Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept

Prasad Kaparaju a, María Serrano a, Anne Belinda Thomsen b, Prawit Kongjan a, Irini Angelidaki a,⁎

a Department of Environmental Engineering, Technical University of Denmark, Building 115, DK-2800 Kgs. Lyngby, Denmark
b Biosystems Department, RISØ-DTU, Building 301, DK-4000, Roskilde, Denmark

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ABSTRACT

The production of bioethanol, biohydrogen and biogas from wheat straw was investigated within a biorefinery framework. Initially, wheat straw was hydrothermally liberated to a cellulose rich fiber fraction and a hemicellulose rich liquid fraction (hydrolysate). Enzymatic hydrolysis and subsequent fermentation of cellulose yielded 0.41 g-ethanol/g-glucose, while dark fermentation of hydrolysate produced 178.0 ml-H2/g-sugars. The effluents from both bioethanol and biohydrogen processes were further used to produce methane with the yields of 0.324 and 0.381 m3/kg volatile solids (VS)added, respectively. Additionally, evaluation of six different wheat straw-to-biofuel production scenario showed that either use of wheat straw for biogas production or multi-fuel production were the energetically most efficient processes compared to production of mono-fuel such as bioethanol when fermenting C6 sugars alone. Thus, multiple biofuels production from wheat straw can increase the efficiency for material and energy and can presumably be more economical process for biomass utilization.

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1. Introduction

Lignocellulosic materials from agriculture and forest management are the largest sources of hexose (C-6) and pentose (C-5) sugars with a potential for the production of biofuels, chemicals and other economic by-products. Progress in this area will not only decouple the food and biofuel production and reduced CO2 emissions but also ensure a more stable and secured energy supply especially in transport sector. Biofuels generated globally from lignocellulosic materials are estimated at about 30 EJ/year, compared to the total energy used world wide of over 400 EJ/year (McKendry, 2002).

Biorefineries for production of several products and by-products such as biofuels, heat and/or electricity have been in focus in the recent years (Chen et al., 2005; Zhang, 2008). In a biorefinery, biomass can be converted to useful biomaterials and/or energy carriers in an integrated manner and thereby it can maximize the economic value of the biomass used while reducing the waste streams produced (Thomsen, 2005). Development of multiple biofuels based biorefinery from lignocellulose is seen as an important possibility to increase the efficiency for materials and energy, and reduce the costs of biomass options to mitigate GHG emissions (Sheehan et al., 2003).

Lignocellulose composed of cellulose (40–50%), hemicelluloses (25–35%) and lignin (15–20%) is extremely resistant to enzymatic digestion. Therefore, a thermochemical pretreatment is usually necessary to disrupt the plant cell wall (lignin) in order to improve enzymatic digestibility (Fan et al., 2006). The thermal pretreatment of biomass results in two main streams; the solid fraction mainly consisting of cellulose (hexose: glucose) (Klinke et al., 2002) and liquid phase (hydrolysate) mainly consisting of hemicellulose (pentose: xylose and arabinose) (Bercier et al., 2007). Hexoses can effectively be converted to bioethanol and the process is carried out with high yield (around 0.4–0.51 g-ethanol/g-glucose) and productivity (up to 1.0 g L−1 h−1) by Saccharomyces cerevisiae or recombiant S. cerevisiae (Hjersted and Henson, 2006; Òghren et al., 2006). Wild S. cerevisiae strains are unable to utilize pentoses. Several recombinant candidates for pentose sugars fermentation have been described and presented as the future solution (Jin and Jeffries, 2004; Ruohonen et al., 2006; Chu and Lee, 2007). Additionally, several new bacterial ethanologenic strains have been reported (Ahiring et al., 1999; Nigam, 2001; Georgieva and Ahiring, 2007). Meanwhile, none of these organisms were as efficient as S. cerevisiae and improvements would certainly be desirable. Often these microorganisms are suffering from low productivities (e.g. Thermoanaerobacter mathranii with productivity of 0.10 g L−1 h−1), low ethanol tolerance and high sensitivity to inhibitors present in the hydrolysates from the pretreatment step (Torry-Smith et al., 2003). Therefore, utilization of hemicellulose remains a challenge to be resolved. One alternative prospect for utilization of hemicellulose is to produce biohydrogen. Biohydrogen production of sugars through anaerobic fermentation is recognized as a very promising, environmental friendly and feasible process (Hawkes et al., 2007). Several studies for utilization of C5 sugars to
bioglucoses and polymeric polysaccharides from the wheat straw have been reported (Lin et al., 2008; Lo et al., 2008; and Wu et al., 2008). The extreme thermophile Caldicellulosiruptor saccharolyticus was used successfully to convert C-5 sugar (xylose) to hydrogen with a relatively high yield of 334.7 ml-H2/g-sugar, accounting to 67% of theoretical yield of 497.6 ml-H2/g-sugar (Kádár et al., 2004).

