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Optimized Production of Second-Generation Bioethanol from a Spent C4 Grass: Vetiver (*Chrysopogon zizanioides*)

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Abstract: Vetiver grass (*Chrysopogon zizanioides*) is well-known for its contaminant phytoextraction potential and its capacity to reduce soil erosion, owing to its massive, dense root system. However, the shoots are not major contributors to either of these processes, and are either not utilized at all or they become part of the waste stream. It is well-recognized that lignocellulosic biomass can serve as a source of raw material to produce second-generation bioethanol. This study investigated the simultaneous saccharification and fermentation (SSF) of acid–alkali pretreated vetiver (VG) shoots by *Saccharomyces cerevisiae*. Vetiver shoots were obtained from three sources: (1) shoots from VG grown in clean potting soil, (2) shoots from VG used for antibiotics phytoextraction from a constructed wetland setup, and (3) shoots from VG used for lead phytoextraction during soil remediation. Bioethanol yield from the shoots from clean soil was the highest (19.58 g/L), followed by the one used for lead phytoextraction (19.50 g/L) and the one used for antibiotics phytoextraction (19.17 g/L). Bioethanol yield and quality obtained from these three VG shoots was superior or similar to other C4 grasses used for bioethanol generation. This study successfully demonstrated that spent vetiver biomass after phytoextraction applications can be repurposed to generate high-quality bioethanol.

Keywords: vetiver shoots; lignocellulosic biomass; waste reuse; dilute acid pretreatment; bioethanol

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1. Introduction

With tremendous energy demand and limited fossil fuel resources, the world is facing pressure to address future energy shortages. Bioenergy production could be a practical solution, which has been studied extensively for the past few decades. "First-generation" bioethanol is produced by fermenting raw materials derived from sugar, whereas "second-generation" bioethanol is produced primarily by fermenting lignocellulosic biomass. "Third-generation" bioethanol is primarily generated from marine organisms such as algae, both microalgae and macroalgae [1]. The properties of microalgae in terms of high carbohydrate and lipid contents, fast growth rate, high contribution to CO_2 mitigation, low land use, and high flexibility in cultivation media make it a well-recognized source for the "third-generation" bioethanol production [2–4]. Irrespective of feedstock sources, fermentation only yields an alcohol-lean broth, rendering it useless for use as fuel or industrial processes. Therefore, bioethanol needs to be refined. Using fractional distillation, it is possible to concentrate bioethanol to a concentration of 95.6 vol% (89.5 mol%), which corresponds to an azeotropic composition with a boiling point of 78.2 °C [5]. In several regions in the U.S., bioethanol has largely replaced methyl tertiary butyl ether as a gasoline additive and oxygenates [6]. Currently, corn starch is used to produce more than 95% of the vehicle-usage bioethanol in the U.S., while the land required for producing these energy feedstocks is considerably large [7]. Land requirement could be significantly minimized if lignocellulosic materials were used for bioenergy production.

Vetiver grass (VG, *Chrysopogon zizanioides*) is a tropical plant that can grow in various environmental conditions, possessing dense root systems which spread into the soil.

Without rhizomes or stolons, VG's root can bind soil beneath the plant to a depth of up to 3 m [8]. The shoots sprout from the bottom of the clump and are long, narrow, and rough. Vetiver also has a high efficiency in absorbing dissolved nutrients [9], so it can proliferate under low nutrient conditions and produce high biomass [10]. In Thailand, 10 types of VG consisting of cellulose (31.85–38.51%), hemicellulose (37.87–42.61%), and lignin (3.67–5.06%) were reported [11].

