Table S1a Coded and uncoded variables for RSM CCD (central composite design) design of experiment using Minitab® 17 to optimize dilute acid pretreatment of vetiver constructed with 5-center points. Optimization of dilute acid pretreatment must be performed under conditions that reduce the release of compounds that are inhibitory to the following stages of ethanol production, such as furfural, 5-hydroxymethyl furfural (5-HMF) and acetic acid. Inhibitory concentrations are as low as 0.1g/L, 0.5g/L, and 2.0g/L, respectively for *Scheffersomyces stipits* [1]. Interaction effects between time and temperature on inhibitor production have been observed [2, 3]. Factors that affect the concentrations of inhibitory byproducts include acid concentration, biomass to acid ratios, time, and temperature [2, 3, 4].

Factors	**Coded Values**	**Uncoded Values**
Time (min)	-1	30
	+1	90
Temperature (°C)	-1	115
	+1	125
% H_2SO_4 (v/v)	-1	1
	+1	10
Biomass:Acid (m/v)	-1	1
	+1	10

Table S1b Factors and levels of treatment investigated for optimization of enzymatic saccharification of vetiver grass for RSM CCD (central composite design) design of experiment using Minitab® 17 constructed with 6-center points. Enzymatic saccharification should be optimized for specific enzymes, enzyme loading, and time, temperature, and pH of treatment [5].

Factors	**Coded Values**	**Uncoded Values**
pH	-1	4
	+1	8
Temperature (°C)	-1	35
	+1	65
Time (hours)	-1	24
	+1	168
Cellulase (FPU/g cellulose)	-1	30
	+1	90
B-glucosidase (pNGU/g cellulose)	-1	30
	+1	90

Table S1c The fermentation parameters investigated for optimal ethanol yield from vetiver grass included 5 continuous factors and 1 categorical factor. Minitab® 17 was used to create the RSM CCD (central composite) design of experiment with 6-center points. Selection of appropriate yeast species is mainly dependent on the monomer content of the biomass [85]. *Saccharomyces cerevisiae* has greater ethanol productivity with preference for glucose from plants with greater cellulose content [6]. *Kluveromyces marxianus, Scheffersomyces* (*Pichia*) *stipitis and Pachysolen tannophilus* show partiality for xylose from plants with more hemicellulose [85]. Factors that influence the ethanol yield from fermentation of hydrolysates include yeast species, time, temperature, pH, and concentration of nutrient amendments and should be optimized for the substrate used for ethanol production [7].

Factors	**Coded Values**	**Uncoded Values**
pH	-1	3
	+1	6
Time (hours)	-1	24
	+1	72
Temperature (°C)	-1	25
	+1	30
Inoculum (%)	-1	5
	+1	15
Nutrient (%)	-1	5
	+1	15
Yeast Species		*Kluyveromyces marxianus*
		Scheffersomyces (Pichia) stipitis
		Pachysolen tannophilus

Equation S2a CCD Regression Equation for Dilute Acid Pretreatment Monosaccharide yield using RSM.

Monosaccharides = 14.404 – 0.193 Time – 0.560 Temperature – 1.416 [Acid] + 5.594 Biomass:Acid + 0.169 Time*Time + 0.078 Time*Temperature – 0.107 [Acid]*[Acid] – 0.006 Biomass:Acid*Biomass:Acid + 0.227 Time*Temperature – 0.534 Time* [Acid] – 0.410 Time*Biomass:Acid – 0.662 Temperature * [Acid] – 0.477 Temperature*Biomass:Acid-0.201 [Acid]*Biomass:Acid

Table S4a 2-way ANOVA for sugar yield vs. factors tested for dilute acid pretreatment optimization. (*) indicates significant treatment effect or interaction.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	16	1660.58	103.79	85.70	0.000
Blocks	2	3.94	1.97	1.63	0.208
Linear	4	1615.07	403.77	333.41	0.000
Time	1	1.78	1.78	1.47	0.232
Temperature	1	15.06	15.06	12.44	0.001*
[Acid]	1	96.19	96.19	79.43	0.000*
Biomass:Acid	1	1502.03	1502.03	1240.30	0.000*
2-Way Interaction	6	38.72	6.45	5.33	0.000
Time*Temperature	1	1.64	1.64	1.36	0.251
Time*[Acid]	1	9.13	9.13	7.54	0.009*
Time*Biomass:Acid	1	10.39	5.39	4.45	0.041*
Temperature*[Acid]	1	14.01	14.01	11.56	0.001*
Temperature*Biomass:Acid	1	7.27	7.27	6.00	0.018*
[Acid]*Biomass:Acid	1	1.29	1.29	1.07	0.307