Another important challenge for biorefineries, is to handle the large amounts of wastewater streams generated by the process. A sustainable solution for removal of the residual organic matter in the effluents from bioethanol and biogas production processes is to convert them to biogas and use the residual effluents as fertilizers in the effluents from bioethanol and biohydrogen processes is to explore the possibility of using wheat straw for the production of bio-ethanol (from cellulose), biogas (from hemicellulose) and biogas (from effluents from bioethanol and biohydrogen production) in an integrated biorefinery concept to improve the overall efficiency of biomass utilization. Wheat straw and its by-products generated during the integrated bioethanol, biogas and biohydrogen production in a biorefinery concept were characterized. The methane potential from wheat straw and its by-products/effluents in a biorefinery process was then determined. Finally, the output energy for six different wheat straw-to-biofuel scenarios was estimated quantitatively to evaluate the efficient utilization of the energy content of wheat straw.

2. Methods

2.1. Raw materials

Wheat straw (Triticum aestivum L.) grown and harvested during summer 2003 in Denmark was obtained from Risø National Laboratory for Renewable Energy, Technical University of Denmark (Denmark). The straw was cut into 1–5 cm pieces on the field by forage harvester and stored in containers at ambient temperature until further use. For chemical analysis and biogas potential assays, wheat straw was milled to <1 mm. The dry matter (DM) content was 90–91% (w/w). The chemical composition of the untreated wheat straw is given in Table 1.

2.2. Pretreatment of wheat straw

Hydrothermal pretreatment of wheat straw carried out in pilot plant (100 kg/h capacity) was described elsewhere (Thomsen et al., 2008). Briefly, wheat straw at the rate of 120–150 kg-DM/h was fed counter currently with water at a flow rate of 400–600 L/h to three serial reactors. The first step being a soaking step was operated at temperature of 80 °C and residence time around 6 min. The pre-soaking wheat straw was then heated up in stage two to approx. 180 °C for 15 min. followed by heating at 190 °C for 3 min. in a third stage. Hydrothermal pretreatment of wheat straw resulted in a liquid fraction called hydrolysate, containing mainly hemicelluloses and a solid fraction rich in cellulose. These fractions were kindly supplied by Dong Energy, Denmark. All materials were collected and stored at −20 °C until further use. Glucose and pentose recovery were calculated as follows:

Glucose recovery(%) = \frac{\text{Total glucose}_{\text{solid fraction}} + \text{Total glucose}_{\text{liquid fraction}}}{\text{Total glucose}_{\text{straw}}} \times 100 \quad (1)

Pentose recovery(%) = \frac{\text{Total pentose}_{\text{solid fraction}} + \text{Total pentose}_{\text{liquid fraction}}}{\text{Total pentose}_{\text{straw}}} \times 100 \quad (2)

