from different handlings, the maximum recoverable energy from DFW was estimated for the two main approaches tested (Figure 1) i.e. the direct fermentation/AD of the whole DFW biomass and separate fermentation/anaerobic digestion of fractions upon extraction. The direct anaerobic digestion of the waste for methane production as well as the initial fermentation towards ethanol/hydrogen production and subsequent anaerobic digestion were taken into account as illustrated in Figure 9.

**Figure 9.** Schematic illustration of the maximum yields of biofuels generated from the valorization of DFW during different approaches.
In order to calculate the stored energy in each biofuel, their energy densities (ED) were used. As such, the ED of ethanol, hydrogen and methane were assumed to be 26.4 kJ/g, 142 kJ/g and 55.5 kJ/g, respectively. The estimated stored energy in the form of ethanol, hydrogen and methane that were produced from the whole DFW and its fractions (extract and residual solids) after the extraction process are summarized below.

As shown in table 5 the maximum total recoverable energy from DFW is quite similar when a two configuration methodology is followed i.e. fermentation and AD of the effluents, regardless if ethanol or hydrogen is produced. However, if the DFW is forwarded to direct AD the energy gain is lower with the relative decrease being approximately 11% and 14% if hydrogen or ethanol is produced, respectively. The energy gain is even higher when fermentation and AD are applied to the different fractions obtained from extraction. As such (table 6) the relative increase of recoverable energy when ethanol is produced from both extract and solids and then methane is produced from the effluents produced is almost 19% compared to that obtained from direct AD of the extract and solids. When hydrogen instead of ethanol is produced, the energy gain is lower, since the relative increase is approximately 12%.

### Table 5: Estimated stored energy per biofuels produced and overall recoverable energy from different fermentation strategies and/or AD of the whole DFW.

<table>
<thead>
<tr>
<th>Biofuel produced from DFW</th>
<th>Stored energy per biofuel</th>
<th>TOTAL recoverable energy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kJ /kg TS of DFW</td>
<td></td>
</tr>
<tr>
<td>EtOH</td>
<td>3374±80</td>
<td>19987±542*</td>
</tr>
<tr>
<td>H₂</td>
<td>2592±55</td>
<td>19205±470**</td>
</tr>
<tr>
<td>CH₄ from effluents</td>
<td>16613±1404</td>
<td></td>
</tr>
<tr>
<td>CH₄ from direct AD</td>
<td>17024±105</td>
<td></td>
</tr>
</tbody>
</table>

(*) in case ethanol and methane are produced, (**) in case hydrogen and methane are produced

### Table 6: Estimated stored energy per biofuels produced and overall recoverable energy from different fermentation strategies and/or AD of the different fraction of DFW after extraction of sugars.

<table>
<thead>
<tr>
<th>Biofuel produced</th>
<th>Stored energy per biofuel</th>
<th>TOTAL recoverable energy</th>
<th>Stored energy per biofuel</th>
<th>TOTAL recoverable energy</th>
<th>TOTAL recoverable energy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>from solids</td>
<td>from extract</td>
<td>from solids+extract</td>
<td></td>
</tr>
<tr>
<td>EtOH</td>
<td>2938±80</td>
<td>15457±307*</td>
<td>1301±106</td>
<td>6060±113*</td>
<td>21495±568*</td>
</tr>
<tr>
<td>H₂</td>
<td>2036±101</td>
<td>14535±412**</td>
<td>632±9</td>
<td>5399±89**</td>
<td>19923±458**</td>
</tr>
<tr>
<td>CH₄ from effluents</td>
<td>12493±1010</td>
<td>4764±224</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₄ from direct AD</td>
<td>13071±37</td>
<td>4414±300</td>
<td>17475±168</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*) in case ethanol and methane are produced, (**) in case hydrogen and methane are produced
4. Conclusions

- Co-cultures of *S. Cereviceae* with either of the C₅ consuming yeasts led to higher ethanol yields in all cases.
- The extraction process was shown to be beneficial for alcoholic fermentation, since higher ethanol yields were obtained during separate fermentation of the soluble sugars and the residual solids.
- Hydrogen production was significantly enhanced when enzymatic hydrolysis of the DFW was applied, but only for the lowest solids loadings i.e. 1% and 2% TSw/v.
- Direct AD of either the whole DFW or each fraction separately led to lower energy recovery compared to that obtained when fermentation and subsequent AD were applied.
- The extraction process and separate bioconversion of the extract and the solid fraction led to higher yields of biofuels and consequently to higher energy recovery.

It should be mentioned that the most viable overall process scheme will be the result of a technoeconomic study, accounting for the extra cost involved in separating fractions and in adding enzymes. The present study is a valuable prerequisite for such an assessment.

Acknowledgments

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Literature