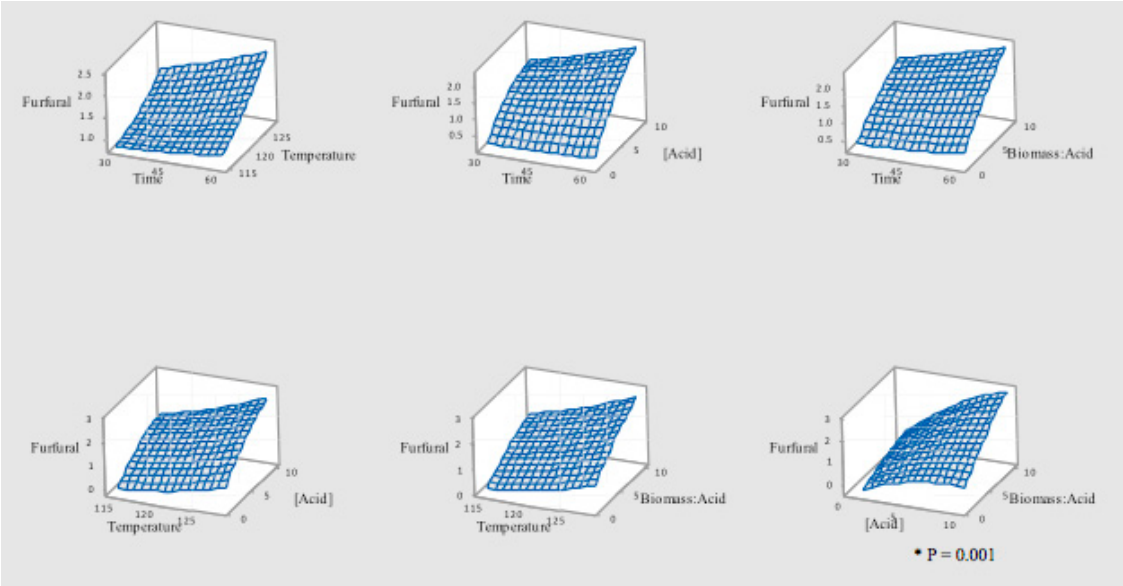

Figure S3a Response surface plots for dilute acid pretreatment sugar yield showing the effects of interactions between investigated factors at varying levels for treatment of vetiver grass. (*) indicates a significant interaction effect between factors, and the p-values for significant interactions are provided below the graphs from 2-way ANOVA. Axis units are: sugars = g/L, time = minutes, temperature = °C, [acid] = %, biomass:acid = %.