2.3. Bioethanol production

Prehydrolysis (liquefaction) and fermentation of solid fiber fraction was treated by first saccharification and subsequently fermentation by Bakers yeast (S. cerevisiae) as described elsewhere (Thomsen et al., 2006). Briefly, the experiment was performed in 200 ml fermentation flasks. Eight grams of the solid fiber fraction from wheat straw was then heated up in stage two to approx. 180 °C for 15 min. followed by heating at 190 °C for 3 min. in a third stage. Hydrothermal pretreatment of wheat straw resulted in a liquid fraction called hydrolysate, containing mainly hemicelluloses and a solid fraction rich in cellulose. These fractions were kindly supplied by Dong Energy, Denmark. All materials were collected and stored at −20 °C until further use. Glucose and pentose recovery were calculated as follows:

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2.4. Biohydrogen production

Another important challenge for biorefineries, is to handle the large amounts of wastewater streams generated by the process. A sustainable solution for removal of the residual organic matter in the effluents from bioethanol and biogas production processes is to convert them to biogas and use the residual effluents as fertilizers in the effluents from bioethanol and biohydrogen processes is to explore the possibility of using wheat straw for the production of bio-ethanol (from cellulose), biogas (from hemicellulose) and biogas (from effluents from bioethanol and biohydrogen production) in an integrated biorefinery concept to improve the overall efficiency of biomass utilization. Wheat straw and its by-products generated during the integrated bioethanol, biogas and biohydrogen production in a biorefinery concept were characterized. The methane potential from wheat straw and its by-products/effluents in a biorefinery process was then determined. Finally, the output energy for six different wheat straw-to-biofuel scenarios was estimated quantitatively to evaluate the efficient utilization of the energy content of wheat straw.
were mixed with 60 ml of a 0.2 M acetate buffer (pH 4.8). Prehy- 
drolysis of the solid fraction was performed at 50 °C for 24 h at 
an enzyme loading of 15 FPU/g DM filter cake using Cellubrix L. 
After liquefaction, the flasks were supplemented with a second 
dose of Cellubrix L enzyme at a loading of 20 FPU/g DM and 
0.2 ml of urea (24%). The suspensions were then inoculated with 
0.2 g yeast after cooling down to room temperature. The flasks 
were sealed with a loop trap filled with glycerol and incubated at 
32 °C for 6–8 h. The CO₂-production was followed by measuring 
the weight loss indicating the ethanol yield (=0.51 g using stillages, the effluents from bioethanol fermentation, obtained 
when hydrogen content in the gas phase during the batch mode 
lysate diluted with BA medium. Continuous mode was started 
the fed-batch reactor was supplemented with a second 
an enzyme loading of 15 FPU/g DM filter cake using Cellubrix L. 

\[
\text{Glucose yield} = \left( \frac{\text{Free glucose after enzymatic hydrolysis}}{\text{Total glucose}} \right) \times 100 
\]

\[ \text{Glucose yield} = \left( \frac{\text{Free glucose after enzymatic hydrolysis}}{\text{Total glucose}} \right) \times 100 \quad (3) \]

2.4. Biohydrogen production

At the same time, hydrolysate was used for biohydrogen 
production through dark fermentation as described in detail by 
Kongjan et al. (2008). The experiment was carried out in a 1 L con- 
tinuously stirred tank reactor (CSTR) reactor with 700 ml working 
volume and hydraulic retention time (HRT) of 72 h. The feed was 
composed of BA medium and 40% hydrolysate solution (1:1 ratio 
V/V). The reactor was first started-up in a batch fed mode with 
140 ml of inoculum from batch cultures and 560 ml of 25% hydro- 
lysate diluted with BA medium. Continuous mode was started 
when hydrogen content in the gas phase during the batch mode 
reached maximum. The reactor temperature was controlled at 
70 °C by circulating hot water inside the reactor jacket.

