Scientific African 3 (2019) e00077

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Hydrolysis of plant biomass at different growth stages using enzyme cocktails for increased fermentable hydrolysates

Henry P. Oamen[a,*], Ebenezer O. Ojo[b,**], Phil J. Hobbs[c,d], Andy Retter[c]

[a] College of Life and Environmental Sciences, School of Biosciences, University of Exeter, Exeter Ex4 4QD, UK
[b] Faculty of Science and Engineering, Osun State University, PMB 4494, Osogbo, Nigeria
[c] Rothamsted Research, North Wyke Research, Okehampton, Devon EX20 2SB, UK
[d] Anaerobic Analytics Ltd., South Zeal, Okehampton, Devon EX20 2JR, UK

ARTICLE INFO

Article history:
Received 23 April 2018
Revised 17 February 2019
Accepted 29 April 2019

Keywords:
Enzymatic hydrolysis
Lignocellulose
Pretreatment
Grass
Taguchi methodology

ABSTRACT

In this experiment, optimum experimental conditions for the enzymatic hydrolysis of grass at different stages of growth were obtained by using the Taguchi methodology. Rye grass silage and three growth stages of Italian rye grass samples were used to determine optimum hydrolysis conditions. Five factors (pretreatment, enzyme composition, incubation temperature, pretreatment time, pH) influencing the hydrolysis process were studied at the individual and interactive levels. All selected experimental factors influenced the hydrolysis of grass. At the individual level, pretreatment of grass with NaOH and enzyme composition had the greatest influence (75% and 14.7% of the variance respectively) on enzymatic hydrolysis. Incubation temperature, pretreatment time and pH had influences of 8.1%, 2.2% and 0.055%, respectively. pH and incubation temperature had the most significant interaction effect (65.6%) on enzymatic hydrolysis. The factors with the least individual influence had the most significant interaction effect on enzymatic hydrolysis. Hydrolysis was improved when optimised conditions were applied to different growth stages of Italian rye grass.

Introduction

The negative impact of fossil fuels combustion has led to an intensive search for plant-based biofuel. Energy stored in the form of polymers as a result of photosynthetic activities in plants, can be converted to soluble fuels. The use of arable crops however adversely affects the food supply chain: hence the need for non-arable crops. Non-food plants such as short rotation coppice (SRC), rye grass, *Miscanthus* and reed canary grass have been used directly as fuels or processed into biofuels [1,2]. Such plants can be grown on relatively low nutrient lands, which require less conditioning as would normally be required for food crops. The chemical and structural nature of the different grasses determines how effectively it can be converted or processed into biofuels through various hydrolytic techniques [3].

* Correspondence to: Department of Plant Biology and Biotechnology, University of Benin, PMB 1154, Benin City, Nigeria
** Corrrespondence to: Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow G1 1RD, UK.
E-mail address: h.oamen@qmul.ac.uk (H.P. Oamen).

https://doi.org/10.1016/j.sciaf.2019.e00077

Table 1
Physical characteristics of substrates used during the experiment.

Substrates	Plot no.	Mean height (mm)	Harvest yield (kg/m^2)	MC (% w/w)	TS (% w/w)	VS (% w/w)
Early cut	1	39.46 ± 0.85	0.16	66.72	33.28	93.50
Regrowth	1	17.33 ± 2.02	0.02	66.17	33.84	91.00
Late cut	2	82.00 ± 1.68	1.69	64.34	35.66	95.50

Abbreviations: T_S, total solids; M_C, moisture content; V_S, volatile solids.

The structural integrity of most plant biomass is maintained by polymers such as cellulose (25–40%), hemicellulose (25–50%) and lignin (10–30%) [4]. The chemical composition of plants changes as the plant grows. Sugar content is higher at the onset of growth with less fibre [5], however, fibre and lignin content increases as the grass matures [6,7]. The complex nature of lignin makes the enzymatic decomposition of grass difficult. Harvest period and frequency of cutting are therefore important considerations for optimised biofuel production to be obtained from the plant biomass [8]. Different methods such as mechanical, physical and chemical pretreatments have been reported to increase the plant biomass surface area and susceptibility to hydrolytic processes [2,9,10].

Reports have shown that the combination of pretreatment methods makes lignocellulose more susceptible to enzyme hydrolysis [11,12]. Enzyme derived from *Trichoderma reesei* have been utilised for lignocellulosic biomass hydrolysis. However, such enzymes are unable to efficiently hydrolyse hemicelluloses and lignin components in lignocelluloses [13]. The optimum pH range for hydrolysis is considered to be within the range of 5.0–6.5 [10]. Hydrolysis of the pretreated lignocellulose to simple sugars typically can use a complex of secreted enzymes derived from filamentous fungi, particularly *Trichoderma sp*. Such enzyme complexes contain high levels of cellulases (endoglucanases and cellobiohydrolases), together with lower amounts of enzymes that attack non-cellulosic polysaccharides such as hemicellulose and pectin.

Efficient enzymatic hydrolysis of pretreated lignocellulosic substrates is often limited by the different process conditions such as substrate loads, pH, temperature, high amount of lignin and product recovery strategy [14]. The hydrolysis may also depend on the enzymes physiochemical properties, thermal stability and specific activities [15]. For enhanced bioconversion of plant biomass, the use of enzyme cocktails have been reported [16]. However, process optimisation, using the statistical analysis of the data that can be gathered from the design of the experiment, will further enhance the bioconversion yield [15] and understanding of process requirements.