VG is widely used for environmental purposes. It is either used for slope stabilization or contaminant phytoextraction [12–14]. The massive and dense root system of VG is responsible for holding the soil particles together and for scavenging contaminants from soil and water [15,16]. However, because of limited translocation, the majority of the contaminants are retained in VG roots during the phytoextraction process. Hence, concentrations of contaminants in VG shoots are typically very low. The high cellulose content of VG shoot, however, makes it a viable raw material for the production of bioethanol. Briefly, cellulose is the main content of lignocellulose, which consists of a linear polymer [17] and a cellulose chain that can be converted into glucose by either chemical or enzymatic digestion. The glucose from cellulose hydrolysis is used for bioethanol fermentation by microbes. Similar to microalgae, VG has a fast growth rate and produces a large quantity of biomass, which provides a sufficient amount of feedstock for bioethanol generation. VG can also be grown in a variety of media, such as in soil, surface water, (either clean or polluted water bodies, or hydroponically, which is another distinct advantage over other feedstock [18].

Overall, bioethanol generation from VG, particularly the spent VG after phytoextraction applications, would likely minimize the use of agricultural land for generating traditional bioethanol feedstock, reduce the cost of producing conventional feedstock, and present a much preferable alternative to landfilling the spent biomass. However, the quality of bioethanol generated from VG and spent VG is unclear. Therefore, the main objective of this study was to produce bioethanol from VG from various sources (i.e., VG grown in clean soil, VG used for phytoextraction of antibiotics, VG used for phytoextraction of lead) and compare the yield and quality of the bioethanol obtained with those of other C4 grasses.

2. Materials and Methods

2.1. Biomass Sources and Preparation

Shoots of VG in this study were obtained from three different sources: shoots from fresh VG that was cultivated in potting soil in the greenhouse [19], shoots from VG that were used for the uptake of antibiotics in our previous study with a ciprofloxacin (CIP) concentration of 80 mg/kg and a tetracycline (TTC) concentration of 98 mg/kg [20], and shoots from VG that was used for the uptake of lead during phytoextraction process in our previous study, which had 11.5 mg/kg lead in their shoots [21]. All three types of VG shoots were washed, sun-dried, and chopped into 1–2 cm pieces before oven-drying (65 °C for 48 h) and milling to small particles (~0.2 mm) in a planetary ball mill following the process in Nagara et al. [22]. The extractives, cellulose, hemicellulose, and lignin content in the VG leaf samples were determined according to the methods described by Yang et al. [23].

2.2. Bioethanol Production

2.2.1. Dilute Acid-Alkali Pretreatment

The processes involved in bioethanol production used in this study are shown in Figure 1. Cellulose (40–60% of the total dry weight), hemicellulose (20–40%), and lignin (10–25%) make up the majority of lignocellulose, the primary component of plant cell walls [5]. Pretreatment comprises of delignification of the feedstock to increase cellulose accessibility at the hydrolysis stage [24]. Dilute acid pretreatment was adopted with minor modifications owing to similarities in the biomass leading to high bioethanol yield [25,26]. About 70 g of milled VG shoots were added to 1 L of 1% (v/v) sulfuric acid. The mixture was autoclaved at 130 °C and 24.5 psi (relative to atmosphere) for 45 min. After acid

pretreatment, the pH of the vetiver slurry was adjusted to 5.0 using 1 M NaOH. Then, the VG shoots were filtered, washed, and dried at 60 °C for 24 h.

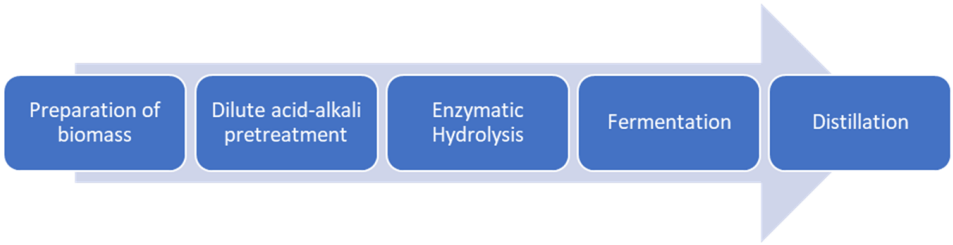

Figure 1. Bioethanol generation process from VG shoots.