2.5. Biological methane potential

Methane production was then investigated comparatively by 
using stillages, the effluents from bioethanol fermentation, obtained 
from the previous experiments in Section 2.3 and from a Swedish 
industrial plant producing ethanol from both wheat straw and from 
grain and the effluents of biohydrogen production. All substrates for 
methane production were also kept at -20 °C until digestion. Bio- 
logical methane potentials were evaluated in batch experiments. 
The experiments were performed in 118 ml serum glass bottles with 
working volume of 40 ml consisting of 30 ml of inoculum and 10 ml of 
substrate. Substrate was diluted with distilled water to attain a 
substrate concentration of 5, 50 or 100%. The headspace in the bottle 
was flushed with pure N₂ for 3–5 min. In addition, 2–3 drops of so- 
dium sulphide was added to ensure anaerobic conditions. The bot- 
tles were sealed with rubber stoppers and aluminium crimps. The 
experiment was conducted in triplicates. The prepared bottles were 
incubated statically at 55 °C for 24 h at an enzyme loading of 15 FPU/g DM filter cake using Cellubrix L. 

\[
B_{\text{vib.}} = \frac{0.415 \text{Carbohydrates} + 0.496 \text{Proteins} + 1.014 \text{Lipids} + 0.373 \text{Acetate} + 0.530 \text{Propionate}}{\text{Carbohydrates} + \text{Proteins} + \text{Lipids} + \text{Acetate} + \text{Propionate}} 
\]

2.6. Analytical methods

Total and soluble Chemical oxygen demand (TCOD and SCOD), 
Total solids (TS), volatile solids (VS), ash content, suspended solids 
(SS), ammonia and total Kjeldahl nitrogen(TKN) were determined 
according to the Standard Methods (APHA, 1998). Standards SCOD 
samples were filtered through glass fiber filter paper (490 mm, 
GF50, Schleicher & Schuell). Lipid extraction was carried out 
through Soxhlet Method. pH was measured using pH meter 
(PHMK92 LAB).

Methane production was measured by gas chromatography 
(GC) using flame ionization detection (FID) fitted with a Porapak 
60/80 mole sievet column. Nitrogen is used as carrier gas with a 
pressure of 2.0 kg/cm². The injection temperature is set to 110 °C. 
The detector and oven temperature is 160 °C.

For VFA and alcohol determination, 1 ml of sample was acidified 
with 50 μL of 34% of phosphoric acid and then centrifuged at 
12,000 rpm for 10 min and measured on GC (Hewlett Packard, HP 
5890 series II) equipped with a flame ionization detector (FID) 
and HP FFAP column (dimensions 30 m × 0.53 mm × 1.0 mm). 
The temperature program for the column was increased from 
50 °C to 190 °C with a rate of 15 °C/min. The temperatures of injec- 
tion port and detector were 200 and 150 °C, respectively. Nitrogen 
was used as the carrier gas at a flow rate of 10 ml/min.

To determine the sugars (glucose, xylose and arabinose) content 
in raw and pretreated straw and liquid fractions Strong acid (72% 
W/W H₂SO₄) hydrolysis of solid fraction and weak acid (4% W/W 
H₂SO₄) of liquid fraction was applied (Thomsen et al., 2006). Sugars 
were quantified on high performance liquid chromatography HPLC 
System HP 1100 (Agilent 1100) equipped with a BioRad Aminex 
HPX-87 H at 63 °C and a refractive index (RI) detector (RID 1362A) using 0.6 ml/min of 4 mM H₂SO₄ as eluent. Manose and gal- 
actose could not be separated clearly from glucose and xylose 
because the manose retention time (10.02 min) was very close to 
the glucose retention time (10.16 min) while, the galactose retention 
time (10.49 min) was also very close to the xylose retention time 
(10.39 min). That is, although manose and galactose could not 
be separately measured, possible presence of these two sugars 
would have been included in the HPLC peaks together with glucose 
and xylose. Additionally, glucose, xylose and arabinose are the main 
monomers in wheat straw, monomers detected were therefore rep- 
resent in total glucose, xylose and arabinose (Klinke et al., 2002; 
Bercier et al., 2007). The analysis detection limits for glucose, xylose 
and arabinose were 0.011, 0.002 and 0.014 g/l, respectively.

Klason lignin in solid fraction was determined as the weight of the 
filter cake (generated during the strong acid hydrolysis of solid 
fraction) from subtracted the ash content. Furfural and 5-hydroxy- 
methyl-2-furaldehyde (HMF) in liquid fraction were measured on 
HPLC used for sugars analysis fitted with ultraviolet (UV) detector 
(G1314A).