In this work, the design of experiment (DoE) approach adopted was based on the Taguchi methodology [17]. The method determines the optimum experimental conditions for the hydrolytic reaction steps while determining maximum estimate of the significant factors. The experimental effort for a full factorial design is minimised and information about the interactions between defined experimental factors can be obtained [17]. This approach has been applied in a number of hydrolysis experiments [18,19]. The current study is aimed at optimising the hydrolysis of rye grass silage and Italian rye grass samples harvested at different growth stages.

Materials and methods

Substrate preparation

The substrates used during the experiment included: rye grass silage and different growth stages of Italian rye grass (see Table 1). All harvested grass samples were planted at the same time on the North Wyke farms with ammonium nitrate fertilizer. Italian rye grass samples were obtained from two plots as follows. The first plot was harvested in May (early harvest) and again in June (regrowth) while the second plot was harvested only once in June (late harvest). These three different samples of Italian rye grass were dried and kept under cold storage at −19 °C. Rye grass silage (92.8% V_s%) was also obtained from North Wyke clamps. All enzymes and reagents used in the experiments were obtained from Novozymes (Bagsvaerd, Denmark) and Sigma respectively.

Determination of yield, moisture content (M_C) total solid (T_S) and volatile solids (V_S) of substrates

Biomass yield was determined by dividing the weight of each sample obtained from a field by the area of harvest. Moisture content (M_C) and total solids (T_S) determination was performed as described by [20]. A known weight (W_k) of each sample was oven dried at 85 °C for 16 h, after which the weight of the dried sample (W_D) was determined. M_C (%) was calculated using the Eq. (1) below:

$$M_C(\%) = \frac{W_k - W_a}{W_k - 100} \tag{1}$$

T_S (%) values were obtained by subtracting the value of M_C from 100% (see Table 1). The volatile solids (V_S) (%) in each substrate was determined by weighing a portion of the dry sample (W_c) into a beaker and heated at a temperature of 550 °C for 16 h in a Gallenkamp series 2 muffle furnace (Fistreem, UK). The weight difference between the remaining ash sample

Table 2
Selected experimental factors and assigned levels for enzymatic hydrolysis of grass.

Serial no.	Factors	Level 1	Level 2	Level 3	Level 4
1	Enzyme	A1	A2	B1	B2
2	Pretreatment (% NaOH)	Steam	0.5	1.0	–
3	Prettime* (min)	15	30	–	–
4	pH	Low (5.0)	High (6.5)	–	–
5	Incubation (min)	40	50	60	–

* Defines the shortened pretreatment time.

Table 3
Enzyme compositions and combinations for hydrolysis of grass.

Enzymes	Set A		Set B		Enzymes compositions
	A1	A2	B1	B2	
NS50013 (mL)	2.4	2.4	3.6	3.6	Cellulase (major), small amounts of beta-glucosidase, xylanase
NS50010 (μL)	480	480	720	720	Beta-glucosidase (major), small amounts of xylanase
NS2202 (μL)	–	225	–	375	Beta-glucanase, xylanase, cellulase, pentosanase, hemicellulase
NS50012 (μL)	–	225	–	375	Multienzyme complex of hemicellulase and pectinase
NS50030 (μL)	–	225	–	375	Endoxylanase

(W_a) and oven-dried sample was determined, and the V_s were calculated using the equation:

$$Vs(\%) = \frac{W_c - W_a}{W_c - 100_{OBJ}} \quad (2)$$

Preparation of citrate buffer and antibiotics

0.1 M sodium citrate buffer was prepared by dissolving each of 14.7 g of sodium citrate and 9.6 g of citric acid in 500 mL of water separately. 400 mL of the sodium citrate solution was added to a beaker and the pH gradually adjusted to 5.0 and then 6.5 respectively with the addition of the citric acid solution. An antibiotic was also prepared by dissolving 0.1 g of tetracycline in 10 mL of 70% ethanol and stored at 4 °C. The antibiotic was necessary to prevent contaminations during the enzymatic hydrolytic step.

Design of experiments (DoE)

DoE approach (L_{16} – orthogonal array) was adopted in this work and these five steps were followed—experimental planning, laboratory investigations and data acquisition, data analysis using modelling tools, and validation based on experimental and modelled results. The overall DoE experiments were performed in two stages—the first was on the rye grass silage and the second was on the cut grass samples.

Experimental planning

For the hydrolytic step, five process parameters (factors) were chosen and ascribed to different levels ranging from level 1–4 as shown in Table 2. Enzyme cocktail with the highest number of level was assigned four, and the cocktail comprised of different combinations of hydrolytic enzymes (see Table 3). Other factors with their corresponding levels are also shown in Table 2. Using the Taguchi approach, experimental design matrix was developed using the L_{16} orthogonal array. The L_{16} (Table 4) shows a total of sixteen experimental trials investigating the degree of interactions among the selected parameters and the overall effects on the outputs. In the design the column represents the factors and the rows the levels attributed to the factors. The degree of freedom was estimated as fifteen (number of experiment $(n) - 1$).