2.2.2. Enzymatic Hydrolysis

Cellulase (60 U/g), β-glucosidase (120 U/g), 400 µL of 2% sodium azide (for antimicrobial activity), and 20 mL of 0.1 M sodium citrate buffer (pH 4.8) were added per gram of milled grass as per the optimized conditions discussed by Geiger et al. [25]. The samples were incubated at 30 °C and shaken at 150 rpm for 132 h. After enzymatic treatment, the reaction mixture was heated at 80 °C for 10 min to stop the enzymatic activity.

2.2.3. Bioethanol Fermentation

Yeast culture: *Saccharomyces cerevisiae* was pre-cultured in yeast extract peptone dextrose (YPD) agar plate (10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose) at 30 °C for 24 h on a rotary shaker at 150 rpm. Yeast cells were centrifuged at 3220× *g* for 10 min and washed with deionized water. For the fermentation of bioethanol, an inoculum containing 10% yeast cells (10^9 cells/mL) was utilized.

Fermentation media: 150 mL of 0.1 M sodium citrate buffer (pH 4.8) was added to acid-pretreated VG samples (10 g) in 250 mL Erlenmeyer flasks, followed by 5 g/L yeast extract, 5 g/L urea, 0.5 g/L $MgSO_4 \cdot 7H_2O$, and 1 g/L KH_2PO_4. The media were autoclaved at 120 °C for 15 min.

2.2.4. Simultaneous Saccharification and Fermentation (SSF)

The short-term commercial production of lignocellulosic bioethanol is greatly improved by fermenting C5 and C6 sugars through simultaneous saccharification and fermentation (SSF). In this study, SSF was performed in 250 mL Erlenmeyer flasks containing 150 mL fermentation medium. Cellulase (8 U/g) and β-glucosidase (120 U/g) were added for saccharification. *S. cerevisiae* was then inoculated at a cell concentration of 22% (*w*/*v*). The mixture was then incubated at 33 °C in an orbital shaker at 150 rpm. Samples were taken after 0, 12, 24, 48, 72, and 104 h, and the amounts of monosaccharides remaining, and bioethanol produced were analyzed by high-performance liquid chromatography (HPLC). All determinations were performed in three biological replications. A distillation procedure was employed to further separate and purify the bioethanol from water during a fermentation conversion based on their distinct volatilities (boiling points of 78.3 °C and 100 °C for bioethanol and water, respectively). Fractional distillation of the fermentation broth was carried out following the process described by Muhaji and Sutjahjo [27]. A Still Spirit Turbo-500 boiler and distiller setup were used to obtain refined bioethanol. Five liters of fermentation broth was continuously boiled at 80 °C, and the condensate bioethanol was collected through the condenser output. This process was continued until there was no condensate from the boiler suggesting that the fermentation broth was converted into an azeotropic mixture, where the alcohol in the vapor phase was not as concentrated as the one in the liquid phase to allow further fractional distillation [28].

Thus, bioethanol can be recovered at a 95% concentration using a condensation process. Bioethanol yield is calculated as the amount of bioethanol produced per unit of substrate utilized as described in the following formula by Zabed et al. [29]:

$$Y_{EtOH} = CV/m$$

where Y_{EtOH} is bioethanol yield (mg/g), C is bioethanol concentration (mg/L), V is the initial volume of liquid medium (L), and m is the mass of the substrate (g).

2.3. Determination of Sugars and Bioethanol

Concentrations of D-glucose, D-galactose, D-xylose, L-arabinose, and bioethanol were determined by the following separation by HPLC using a BioRad (Hercules, CA, USA) Aminex HPX-87H column (300 × 7.8 mm) at 65 °C with 5 mM H_2SO_4 as the mobile phase, at a flow rate of 0.6 mL/min and an injection volume of 10 µL (Figure S1 in Supplementary Information). Samples were appropriately diluted in deionized water prior to injection. The quality of bioethanol generated by VG from different sources was analyzed using various ASTM methods.

2.4. Quality Analysis of Bioethanol

Ethanol was extracted from the fermentation broth and then purified to assess its suitability for use as a transportation fuel. The distillate was refined via four rounds of filtration, and then ASTM procedures were used to evaluate its physicochemical qualities following the protocols by Bint-E-Naser et al. [30], Mass et al. [31], Yüksel and Yüksel [32] and Sutjahjo [27].