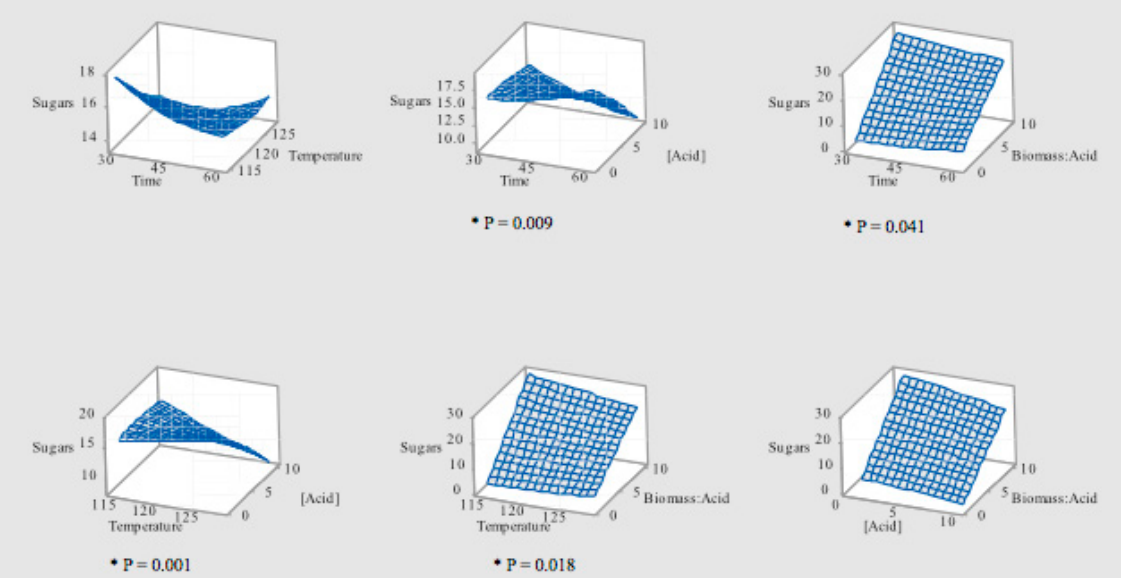

Figure S3b Response surface plots for dilute acid pretreatment furfural production, showing the effects of interactions between investigated factors at varying levels for treatment of vetiver grass. (*) indicates a significant interaction effect between factors, and the p-values for significant interactions are provided below the graphs from 2-way ANOVA. Axis units are: sugars = g/L, time = minutes, temperature = °C, [acid] = %, biomass:acid = %, furfural = g/L.

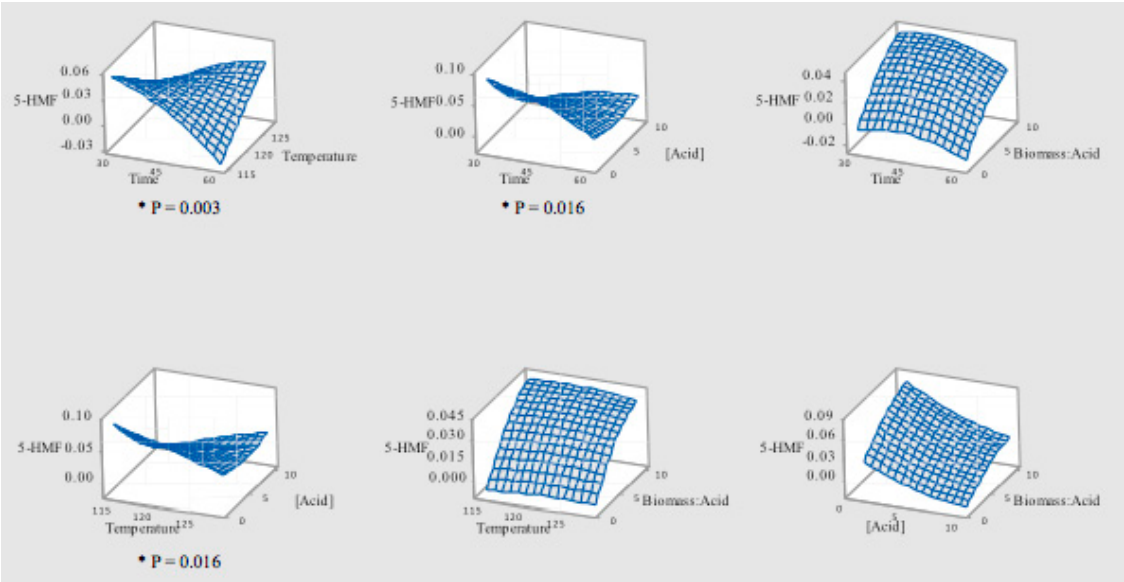

Figure S3c Response surface plots for dilute acid pretreatment 5-hydroxymethyl furfural production, showing the effects of interactions between investigated factors at varying levels for treatment of vetiver grass. (*) indicates a significant interaction effect between factors, and the p-values for significant interactions are provided below the graphs from 2-way ANOVA. Axis units are: sugars = g/L, time = minutes, temperature = °C, [acid] = %, biomass:acid = %, 5-HMF = g/L. 5-HMF = 5-hydroxymethyl furfural.