Phenolic compounds derived from pretreatment of wheat straw 
were quantified by GC equipped by FID. Compounds were firstly 
isolated from the liquid fraction at pH 2 by solid-phase extraction 
on polystyrene divinylbenzene polymer columns (Klinke et al., 
2002).

2.7. Energy output of a biorefinery concept

Six different wheat straw to biofuel energy production scenarios 
were considered to evaluate the most energetically efficient pro-
straw → Bioethanol → Biohydrogen → Biogas. Data on the chemical composition and bioethanol, biohydrogen, methane yields of substrate and/or effluent from each process obtained in the present study were used to estimate quantitatively the energy output for each scenario. Energy was expressed in MJ.

Mass flow in the studied biorefinery concept was calculated based on the amount of sugars and their conversion to different biofuels/products (Fig. 1). Pretreatment often results in loss of dry matter. This can be due to formation of gasses like CO₂ and acetic acid at high temperature and/or loss of dry matter along with water as biomass is washed with water in order to remove the inhibitory materials and/or other water-soluble hemicellulose produced during thermal hydrolysis. Losses of pretreated wheat straw through steam explosion was reported to be up to 20% (Bjerre and Schmidt, 1997). In the present study, a dry matter loss of 5% was assumed, as the system was well closed. From the remaining dry matter, around 75% of the dry matter was transferred to the solid fraction containing mainly cellulose and lignin and the remaining 25% of the dry matter was retained in the liquid fraction.

Finally, the energy output for each studied scenario was estimated by multiplying the amount of individual biofuel produced with its lower calorific value (LCV). The LCV for ethanol, methane, hydrogen, dry lignin and wheat straw were 26.72, 50.1, 122, 20.95 and 19.1 MJ/Kg, respectively (ORNL, 2006).

3. Results and discussion

3.1. Characteristics of wheat straw and its by-products/effluents in a biorefinery

3.1.1. Wheat straw

The chemical composition of wheat straw and effluents formed during bioethanol and biohydrogen fermentation of the pretreated straw streams; i.e. solids fraction or liquid fraction (hydrolysate), respectively, are shown in Table 2. Untreated wheat straw had dry matter content of 91.6%. Cellulose, hemicellulose and lignin contents were 35.9, 23.9, and 19.3 g/100 g-DM, respectively. These results are in accordance with the typical composition of wheat straw reported Thomsen et al. (2008).

3.1.2. Solid and hydrolysate fraction

Composition of fiber fraction (Table 1) showed that hydrothermal pretreatment affected the degradation and solubilization of individual sugars. Glucan and xylose were noticed while arabinan was found in trace amount. The solid fraction had rather high DM of 32.5%-TS. It has been stated that a high DM concentration of 32.5%-TS in the solid fraction could be possible to perform ethanol fermentation (Jorgensen et al., 2007). The high DM concentration (>20%-TS) in the solids fraction would give sugar and ethanol concentration above 8% and 4% w/w, respectively during the enzymatic hydrolysis and fermentation (Larsen et al., 2008). Significant reduction of the distillation costs could be thereby achieved when the ethanol from fermentation broth is above 4% (w/w) (Zacchi and Axelson, 1989).

Analysis of hydrolysate (Table 1) revealed that xylose was the main sugar accounting for 72.9% of sugars which could be converted to hydrogen in the subsequent dark fermentation. Glucose and arabinose were also found in the hydrolysate but at a very low concentration. The low glucose concentration in hydrolysate was probably due to its crystalline and thermally stable structure. The relatively low concentration of degradation products such as acetic acid, hydroxymethylfurfural (HMF) furfural and phenolic compounds, which were only found in the hydrolysate (Table 1) could be an advantage for the subsequent fermentation. These compounds generated during the hydrothermal pretreatment of straw are considered to be inhibitory to most microorganisms (Thomsen et al., 2006). The rather low pH of hydrolysate (pH = 4.9) was properly due to high amount acetic acid content in hydrolysate, accounting for 80% of total VFAs. Acetic acid was released from the hydrolysis of acetyl groups contained in the hemicellulose.
celluloses. HMF and furfural are derived from the degradation of glucose and xylose derived from hemicellulose, respectively, whereas, phenolic compounds result from partial lignin degradation (Klinke et al., 2002). Vanillin, 2-furoic acid, coumaric acid and ferulic acid were identified as the main phenolics and occurred in a concentration of 0.101 g/l in this study.