2.5. Statistical Analysis

All sample analysis was performed in triplicates and the data were statistically analyzed using the JMP statistical software package (JMP 10, SAS Institute Inc.). The Tukey–Kramer HSD test was carried out to test whether group mean pairs were significantly different at $\alpha = 0.05$.

3. Results and Discussion

3.1. Characterization of Vetiver Grass

Figure 2 shows the percent composition of the extractives, cellulose, hemicellulose, and lignin in VG that were obtained from three different sources. Holocellulose, including cellulose and hemicellulose, was present in much larger amounts (~70%) than extractives (~10%) and lignin (~15%). In terms of those four compositions, there was no significant difference ($p < 0.05$) among the VG from different sources, indicating that the lignocellulosic composition of VG may not be significantly affected by the accumulation of antibiotics or lead during phytoextraction processes.

Restiawaty and Dewi reported that VG shoots had high contents of hemicellulose (34.55% *w*/*w*), cellulose (31.39% *w*/*w*), and lignin (17.58% *w*/*w*) [26], which is similar to the current study. By comparing VG composition reported previously (Table 1), we found that the contents of cellulose, hemicellulose, and lignin in the VG in this study were similar to the previous studies. The overall composition was around the mean of the ranges for individual components—cellulose (30–38.51%), hemicellulose (31.5–42.61%), and lignin (3.67–17.58%) (Table 1). When VG in this study was compared to other C4 grasses, it showed higher percentages of cellulose, hemicellulose, and lignin than those in sorghum. The cellulose content of VG was similar to switchgrass and miscanthus, and lower than wheat straw. Both hemicellulose and lignin contents in VG were higher than those in sorghum, miscanthus, and wheat straw (Table 1), suggesting that VG (regardless of the source) can be employed for bioenergy production instead of being landfilled to form a circular economy model.

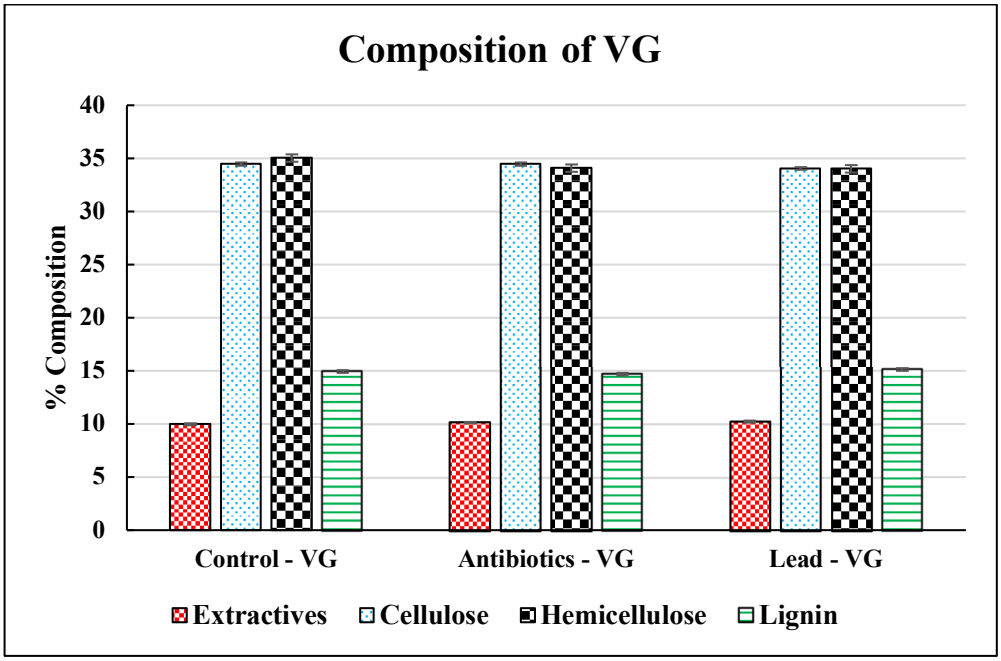

Figure 2. Lignocellulosic composition of VG from different sources.