Data analytics and model development

The experimental design and experimental data shown in Table 4 were transferred to Qualitek-4 software (Nutek Inc., MI) for further processing. The software is a predictive tool for efficient evaluation of the different interactions between the input factors and their overall effects on the process outputs. The software further helps to identify the optimum conditions and equally predicts process outcomes at other chosen conditions. The statistical capabilities also help establish ANOVA correlation indicating significant factors and process robustness.

Steam and alkali pretreatment of samples

5% (w/v) feedstock samples in water were prepared to a working volume of 800 mL in two flasks. The first flask was autoclaved at 121 °C for 15 min and the second for 30 min. The autoclaved suspensions were filtered, and the grass residues were oven-dried at 60 °C for 16 h. For the pretreatment step with NaOH, two flasks each of 5% (w/v) feedstock samples in 0.5% (w/v) and 1% (w/v) NaOH solution were prepared to a working volume of 600 mL. Two of the flasks each containing

Table 4
Experimental layout (L-16) for optimising hydrolysis conditions of grass.

Sample no.	Factor levels					Sugar concentration (g/5 g)	
	1	2	3	4	5	Glucose	Xylose
1	1	1	1	1	1	1.14	0.54
2	1	2	1	2	2	0.98	0.4
3	1	3	2	1	3	1.47	1.13
4	1	1	2	2	1	0.71	0.42
5	3	1	1	2	3	0.66	0.26
6	3	2	1	1	1	1.13	1.16
7	3	3	2	2	1	1.75	0.94
8	3	1	2	1	2	1.03	0.6
9	2	1	2	1	1	0.69	0.38
10	2	2	2	2	3	0.42	0.21
11	2	3	1	1	2	2.26	1.56
12	2	1	1	2	1	0.45	0.15
13	4	1	2	2	2	1.04	0.78
14	4	2	2	1	1	1.56	1.56
15	4	3	1	2	1	1.79	1.18
16	4	1	1	1	3	1.32	0.89

Table 5
Enzymatic hydrolysis optimum conditions and performance.

Serial no.	Factor	Level description	Level	Contribution
1	Enzyme	B2	4	0.275
2	Pretreatment	1.0% NaOH	3	0.668
3	Prettime (min)	15	1	0.066
4	pH	6.5	2	0.010
5	Incubation	50	2	0.178
Total contribution from all factors				1.197
Current grand average of performance				1.146
Expected result at optimum condition				2.343

0.5% (w/v) and 1% (w/v) NaOH were autoclaved at 121 °C for 15 min while the remaining two flasks also containing 0.5% (w/v) and 1% (w/v) NaOH were autoclaved at 121 °C for 30 min. The autoclaved suspensions were filtered, and the grass residues were oven-dried at 60 °C for 16 h. The pretreatment procedures above were also conducted for each of the Italian rye grass samples. The weights of all the pretreated substrates obtained after drying is presented in Fig. 1.

Enzymatic hydrolysis step

The enzyme cocktails shown in Table 3 (A1, A2, B1 and B2) were prepared by adding different volumes of enzymes mixes (NS50013, NS50010, NS2202, NS50012 and NS50030). To make A1 and B1, certain mixes (NS2202, NS50012 and NS50030) were not included whereas these mixes were added in different volumes in preparing A2 and B2. Rye grass silage was used to configure the experimental conditions, which was later applied to the cut grass samples. Enzymatic hydrolysis was carried out in a 60 mL labelled syringe. Each setup contained 5% (w/v) pretreated rye grass silage in 0.1 M sodium citrate buffer and 180 µL of tetracycline solution inoculated with the various enzyme mixtures. Each syringe was fitted with rubber tubing and clip for ease of collection of hydrolysates. The syringes were placed in incubators operated at various temperatures as shown in Table 1. The syringes were placed in an inverted position so as to avoid leakage of the contents. 2 mL of hydrolysate was collected after 16, 24, 40, 48 and 72 h of experiment start time and analysed. The data obtained was utilised to determine the optimum experimental conditions for hydrolysis (Table 5).

Validation of results

To validate the result obtained in the optimisation step, the procedure above was performed for the cut grass samples using the optimum condition determined from using rye grass silage. Only the pretreatment (steam, 0.5% or 1% NaOH) was altered in the experiment with the growth stages (see Table 6). The results were compared for process robustness.

Analytical procedure

The Thermo Electron High Performance Liquid Chromatography (HPLC) was used for sugar analysis using a Bio Rad Aminex Fermentation Monitor column (150 mm × 7.8 mm Cat. #125–0115). The mobile phase used consisted of 70 µL of H_2SO_4 in 1 L of water, with a flow rate of 0.5 mL/min. Data analysis and the optimum conditions for hydrolysis were determined using the Qualitek-4 software (Nutek Inc., MI) with 'bigger is better' quality characteristic. The Qualitek-4 software analysis also revealed individual factor influences and factor interactions on hydrolysis.