Glucose and pentose recovery were rather high around 95% and 83% respectively (Thomsen et al., 2008), indicating that pretreatment conditions were effective in releasing these sugars from cellulose and hemicelluloses consisted in the straw.

3.1.3. Stillage
Stillage is the residue obtained after distillation of the ethanol fermentation broth. The carbohydrate and COD content in Swedish stillage was slightly higher than that of Risø stillage (Table 1). The difference in COD and carbohydrate content between the studied stillages could be due to differences in the feedstock used, pretreatment condition and ethanol extraction/distillation process. Risø stillage was obtained from lab-scale fermentation of solid fraction (32.5% DM) originated from hydrothermally pretreated wheat straw. Ethanol was extracted through vacuum distillation to 10% concentration. On the other hand, Swedish stillage was obtained from an industrial plant producing ethanol from wheat straw and grains as sugar feedstock. The high COD values in the present study were within the range reported for similar stillages (Wilkie et al., 2000). Ethanol concentration in both stillages ranged between 0.8 and 2.34 g/l indicating that the distillation was more efficient in the full-scale process. The high concentration of sugars in Swedish stillage could be due to incomplete fermentation of the sugars. Low amounts of 5-HMF in Swedish stillage while the concentration of furfural and 5-HMF in Risø stillage were below the detection limit (Table 1). Nevertheless, the concentration of 5-HMF in Swedish stillage was below the threshold level that could cause inhibition during methanogenesis (Torry-Smith et al., 2003). The high nitrogen content of Swedish stillage (6 g/l) stands out and inhibition during methanogenesis (Torry-Smith et al., 2003). The methane yield obtained from solids fraction, hydrolysedate and H₂ effluent were more or less the same and were 29% higher than that of wheat straw yield, while the hydrothermally pretreated straw had a specific methane yield of 297 ml/gVS added. This yield was on the higher side of 146 and 267 ml/gVS added achieved from mesophilic digestion of wheat straw (Demirbas, 2006). The higher yield achieved in the present study could be due to the maceration of the straw before incubation and also due to the incubation at thermophilic temperatures (55 °C). The thermophilic anaerobic digestion could improve the hydrolysis of the organic matter (Kuo and Cheng, 2007). The methane yields obtained from solids fraction, hydrolysedate and H₂ effluent were more or less the same and were 29% higher than that of wheat straw yield, while the hydrothermally pretreated straw produced 396 ml/gVS added.

3.1.4. Hydrogen effluent
Effluent from biohydrogen production contained low VS (2.8%) and lignin-related compounds (0.015 g/l) while furfurals and 5-HMF levels were below the detection limit. These results suggest that inhibitory products such as furans and phenolics present in the lignocellulosic hydrolysate might have been degraded to other intermediates during anaerobic treatment (Torry-Smith et al., 2003). Additionally, it has been previously proved that the residual organic matter contained in the hydrogen effluent could be served as an ideal substrate for methane production by anaerobic digestion (Liu et al., 2006).

3.2. Bioethanol, biohydrogen and biogas production in a biorefinery concept
3.2.1. Bioethanol production
SSF of the pretreated solid fraction was performed with Cellubrix L. Enzyme and baker’s yeast respectively after adjusting the pH to 4.8. Glucose yield_solid fraction was around 90%. Ethanol production started immediately without any lag phase. Results showed that 60% of ethanol theoretical yield (0.51 g-ethanol/g-glucose) was achieved within the first 50 h and further increased to 0.41 g-ethanol/g-glucose after 6 days, accounting for 80% of ethanol theoretical yield. No inhibition was found during the fermentation.