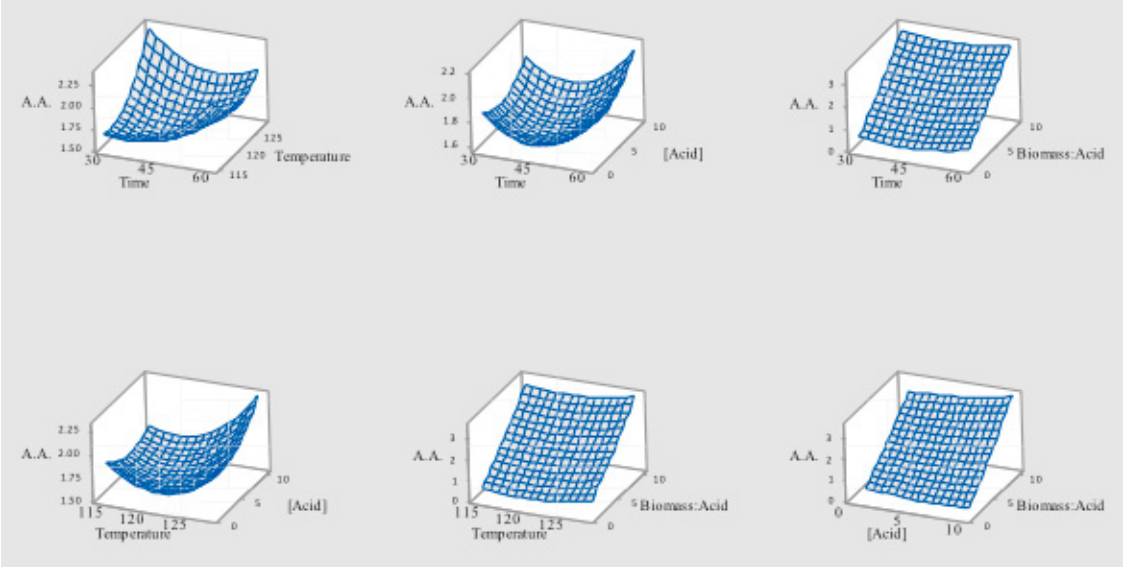

Figure S3d Response surface plots for dilute acid pretreatment acetic acid production, showing the effects of interactions between investigated factors at varying levels for treatment of vetiver grass. (*) indicates a significant interaction effect between factors, and the p-values for significant interactions are provided below the graphs from 2-way ANOVA. Axis units are: sugars = g/L, time = minutes, temperature = °C, [acid] = %, biomass:acid = %, A.A. = g/L. A.A = acetic acid.

Equation S2b CCD Regression Equation for glucose yield from Enzymatic Hydrolysis yield using RSM:

Glucose = 10.49 - 0.85 pH + 0.114 Temperature (C) -0.0343 Cellulase (FPU/g cellulose) - 0.0003 B-galactosidase (pNPGU/g cellulose) - 0.0018 Time (hrs) + 0.0092 pH*pH - 0.00161 Temperature (C)*Temperature (C)
+ 0.000222 Cellulase (FPU/g cellulose)*Cellulase (FPU/g cellulose) + 0.000165 B-galactosidase (pNPGU/g cellu)
*B-galactosidase (pNPGU/g cellulose) - 0.000075 Time (hrs)*Time (hrs)
+ 0.0092 pH*Temperature (C) + 0.00531 pH*Cellulase (FPU/g cellulose)
+ 0.00358 pH*B-galactosidase (pNPGU/g cellulose) - 0.00176 pH*Time (hrs)
- 0.000510 Temperature (C)*Cellulase (FPU/g cellulose) - 0.000769 Temperature (C)*B-galactosidase (pNPGU/g cellulose) + 0.000342 Temperature (C)*Time (hrs) - 0.000335 Cellulase (FPU/g cellulose) *B-galactosidase (pNPGU/g cellulose)
+ 0.000148 Cellulase (FPU/g cellulose) *Time (hrs) + 0.000126 B-galactosidase (pNPGU/g cellulose)*Time (hrs)