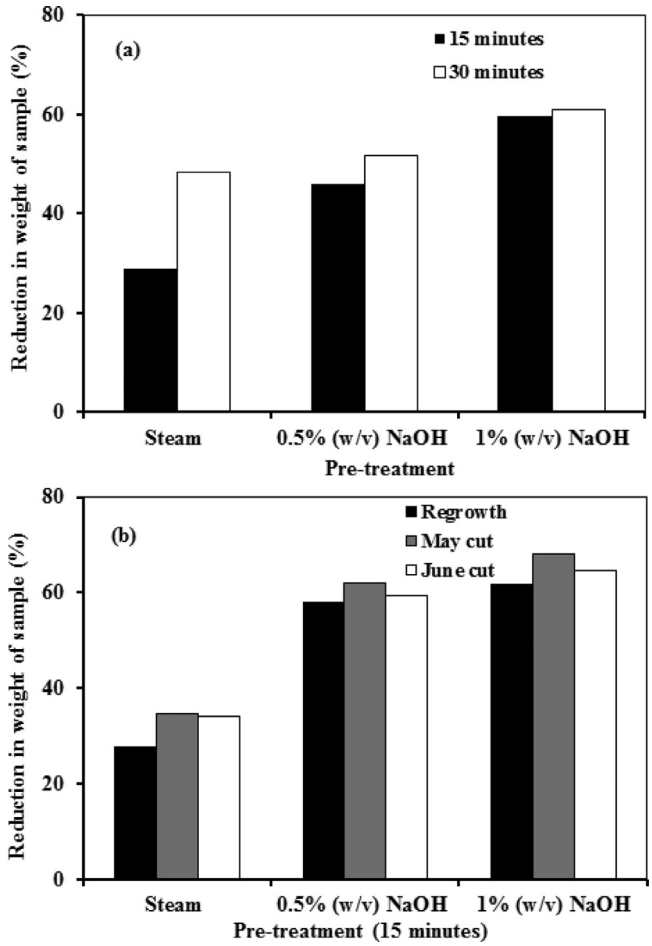

Fig. 1. Percentage weight reduction of samples after pretreatment (a) rye grass silage (b) growth stages. (Conditions of experiment—samples were pre-treated with steam and NaOH as depicted in (a) after which they were dried and weighed. The pretreatment for the growth stages (b) was done for 15 min as this was confirmed as the optimum pretreatment time using rye grass silage).

Table 6
Experimental layout for Italian rye grass samples.

Sample no.	Substrate	Factors					Sugar concentration (g/5 g)	
		1	2	3	4	5	Glucose	Xylose
1	Regrowth	3	2	1	2	2	2.3	1.17
2	Early cut	3	2	1	2	2	1.27	0.63
3	Late cut	3	2	1	2	2	1.29	0.71
4	Regrowth	3	3	1	2	2	2.3	1.08
5	Early cut	3	3	1	2	2	1.11	0.56
6	Late cut	3	3	1	2	2	1.19	0.62
7	Regrowth	3	1	1	2	2	1.37	1.06
8	Early cut	3	1	1	2	2	0.79	0.67
9	Late cut	3	1	1	2	2	0.57	0.59

Results and discussion

Effect of pretreatment methods on substrates physical properties

The physical properties of the harvested biomass of Italian rye grass were quantified as shown in Table 1, which also includes the measured mean heights for the early and late harvest doubled over a period of eighteen days between harvests respectively. The total height of the regrowth from the stumps of early cut was 60% less than the mean height between the early and late cut. However, comparison of the harvest yield for the early and late cut resulted in ten-fold increase respectively. The regrowth had significantly low hydrolysate yield of approximately hundred fold lower compared to the

Table 7
ANOVA table.

Factor	DOF (f)	Sum of squares (S)	Variance (V)	F-ratio	Pure sum (S')	Percentage P (%)
Enzyme	3	0.476	0.158	9520.00	0.475	14.665
Pretreatment (min)	2	2.433	1.216	72,990.61	2.432	74.965
Prettime (min)	1	0.071	0.071	4293.18	0.071	2.204
pH	1	0.001	0.001	108.31	0.001	0.055
Incubation	2	0.262	0.131	7889.07	262.000	8.101
Other/error	6	0.001	–	–	–	0.010
Total	15	3.245	–	–	–	100.000

late cut. Based on the time of cut, total solids, moisture content and the volatile solids contents of the biomass can be estimated. An overall trend shows similar percentage w/w of M_c, T_s and V_s for the early cut and regrowth. However, late cut was slightly higher for the three properties investigated (see Table 1). For the pretreatment of biomass prior to hydrolytic steps, the percentage loss in weight of samples were estimated as shown in Fig. 1 (a and b).

For both rye grass silage and the Italian rye grass samples, the highest dosage of NaOH (1% w/v) resulted in the highest percentage mass reduction while steam-pretreated samples were generally low at 15 min reaction time. For the rye grass silage (Fig. 1a) in particular, steaming for an extended time resulted in significant rise in sample weight reduction. Comparing the cut biomass samples pretreated with different amount of NaOH for 15 min demonstrated no significant difference between the effect of increasing concentration from 0.5 to 1% w/v NaOH. Nonetheless, weight of steam-treated samples was significantly lower. In general, the Italian rye growth samples had greater percentage weight loss when compared with rye grass silage samples having the same pretreatment. This may have been because the rye grass silage fermentation involves hydrolysis of the grasses at an acidic pH. Similar observations have been reported for NaOH hydrolysis of switch grass [21]. Hemicelluloses, lignin and cellulose are efficiently reduced by NaOH pretreatment [22].