Table 1. Comparison of the main composition of VG and other C4 grasses from previous reports.

C4 Grasses	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
Vetiver	34.48	35.07	14.34	This study
Vetiver	34.49	34.12	14.69	[20]
Vetiver	34.06	34.05	15.12	[21]
Vetiver	31.39	34.55	17.58	[26]
Vetiver	32.6	31.5	17.3	[10]
Vetiver	31.85−38.51	37.87–42.61	3.67–5.06	[11]
Vetiver	30–35	40	10	[33]
Wheat straw	49	34	6.5	[34]
Sorghum	26.3	20	7	[35]
Switchgrass	29.5–37.8	21.5–27.4	13.9–21.1	[36]
Miscanthus	32.71	34.86	8.9	[37]

3.2. Sugar Release from Vetiver Grass

Hemicellulose produces several pentoses and hexoses, and cellulose hydrolyzes to provide glucose if properly catalyzed [38]. Hemicellulase and cellulase are the two enzymes that could break down the bonds in hemicellulose and cellulose, respectively. Since high concentrations of hemicellulose (~35%, *w*/*w*) and cellulose (~35%, *w*/*w*) were present in VG from all three sources (Figure 2), sugars were produced as expected. The water-soluble fractions of the extractable monosaccharides (i.e., galactose, glucose, arabinose, and xylose) are shown in Figure 3. For all VG types, xylose was the dominating monosaccharide (44.21–47.84%), followed by arabinose (15.41–16.34%), glucose (11.08–13.56%), and galactose (6.51–7.88%). The high sugar production was probably due to the two-stage acid–alkali pretreatment, which produced higher sugar contents than the single-stage acid or alkali pretreatment [39]. The fine particle size (~0.2 mm) of the milled VG biomass utilized in this study enhanced the surface area of biomass and aided in the breakdown of hemicelluloses. The finding from sugar production indicated that hemicelluloses in VG

were dominated by arabinoxylan, a commonly found sugar in the cell walls of plants [40]. Glucose showed up in significant amounts (>10%), probably as glucan, while galactose may represent end-group residues in the samples' side chains [41].

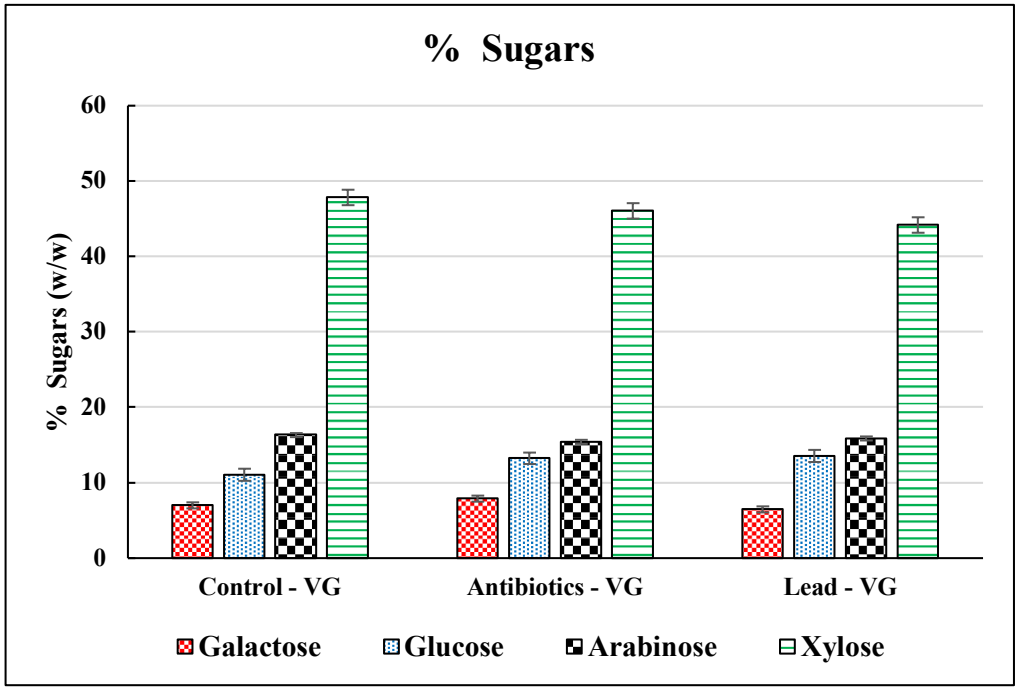

Figure 3. Reducing sugars in different sources of VG.