Table S4b Statistical analysis by 2-way ANOVA for influence of treatment factors on glucose concentrations from enzymatic saccharification of vetiver biomass are shown below. (*) indicates significant treatment effects or interaction effects.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	21	86.772	4.1320	1.10	0.380
Blocks	1	7.112	7.1122	1.90	0.175
Linear	5	30.105	6.0210	1.61	0.178
pH	1	1.523	1.5230	0.41	0.527
Temperature	1	13.685	13.6854	3.65	0.063
Cellulase	1	2.215	2.2145	0.59	0.446
B-glucosidase	1	1.323	1.3234	0.35	0.555
Time	1	11.359	11.3588	3.03	0.089
2-Way Interaction	10	27.667	2.7667	0.74	0.685
pH*Temperature	1	2.448	2.4476	0.65	0.423
pH*Cellulase	1	3.245	3.2449	0.87	0.357
pH*B-glucosidase	1	1.475	1.4749	0.39	0.534
pH*Time	1	2.055	2.0554	0.55	0.463
Temperature*Cellulase	1	1.688	1.6882	0.45	0.506
Temperature*B-glucosidase	1	3.830	3.8295	1.02	0.318
Temperature*Time	1	4.373	4.3734	1.17	0.286
Cellulase*B-glucosidase	1	2.910	2.9101	0.78	0.383
Cellulase*Time	1	3.283	3.2832	0.88	0.354
B-glucosidase*Time	1	2.360	2.3599	0.63	0.432

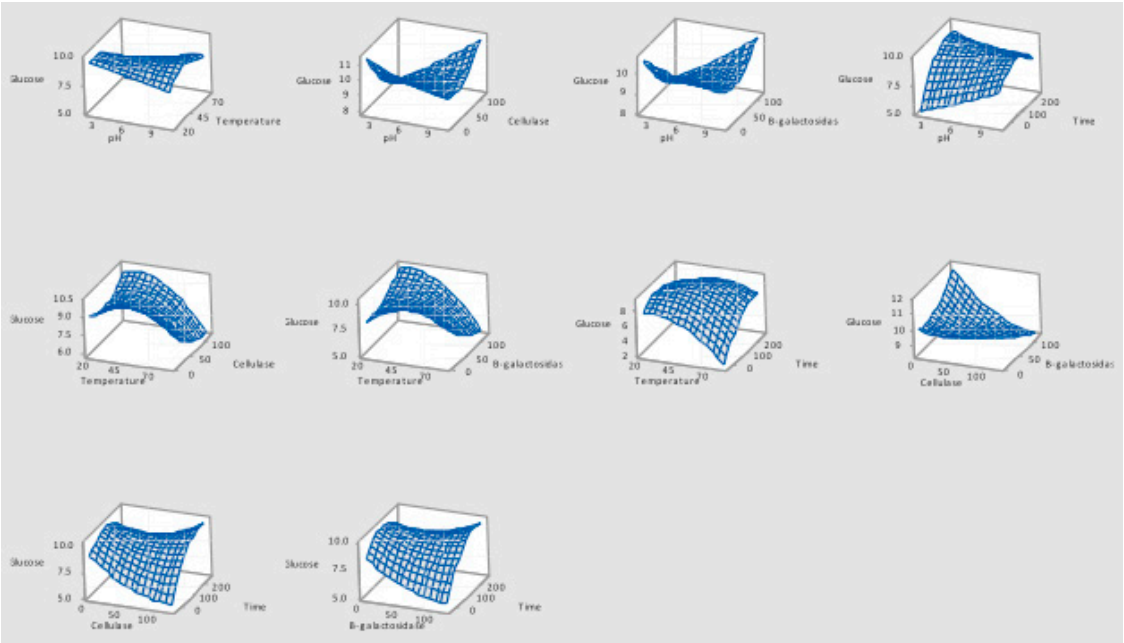

Figure S3e Response surface plots for glucose yield by enzymatic hydrolysis of vetiver biomass, showing the effects of interactions between investigated factors at varying levels for treatment of vetiver biomass. Units for axis are: time = hours, temperature = °C, cellulase = FPU/g cellulose, β-glucosidase = pNPGU/g cellulose, glucose = g/L.