Quantification of hydrolysates

Based on the L_{16} orthogonal array experimental design layout, each factor was attributed to a number 1–5, and the factor levels also varied from level 1 to 4 as shown in Table 2. An experimental layout was generated, and the corresponding concentration of glucose and xylose measured (in g/5 g of original substrate) in the hydrolysates collected after 72 h and recorded for the rye grass silage (see Table 4). The lowest glucose and xylose concentrations (0.42 g/5 g and 0.21 g/5 g respectively) were observed in sample number 10 (enzyme level 2, pretreatment level 2, pretreatment time level 2, incubation temperature level 3, and pH level 2). The glucose concentration ranged from 0.42 g/5 g to 2.26 g/5 g while xylose concentration ranged from 0.15 g/5 g to 1.56 g/5 g. Overall, the recorded glucose: xylose ratio was approximately 2:1. This probably suggests that the rye grass silage had higher concentration of cellulose compared to the hemicellulose. This was expected considering that all the enzyme compositions used contains cellulase and beta-glucosidase as major components. These enzymes are particularly suited to release of glucose from lignocellulose.

Table 6 shows the glucose and xylose concentrations observed in hydrolysates collected from the various growth stages after 72 h of experiment. Regrowth at 0.5% (w/v) and 1% (w/v) NaOH pretreatment and 15 min pretreatment time had the highest values (2.304 g/5 g, 2.297 g/5 g; 1.174 g/5 g, 1.083 g/5 g) of glucose and xylose concentrations respectively.

The lowest concentration of glucose (0.569 g/5 g) was observed in June cut, while the lowest concentration of xylose (0.555 g/5 g) was observed in May cut, both pretreated with steam and 1% (w/v) NaOH respectively for 15 min. Nearly all samples pretreated with steam had lower concentrations of glucose and xylose compared with samples pretreated with NaOH solution for each growth stage sample considered. This observation is in agreement with [21]. This result further confirms the observation from the sample weight measured after pretreatment (Fig. 1a and b), suggesting steam as less efficient at delignification at shorter reaction times compared to NaOH [23]. The ratio of glucose to xylose in the growth samples was mostly 2:1 except in steam pretreated samples.

The higher degree of lignin degradation (see Fig. 1) may have been responsible for the higher glucose and xylose concentrations observed in hydrolysates obtained from samples pretreated with 1% (w/v) NaOH solution (see Table 4 and Table 6). Similar result has been reported by [23]. Lignin increases in content with age of the grass. Lignin degradation therefore enhances enzyme access to cellulose in grass [7,24]. NaOH pretreatment can reduce cellulose crystallinity and lignocellulose recalcitrance [25] by up to 86% at optimum conditions of pretreatment and 100% lignin removal at 1% concentration for 30 min [26]. The regrowth and the second cut samples contain younger leaves and stem and perhaps less lignin. These samples are therefore likely to have higher degradation with NaOH pretreatment [27] when compared with late cut samples. Mild alkali treatment can reduce inhibitory product formation (Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimising their effects [28]).

Statistical analysis

The effect of factor variability on enzymatic hydrolysis of grasses was studied using ANOVA. The variability attributed to each factor is shown in column seven of Table 7. Statistical analysis revealed that pretreatment had the highest percentage impact (74.9%) on enzymatic hydrolysis, followed by enzyme mixture (14.7%). The pH levels of the buffer had the least impact (0.055%) on enzymatic hydrolysis during the experiment. pH has also been shown to have significantly reduced