3.2.2. Biohydrogen production
Results from the lab-scale semi-continuous experiments affirmed that wheat straw hydrolysate (xylose) can be used as substrate for biohydrogen production through dark fermentation in CSTR. No methane was detected indicating the absence of methanogens. Hydrogen yield from wheat straw hydrolysate was around 178.0 ml-H₂/g-sugars with a SCOD removal around 72% (Kongjan et al., 2008). These results are in accordance to other reports demonstrating hydrogen production from paper sludge hydrolysate. This yield was lower than the yield of 334.7 ml-H₂/g-sugar which was converted from xylose by using the extreme thermophile of C. saccharolyticus (Kádár et al. (2004). The hydrogen yield in our investigation was low, as the main part of sugars contained in the straw (i.e. glucose) was used for ethanol production, and only the non-utilized residual sugars were reserved for hydrogen production. Combination of hydrogen and methane production from the residual organic matter not utilized for ethanol would increase the overall efficiency of ethanol production facilities. However, with sufficient acclimatization, the process could be optimized for hydrogen production in continuous mode.

3.2.3. Biological methane potential
Results from the biological methane potential assays are summarized in Table 2. The experiment was carried for 60 d. Methane production started immediately in all assays and reached maximum after 15–30 d (data not shown). Mean methane yields (Bₘ) for the studied substrates ranged from 297 to 485 ml/gVS added. These yields were 68–92% of the theoretical yields (Bₘ). Untreated straw had a specific methane yield of 297 ml/gVS added. This yield was on the higher side of 146 and 267 ml/gVS added achieved from mesophilic digestion of wheat straw (Demirbas, 2006). The higher yield achieved in the present study could be due to the maceration of the straw before incubation and also due to the incubation at thermophilic temperatures (55 °C). The thermophilic anaerobic digestion could improve the hydrolysis of the organic matter (Kuo and Cheng, 2007). The methane yields obtained from solids fraction, hydrolysedate and H₂ effluent were more or less the same and were 29% higher than that of wheat straw yield, while the hydrothermally pretreated straw produced 396 ml/gVS added. This was probably due to the improvement of COD solubilization and anaerobic biodegradability from the pretreatment of wheat straw.

Between the two studied stillages, Swedish stillage had higher methane yield (485 ml/gVS added) than Risø stillage (324 ml/gVS added). However, methane yields for wheat stillage reported in the literature ranged between 380 ml/gVS added and 529 ml/gVS added (Torry-Smith et al., 2003). The low methane yields obtained for Risø stillage in the present study was probably due to inhibition e.g. compounds such as acetovanillone and syringic acid that were noticed at high concentration in this substrate. Previous studies have suggested that high levels of potassium, metals and sulfate in addition to phenolic compounds that are produced during whole ethanol production process can inhibit methanogenesis (Wilkie et al., 2000). The high biodegradability noticed for Swedish stillage could be due to some statistical deviations in the analytical procedures resulting in underestimating the theoretical yield. It was however clear that high methane conversion efficiency could be achieved.

The high concentration of substrate could inhibit to the methane yields of the studied substrates (Data not shown). No process inhibition was noticed for Swedish stillage when incubated with two times dilution of substrate while inhibition was noticed with no dilution of substrate. This inhibition was properly caused over-
load of organic matter and nitrogen of which were contained in high concentration in the Swedish stillage (Table 1).

3.3. Mass balance and energy output

The mass flow within the two main studied biorefinery alternatives is shown in Fig. 1. Mass balance is expressed in terms of DM. During hydrothermal pretreatment of one ton of wheat straw, 75% of the DM was transferred to solid fraction and the remaining 25% of input was extracted as hydrolysate. Fig. 2 presents six scenario proposed to optimize wheat straw use and different contribution of individual biofuel in each scenario. It should be noted that the present study is a very simplified analysis where the energy requirements for biofuels production such as farming, harvesting, transporting, feedstock processing, fermentation, alcohol recovery, alcohol purification process, biogas process, etc. were not considered but it could be used as an important input for an in depth life cycle assessment.