3.3. Bioethanol Production from Vetiver Grass

As seen in Figure 4, the time profile of the SSF exhibits a growth pattern until 72 h of fermentation, and there is a drop in the concentration of bioethanol past that up to the end of the experiment at 104 h. All three sets of VG had similar concentrations, most of the time points barring the 48 h mark. The increase in bioethanol concentration can also be attributed to the potential drop in the sugar concentrations. As suggested by Das et al. [42], there is an inverse relationship between the bioethanol and sugar content of the lignocellulosic biomass. A substantial decrease in the bioethanol concentration after 72 h was observed owing to the accumulation of reducing sugars in the fermentation media.

The findings of this study show that fresh VG shoots produced the highest bioethanol concentration (279.76 mg/g), followed by the spent VG used for lead phytoextraction (278.57 mg/g) and antibiotics phytoextraction (273.90 mg/g). This corresponded well with the holocellulose contents with the highest in fresh VG shoots (69.55%, Figure 2). Although the bioethanol production rates for the VG used for lead and antibiotic phytoextraction were slightly lower, they were not significantly different from those for fresh VG, indicating that the presence of lead or antibiotics in the biomass would not significantly affect the bioethanol production process. In this study, the average bioethanol yields from VG were higher than the bioethanol yield from dwarf Napier grass (121 mg/g) but lower than that from Kans grass (460 mg/g) (Table 2). Although *S. cerevisiae* was the common microbial species in all these cases, the higher yield in Kans grass could be associated with the independent process of saccharification and acid pretreatment. It is also observed that *S. cerevisiae* is not the highest-yielding fermentation microorganism, and better yields are observed when used in conjunction, as seen in the results of Eliana et al. [43] and Xiang et al. [44]. Genetically modified versions of *S. cerevisiae* have also proven effective in similar operating conditions [44,45].

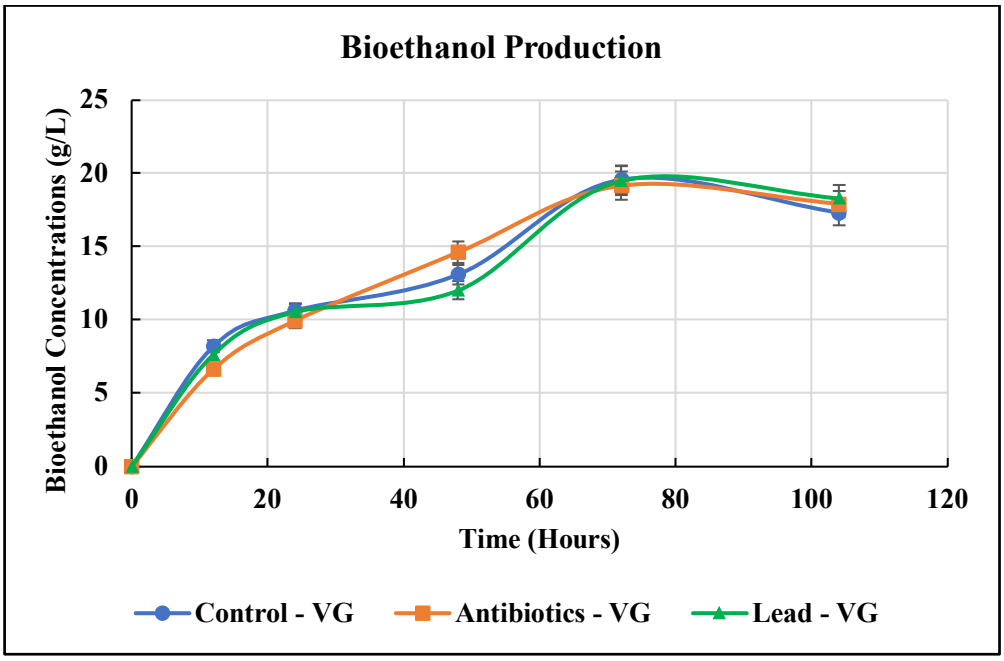

Figure 4. Bioethanol production from different sources of VG.