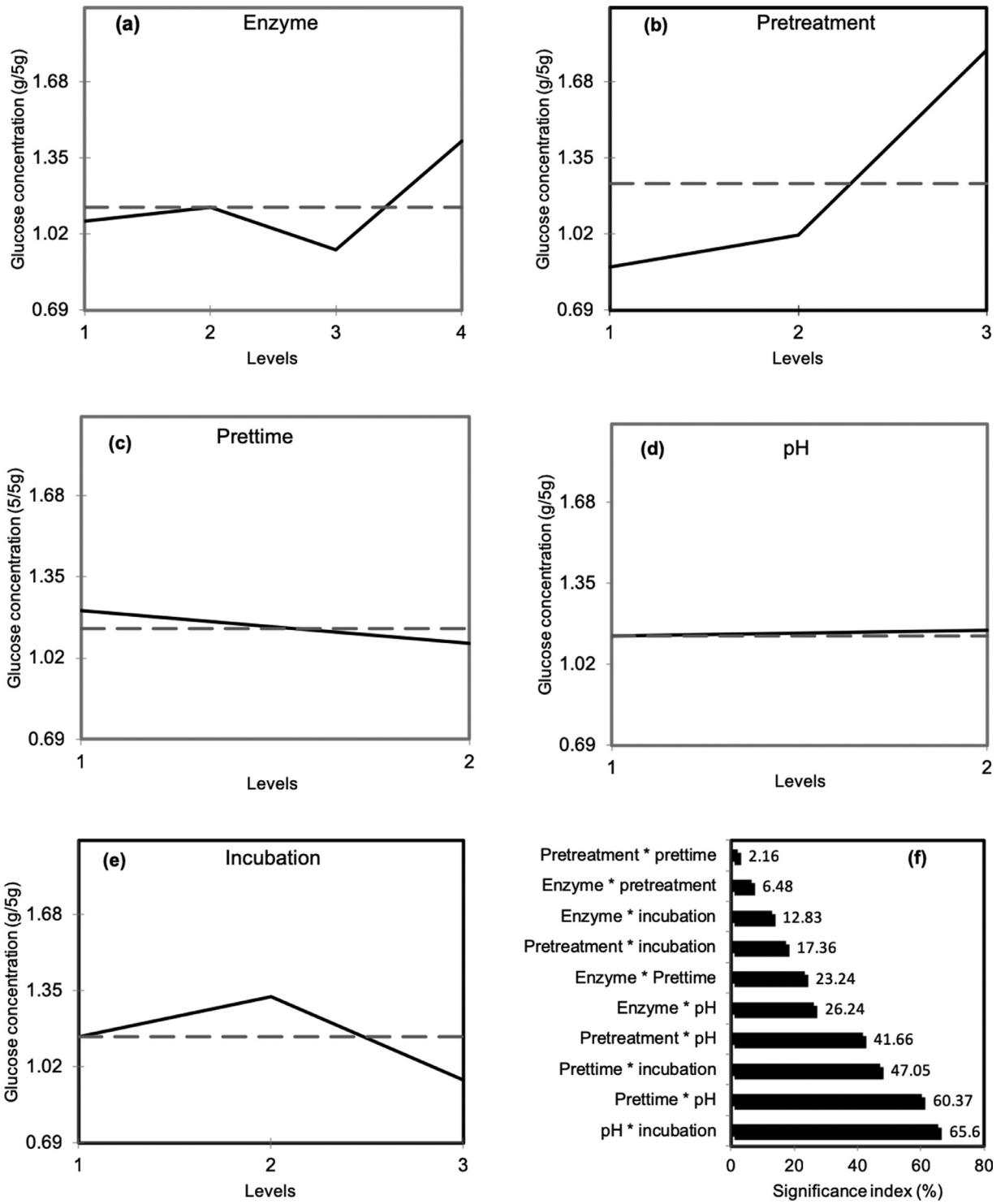

Fig. 2. Impact of factors on enzymatic hydrolysis of rye grass silage. The Figs. (A–E) shows the impact of each factor on enzymatic hydrolysis of italian rye grass and (F) the impact of factors interaction on hydrolysis.

impact on xylanase production in *Aspergillus terrus* [29]. Overall, the percentage impact of enzyme set and pretreatment type together represent approximately 85% of total impact of all the factors under consideration. This indicates the significance of these two factors to optimisation of enzymatic hydrolysis of grass.

The influence of individual factors on enzymatic hydrolysis of grass

Fig. 2 provides an understanding of the main influence of each factor on the enzymatic hydrolysis of grasses. To study this, glucose concentration in hydrolysates was used in the analysis. Variation in enzyme set from level 1 (A1) to 2 (A2) had little effect on hydrolysis, however, variation from level 3 (B1) to 4 (B2) had significant influence on hydrolysis (Fig. 2a). The result suggests that the wider variability (Table 3) in enzyme composition of B2 may be responsible for differences in the degree of hydrolysis observed between treated substrates. Pectinases and hemicellulases (present in enzyme set B) are known to increase the access of cellulases to cellulose [9] and therefore may have had an additional effect on the degree of hydrolysis. Pretreatment with 1.0% w/v NaOH (level 3) greatly influenced enzymatic hydrolysis [26,30] while steam pretreatment (level 1) had the least effect on enzymatic hydrolysis (Fig. 2b). Pretreatment for 15 min (level 1) had the greatest influence on enzymatic hydrolysis (Fig. 2c) while a change in pH from 5.0 (level 1) to 6.5 (level 2) had no significant influence (Fig. 2d). The incubation temperature with the highest influence on enzymatic hydrolysis was 50 °C (level 2) while 60 °C (level 3) had the least influence (Fig. 2e).

The interactions of factors on enzymatic hydrolysis

An understanding of the interaction of factors is important in the design of optimum hydrolytic conditions for an experiment. Fig. 2f shows the interactions among the individual factors selected during the experiment and the effect of such interactions i.e. the severity index (SI) on enzymatic hydrolysis. SI is normally presented on a scale of 0–100%. The greatest interaction SI (65.6%) on enzymatic hydrolysis was observed between the pH and incubation temperature followed by interaction between pretreatment time and pH (60.37%). Interaction between pretreatment time and incubation had SI index of 47.05%. Pretreatment and pretreatment time had the least observed SI (2.16%) of all the SI observed. An interesting observation was that the factors with the least individual percentage impact pH (0.06%) and incubation (8.10%) had the greatest SI. Overall highest SI was mostly observed when the factors with the least individual percentage impact on hydrolysis interacted with other experimental factors. This probably suggests that these factors are also critical for optimising enzymatic hydrolysis and as such were indispensable factors. Similar effect was observed when various factors were combined to optimise anaerobic digestion using the same methodology [19].