Equation S2c CCD Regression Equation for glucose yield from ethanol yield using RSM:

Scheffersomyces stipitis: Ethanol (g/L) = -6.61 - 0.031 pH + 0.0160 Time (hr)
+ 0.388 Temperature (C) + 0.0084 Inoculum (%) - 0.178 Nutrients (%) - 0.0274 pH*pH -
0.000086 Time (hr)* Time (hr) - 0.00835 Temperature (C) *Temperature (C) -
0.00219 Inoculum (%)*Inoculum (%) + 0.00369 Nutrients (%)*Nutrients (%)
+ 0.00182 pH*Time (hr) + 0.0197 pH*Temperature (C) - 0.00082 pH*Inoculum (%)
+ 0.0139 pH*Nutrients (%) - 0.000214 Time (hr)*Temperature (C)
- 0.000135 Time (hr)*Inoculum (%) + 0.000993 Time (hr)*Nutrients (%)
+ 0.00411 Temperature (C)*Inoculum (%) + 0.00791 Temperature (C) *Nutrients (%)
+ 0.00381 Inoculum (%)*Nutrients (%)

Kluyveromyces marxianus: Ethanol (g/L) = -3.22 - 0.420 pH + 0.0077 Time (hr)
+ 0.335 Temperature (C) - 0.0570 Inoculum (%) - 0.226 Nutrients (%) - 0.0274 pH*pH -
0.000086 Time (hr)*Time (hr) - 0.00835 Temperature (C) *Temperature (C) -
0.00219 Inoculum (%)*Inoculum (%) + 0.00369 Nutrients (%)*Nutrients (%)
+ 0.00182 pH*Time (hr) + 0.0197 pH*Temperature (C) - 0.00082 pH*Inoculum (%)
+ 0.0139 pH*Nutrients (%) - 0.000214 Time (hr)*Temperature (C) -
0.000135 Time (hr)*Inoculum (%) + 0.000993 Time (hr)*Nutrients (%)
+ 0.00411 Temperature (C)*Inoculum (%) + 0.00791 Temperature (C) *Nutrients (%)
+ 0.00381 Inoculum (%)*Nutrients (%)

Pachysolen tannophilus: Ethanol (g/L) = -5.71 - 0.318 pH + 0.0155 Time (hr)
+ 0.397 Temperature (C) - 0.0232 Inoculum (%) - 0.165 Nutrients (%) - 0.0274 pH*pH-
0.000086 Time (hr)*Time (hr) 0.00835 Temperature (C) *Temperature (C)-
0.00219 Inoculum (%)*Inoculum (%)
+ 0.00369 Nutrients (%)*Nutrients (%)+ 0.00182 pH*Time (hr)
+ 0.0197 pH*Temperature (C) - 0.00082 pH*Inoculum (%)
+ 0.0139 pH*Nutrients (%) - 0.000214 Time (hr)*Temperature (C)
- 0.000135 Time (hr)*Inoculum (%) + 0.000993 Time (hr)*Nutrients (%)
+ 0.00411 Temperature (C)*Inoculum (%)+ 0.00791 Temperature (C)
*Nutrients (%) + 0.00381 Inoculum (%)*Nutrients (%)