Table 2. Comparison of bioethanol yield of VG and other C4 grasses previously reported.

C4 Grasses	Microorganisms	Mechanisms	Bioethanol Yields	References
Vetiver (fresh)	*S. cerevisiae*	SSF	279.76 mg/g or 19.58 g/L	This study
Vetiver (antibiotics contamination)	*S. cerevisiae*	SSF	273.90 mg/g or 19.17 g/L	This study
Vetiver (lead contamination)	*S. cerevisiae*	SSF	278.57 mg/g or 19.50 g/L	This study
Dwarf Napier grass (*Schumach*)	*S. cerevisiae* NBRC 2044	SSF	121 mg/g	[46]
Kans grass (*Saccharum spontaneum*)	*S. cerevisiae*	SAF	460 mg/g	[47]
Switch grass (*Panicum virgatum*)	*S. cerevisiae* 424 A (LNH-ST)	SSCF	32.1 g/L	[45]
Elephant grass (*Pennisetun purpureum*)	*Aspergillus niger and S. cerevisiae*	SSF	23.4 g/L	[43]
Switchgrass (*Panicum virgatum*)	*Kluyveromyces marxianus IMB3*	SSF	22.5 g/L	[48]
Mission grass (*Pennisetum polystachion*)	*S. cerevisiae* TISTR 5596	SHF	16 g/L	[49]
Thatch grass (*Hyparrhenia rufa*)	*Zymomonas mobilis*	SSF	8.8 g/L	[42]

Table 2. *Cont.*

C4 Grasses	Microorganisms	Mechanisms	Bioethanol Yields	References
Sea grass (*Cymodocea serrulata*)	*S. cerevisiae*	SSF	0.047 mL/g	[50]
Cocksfoot grass (*Dactylis glomerata*)	*Pichia stipitis CBS 6054*	SSF	158 mL/kg	[51]
Giant miscanthus (*Miscanthus giganteus*)	*Scheffersomyces* (*Pichia*) *stipitis* CBS 6054	SSF	12.1 g/L	[52]
Rice grass (*Spartina* spp.)	*Trichoderma reesei* SEMCC 3.217 and *S. cerevisiae* SEMCC 2.157	SSF	28.1 g/L	[44]

SSF = Simultaneous Saccharification and Fermentation; SSCF = Simultaneous Saccharification and Co-Fermentation; SAF = Saccharification; SHF = Separate Hydrolysis and Fermentation.

The yield of bioethanol from VG was typically higher than that from microalgae, a well-recognized biomass source for "third-generation" bioethanol production. Bibi et al. [53] reported bioethanol production of 14.9 g/L using freshwater green microalgae *Closteriopsis acicularis*. Bioethanol production was reported to be 11.7 g/L for a carbohydrate-rich microalgae *Chlorella vulgaris* FSP-E [54]; a lower yield of bioethanol (3.83 g/L) was reported by Harun et al. [55].

The values of lignin content of the different grasses directly correlate with the bioethanol yield. The various pretreatment methods are responsible for breaking through the lignin, accessing the holocellulosic material, and converting it to sugars and bioethanol. As inferred from Figure 4 earlier, if fewer sugars are accumulated at the end of the process, it would favor bioethanol production. Easier digestion of VG is made possible by the increase in the surface area resulting from milling the dried shoots. In addition, a bigger mesh size would result in less sugar being produced from the breakdown of carbohydrates.