The Combustion of wheat straw produced 16503 MJ of useful energy and was considered as the reference scenario (Scenario 1). Among the studied scenarios, highest energy output (10452 MJ) was obtained when hydrothermally pretreated wheat straw was used for biogas production (Scenario 3). Untreated wheat straw gave an energy output when converted to biogas (Scenario 2) which was approx. 10% lower than that of Scenario 3. Production of bioethanol alone (Scenario 4) was the least energy efficient process (3572 MJ). Finally, production of multiple biofuels in Scenario 5 or 6 yielded resulted in higher amount of energy (9100 and 9364, respectively), compared to ethanol production alone. Despite a low energy output from using of hydrolysate (C-5 sugars) for biogas or hydrogen in scenario 6, subsequent biogas production from its effluent could improve the overall energy yield of this system, enabling the multiple biofuels. Although relatively small amount of hydrogen was produced, it would be advantageous to use a mixture of methane hydrogen than methane alone in Internal Combustion Engines. Even the addition of a small amount of hydrogen to methane (5–30% by volume that means ~1.5–10% by energy) would lead to many advantages, due to the specific physical and chemical properties of the two fuels. Methane has a slow flame speed, while hydrogen has eight times higher flame speed which significantly improves the efficiency of the gas combustion in motors. Furthermore, addition of even small amounts of hydrogen decreases the air/fuel ratio (lambda) resulting in a much more stable combustion of methane.

The stillage contained with lignin content of 24.6 g/100 g-DM could partially be re-circulated as it still contained active enzymes. Lignin, which is not affected the distillation process, could possibly be separated by a decanter centrifuge or a filter press from stillage and used as a solid biofuel through combustion in a CHP plant where wet lignin can be fed directly to highly efficient fired-power plant (Larsen et al., 2008). The produced energy of 3833 MJ/ton-wheat straw could be used either in the pretreatment of wheat straw or internally for energy requirements of the biorefinery. This choice would only be viable when cheap excess energy is available, such as in connection to power plants, as the requirements for separating lignin and evaporating moisture content would make decrease the final energy output. However, if cheap excess energy is not available burning lignin would have a negative energy balance of −1490 MJ/ton-wheat straw.

Despite low energy output from production of biofuels such as ethanol, and mixture of hydrogen and methane compared to incineration of biomass, there are several advantages for conversion of biomass to biofuels, compared to converting biomass to heat and electricity by incineration. There is a large demand for liquid fuels in the transportation sector in which bioethanol can be directly used. CO2 emissions from transportation are accounting for a significant part of the total GHG emissions with upto 30% in industrialized countries (Alavandi and Agrawal, 2008; Sheehan et al., 2003). Additionally, the digested material from biorefinery process could be used as an excellent and sustainable fertilizer for cultivation of crops. While, incineration of straw is not a recycling process, since nutrients are not retained and recycled back to soils and soil is deprived by structural material. Moreover, combustion of raw straw is connected with serious problems such as fly ash disposal and super heater corrosion due to the high content of potassium chloride in straw. Although biofuels production from lignocellulosic feedstock is not yet practiced in advanced concepts like biorefinery could emerge in the future for both, biofuel production and environmental protection.

4. Conclusions

Sugars released from wheat straw by hydrothermal pretreatment were used for biofuels (bioethanol, biohydrogen and biogas) production based biorefinery successfully. Energy output from the biogas production of pretreated wheat straw was the most energetically efficient and approximately 10% higher than that of untreated wheat straw. It is questionable whether the energy used during pretreatment of straw would be converted by the extra methane produced. Additionally, it was not taken into account that possible mass-losses might occur during the pretreatment process, such as evaporation of volatile compounds, which of course would decrease extra methane potential that would be achieved in the pretreated straw and thereby minimise the advantage of pretreatment.

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