Optimum conditions for enzymatic hydrolysis of grass biomass

The optimum conditions for enzymatic hydrolysis and their respective performance as determined by the Taguchi design of the experiment is shown in Table 5. The predicted optimum conditions for enzymatic hydrolysis were: enzyme set B2, 1.0% NaOH pretreatment for duration of 15 min with a pH 6.5 and at an incubation temperature of 50 °C. In terms of contribution of each factor at optimum conditions and performance, 1.0% (w/v) NaOH pretreatment had the highest contribution (0.668%) followed by enzyme set B2 (0.275%). The lowest contribution was from pH (0.010%). The expected result at optimum conditions was 2.34 g/5 g, the total contribution from all factors was 1.20 g/5 g and the current grand average of performance was 1.15 g/5 g. The highest yield of glucose (2.26 g/5 g) obtained during the optimisation step with rye grass silage as shown in Table 3 was obtained when three of the predicted optimum conditions above (pretreatment level 3, pretreatment time level 1, and incubation level 2) were combined. When three of the predicted optimum conditions (pretreatment time level 1, incubation level 2 and pH level 2) and enzyme set (B1) were combined but with variation in pretreatment, a higher concentration of glucose (2.3 g/5 g) was observed for both the growth stage samples no 1 and 4 corresponding to regrowth sample (Table 6). This suggests that a combination of all the predicted optimum conditions improved hydrolysis. Hydrolysis is a critical stage in lignocellulosic biofuel production process; therefore, the optimum conditions we have determined here will improve the final product output if applied on an industrial scale.

Conclusion

Hydrolysis is a crucial stage for grass biomass bioconversion. Therefore, the focus of this work was to identify the best combination of process conditions to ensure optimum sugar yield. The optimum experimental conditions for grass biomass hydrolysis were determined. Pretreatment with 1% (w/v) NaOH and enzyme set B2 (comprising majorly cellulase, beta-glucosidase and some amounts of xylanase, pentosanase, hemicellulase and endoxylanase) were identified as the most significant process factors in enzymatic hydrolysis of grass. The combination of some key factors at the optimum levels yielded glucose concentrations from silage (2.263 g/5 g) and growth stages (2.304 g/5 g and 2.343 g/5 g) near predicted optimum values. pH and incubation, which both had the lowest individual impacts on hydrolysis, had the most significant interaction influence (65.6%). It is expected that the application of these hydrolysis conditions will go a long way to increase the output as well as impacting positively on the cost of biofuel production.

Acknowledgements

This work was conducted at Rothamsted Research, NorthWyke in collaboration with the University of Exeter. The authors wish to really thank Dr. Sreenivas Rao Ravella for his huge support and contribution during the project. The authors wish to

also thank the Commonwealth Scholarship Commision in the UK. The fund was received in a form of scholarship to pursue a Master's degree. The article was an outcome of a research undertaken during the Master's study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2019.e00077.