Table S4c Interaction effects between treatment factors on ethanol production from vetiver hydrolysates were statistically analyzed by 2-way ANOVA and alpha = 0.05. (*) denote a significant treatment factor or combination of factors.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	34	92.063	2.7077	17.16	0.000
Blocks	2	1.472	0.7358	4.66	0.011
Linear	7	74.127	10.5896	67.10	0.000
pH	1	7.054	7.0535	44.69	0.000*
Time	1	5.412	5.4123	34.29	0.000*
Temperature	1	2.453	2.4533	15.54	0.000*
Inoculum (%)	1	6.696	6.6961	42.43	0.000*
Nutrients (%)	1	25.320	25.3200	160.43	0.000*
Yeast	2	27.192	13.5960	86.15	0.000*
2-Way Interaction	20	14.449	0.7225	4.58	0.000
pH*Time	1	0.412	0.4121	2.61	0.109
pH*Temperature	1	0.524	0.5236	3.32	0.071
pH*Inoculum (%)	1	0.004	0.0036	0.02	0.880
pH*Nutrients (%)	1	0.212	0.2119	1.34	0.249
pH*Yeast	2	7.902	3.9509	25.03	0.000*
Time*Temperature	1	0.016	0.0158	0.10	0.753
Time*Inoculum (%)	1	0.025	0.0250	0.16	0.691
Time*Nutrients (%)	1	0.276	0.2763	1.75	0.188
Time*Yeast	2	1.087	0.5436	3.44	0.035*
Temperature*Inoculum (%)	1	0.253	0.2532	1.60	0.208
Temperature*Nutrients (%)	1	0.190	0.1899	1.20	0.275
Temperature*Yeast	2	0.600	0.2999	1.90	0.154
Inoculum (%)*Nutrients (%)	1	0.176	0.1760	1.11	0.293
Inoculum (%)*Yeast	2	2.311	1.1555	7.32	0.001*
Nutrients (%)*Yeast	2	0.462	0.2312	1.46	0.235

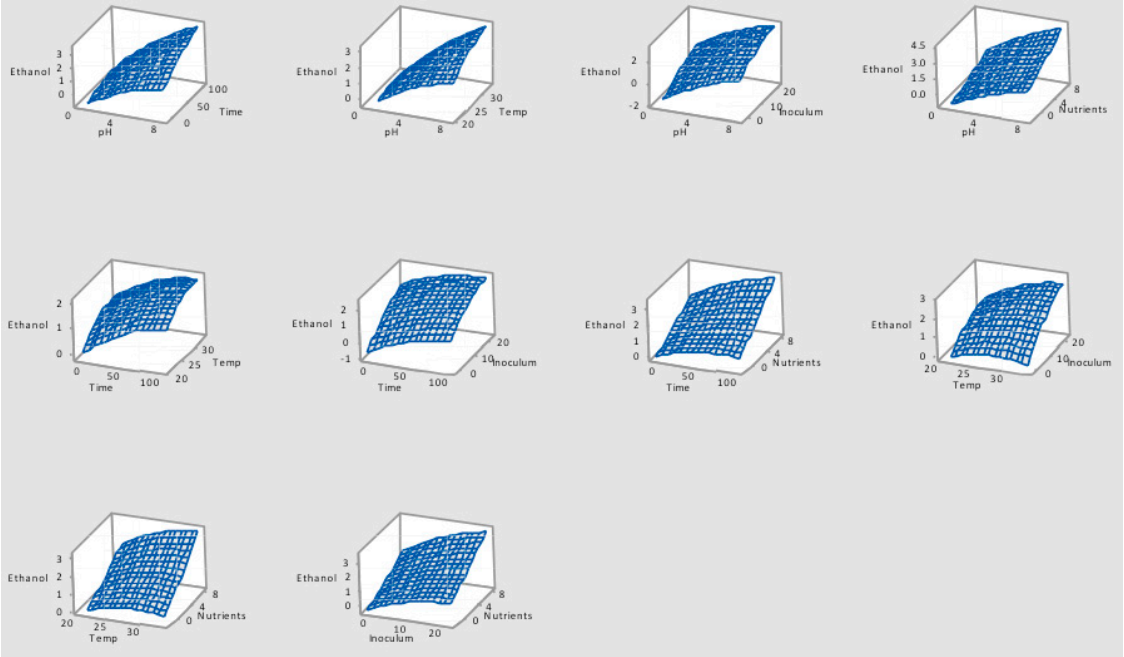

Figure S3f Response surface plots for fermentation factors and their impact on ethanol production from vetiver hydrolysates, showing the effects of interactions between investigated factors at varying levels for treatment of vetiver grass. Units for axis are: time = hours, temperature = °C, inoculum = %, nutrients = %, ethanol = g/L

Figure S4. Vetiver plants growing in columns in the greenhouse on day 60. Control columns are on the left and experimental columns are on the right. The columns in the foreground are abiotic columns.

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