References

[1] J.D. Murphy, N.M. Power, An argument for using biomethane generated from grass as a biofuel in Ireland, Biomass Bioenergy 33 (2009) 504–512, doi:10.1016/j.biombioe.2008.08.018.
[2] A.T.W.M. Hendriks, G. Zeeman, Pretreatments to enhance the digestibility of lignocellulosic biomass, Bioresour. Technol. 100 (2009) 10–18, doi:10.1016/j.biortech.2008.05.027.
[3] J. Mata-Alvarez, S. Macé, P. Llabrés, Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives, Bioresour. Technol. 74 (2000) 3–16, doi:10.1016/S0960-8524(00)00023-7.
[4] R.L. Howard, E. Abotsi, R.E.L. Jansen van, S. Howard, Lignocellulose biotechnology: issues of bioconversion and enzyme production, Afr. J. Biotechnol. 2 (2003) 602–619, doi:10.5897/AJB2003.000-1115.
[5] T. Amon, B. Amon, V. Kryvoruchko, W. Zollitsch, K. Mayer, L. Gruber, Biogas production from maize and dairy cattle manure-influence of biomass composition on the methane yield, Agric. Ecosyst. Environ. 118 (2007) 173–182, doi:10.1016/j.agee.2006.05.007.
[6] M.C. Chang, Harnessing energy from plant biomass, Curr. Opin. Chem. Biol. 11 (2007) 677–684, doi:10.1016/j.cbpa.2007.08.039.
[7] A.-S. Nizami, N.E. Korres, J.D. Murphy, ChemInform Abstract: the integrated process for the production of grass biomethane, ChemInform Environ. Sci. Technol. Dep. Civ. Environ. Eng. 40 (2009) 8496–8508 Univ. Coll. Cork., doi:10.1002/chin.200952269.
[8] T. Callaghan, G. Lawson, R. Scott, Scott, the Potential of Natural Vegetation as a Source of Biomass Energy, Elsevier Applied Science Publishers, London, 1985.
[9] U.S. Aswathy, R.K. Sukumaran, G.L. Devi, K.P. Rajasree, R.R. Singhania, A. Pandey, Bio-ethanol from water hyacinth biomass : an evaluation of enzymatic saccharification strategy, Bioresour. Technol. 101 (2010) 925–930, doi:10.1016/j.biortech.2009.08.019.
[10] A.J. Ward, P.J. Hobbs, P.J. Holliman, D.L. Jones, Optimisation of the anaerobic digestion of agricultural resources, Bioresour. Technol. 99 (2008) 7928–7940, doi:10.1016/j.biortech.2008.02.044.
[11] D. Gregg, J.N. Saddler, A techno-economic assessment of the pretreatment and fractionation steps of a biomass-to-ethanol process, Appl. Biochem. Biotechnol. 57–58 (1996) 711–727, doi:10.1007/BF02941753.
[12] H.B. Klinke, B.K. Ahring, A.S. Schmidt, A.B. Thomsen, Characterization of degradation products from alkaline wet oxidation of wheat straw, Bioresour. Technol. 82 (2002) 15–26, doi:10.1016/S0960-8524(01)00152-3.
[13] A. Berlin, V. Maximenko, N. Gilkes, J. Saddler, Optimization of enzyme complexes for lignocellulose hydrolysis, Biotechnol. Bioeng. 97 (2007) 287–296, doi:10.1002/bit.21238.
[14] H. Jørgensen, J.B. Kristensen, C. Felby, Felby, Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities, Biofuels Bioprod. Biorefin. 1 (2007) 119–134, doi:10.1002/bbb.4.
[15] G. Banerjee, S. Car, J.S. Scott-craig, M.S. Borrusch, J.D. Walton, Rapid optimization of enzyme mixtures for deconstruction of diverse pretreatment / biomass feedstock combinations, Biotechnol. Biofuels 3 (2010) 22, doi:10.1186/1754-6834-3-22.
[16] F.M. Reyes-sosa, D. Bruno, Minireview, Enzymatic Hydrolysis of Biomass from Wood, Microb. Biotechnol. 9 (2) (2016) 149–156, doi:10.1111/1751-7915.12346.
[17] R.S. Rao, C.G. Kumar, R.S. Prakasham, P.J. Hobbs, The Taguchi methodology as a statistical tool for biotechnological applications: a critical appraisal, Biotechnol. J. 3 (2008) 510–523, doi:10.1002/biot.200700201.
[18] R.S. Prakasham, C. Subba Rao, R. Sreenivas Rao, P.N. Sarma, Enhancement of acid amylase production by an isolated *Aspergillus awamori*, J. Appl. Microbiol. 102 (2007) 204–211, doi:10.1111/j.1365-2672.2006.03058.x.
[19] N. Adu-Gyamfi, S.R. Ravella, P.J. Hobbs, Optimizing anaerobic digestion by selection of the immobilizing surface for enhanced methane production, Bioresour. Technol. 120 (2012) 248–255, doi:10.1016/j.biortech.2012.06.042.
[20] Z.Z. Asam, T.G. Poulsen, A.S. Nizami, R. Rafique, G. Kiely, J.D. Murphy, How can we improve biomethane production per unit of feedstock in biogas plants? Appl. Energy 88 (2011) 2013–2018, doi:10.1016/j.apenergy.2010.12.036.
[21] K.C. Nlewem, M.E. Thrash, Thrash, Comparison of different pretreatment methods based on residual lignin effect on the enzymatic hydrolysis of switchgrass, Bioresour. Technol. 101 (2010) 5426–5430, doi:10.1016/j.biortech.2010.02.031.
[22] M. Cui, J. Shen, Effects of acid and alkaline pretreatments on the biohydrogen production from grass by anaerobic dark fermentation, Int. J. Hydrogen Energy 37 (2012) 1120–1124, doi:10.1016/j.ijhydene.2011.02.078.
[23] C. Eliana, R. Jorge, P. Juan, R. Luis, Effects of the pretreatment method on enzymatic hydrolysis and ethanol fermentability of the cellulosic fraction from elephant grass, Fuel 118 (2014) 41–47, doi:10.1016/j.fuel.2013.10.055.
[24] S.D. Mansfield, C. Mooney, J.N. Saddler, Substrate and enzyme characteristics that limit cellulose hydrolysis, Biotechnol. Prog. 15 (1999) 804–816, doi:10.1021/bp9900864.
[25] M.J. Taherzadeh, K. Karimi, Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review, Int. J. Mol. Sci. 9 (2008) 1621–1651, doi:10.3390/ijms9091621.
[26] Z. Wang, D.R. Keshwani, A.P. Redding, J.J. Cheng, Sodium hydroxide pretreatment and enzymatic hydrolysis of coastal Bermuda grass, Bioresour. Technol. 101 (2010) 3583–3585, doi:10.1016/j.biortech.2009.12.097.
[27] M. Seppälä, T. Paavola, A. Lehtomäki, J. Rintala, Biogas production from boreal herbaceous grasses—specific methane yield and methane yield per hectare, Bioresour. Technol. 100 (2009) 2952–2958, doi:10.1016/j.biortech.2009.01.044.
[28] L.J. Jönsson, C. Martín, Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects, Bioresour. Technol. 199 (2016) 103–112, doi:10.1016/j.biortech.2015.10.009.
[29] G.S. Lakshmi, C.S. Rao, R.S. Rao, P.J. Hobbs, R.S. Prakasham, Enhanced production of xylanase by a newly isolated *Aspergillus terreus* under solid state fermentation using palm industrial waste: a statistical optimization, Biochem. Eng. J. 48 (2009) 51–57, doi:10.1016/j.bej.2009.08.005.
[30] F. Carvalheiro, L.C. Duarte, F.M. Girio, Hemicellulose biorefineries: a review on biomass pretreatments, J. Sci. Ind. Res. 67 (2008) 849–864, doi:10.1016/j.talanta.2015.06.045.