3.4. Quality Analysis of Bioethanol

Results indicated that there was no significant difference among those three sources of VG in terms of bioethanol content, density, freezing points, and all other tested parameters. Because the resulting bioethanol had no lead or antibiotics, the spent VG shoot biomass after phytoextraction applications can also be used for high-quality bioethanol generation. The values of all parameters, except the freezing point, of the bioethanol from VG were comparable to the standard bioethanol [32,56]. The ethanol contents of VG bioethanol ranged from 98.83 to 98.89%, a slightly higher value originating from the fresh VG. Although these values were slightly lower than the standard values, they were higher than those reported in the literature. Specifically, with a 4% HNO_3 pretreatment and distillation, the bioethanol contents generated from *Miscanthus sacchariflorus* (a C4 perennial grass) ranged from 89.9 to 92.5% [57]. Azeke et al. [58] reported an even lower range from 44 to 72.6% using elephant grass (*Pennisetun purpureum*) as the feedstock to produce bioethanol. The higher freezing point (i.e., −94 °C) of VG bioethanol than the standard bioethanol (i.e., −114 °C) could be induced by the impurities in the VG bioethanol. Some commonly identified impurities, such as ethyl acetate and crotonaldehyde [57], present higher freezing points (−84 and −76 °C, respectively), which increases the overall freezing point of the bioethanol. The existence of impurities also corresponds to the slightly lower bioethanol contents from VG than the standard bioethanol (Table 3). The bioethanol content in the purified form also correlates to the concentration in the fermentation media, so a better choice of fermentation media may lead to a higher yield and of higher quality.

Table 3. Comparison of bioethanol quality of VG with standards.

Parameter	ASTM Test Methods	Control—VG	Lead—VG	Antibiotics—VG	Standard
Ethanol Content (%)	ASTM D 5501	98.89	98.85	98.83	99–100
Density at 25°C (g/mL)	ASTM D 4052	0.766	0.784	0.774	0.79
Freezing Point (°C)	ASTM D 2386	−94	−94	−94	−114
Boiling Point (°C)	ASTM D 5399-09	78.3	78.4	78.4	78
Flash Point (°C)	ASTM D 93	12.8	12.7	12.7	13
API gravity (°)	ASTM D 4052	52.3	51.9	53.1	52
Calorific Value (MJ/kg)	ASTM D 2014-96	31.26	30.68	33.09	
Heat of vaporization (kJ/kg K)	ASTM E 2071	278	282	279	289
Reid vapor pressure (kPa)	ASTM D 323-99a	12.67	11.96	13.24	14.25
Viscosity (cSt)	ASTM D 88-94	1.01	1.01	1.04	1.03
Cu strip corrosion at 50 °C	ASTM D 130-04	1a	1a	1a	1a
ASTM distillation IBP (°C)	ASTM D 86-04b	80.5	80.6	81	81
Sulfur content (wt%)	ASTM D 3177-89	0.03	0.03	0.03	0.03
Water content (%)	ASTM D 95-70	1.11	1.15	1.17	0–1
ASTM color	ASTM D 1500-03	None	None	None	None
Research Octane Number	ASTM D 2699	107	108	108	108

The density of bioethanol is much lower than water's, while the American Petroleum Institute gravity (API gravity) values for all three feedstocks were in the range of 51.9–53.1, which is much higher than the value of water, indicating that the fluid can be easily transported through barrels and pipelines [30]. Viscosity is the measure of resistance to flow, and the viscosity of these VG is observed to be twice that of gasoline-based fuels. All three samples of VG gave a slight tarnish (1a) to the copper strip and were colorless overall. The Research Octane number meets the standard value of 108 and suggests that this bioethanol can be blended with traditional fluid and used as E10 fuel.

3.5. Future Perspectives

Due to the rising demand for bioethanol in the world market, it is critical to provide a consistent supply of inexpensive and plentiful feedstocks for generating high-quality bioethanol at competitive prices. By investigating the simultaneous saccharification and fermentation of acid–alkali pretreated vetiver shoots by *Saccharomyces cerevisiae*, this study demonstrated that the spent shoot biomass of VG involved in contaminant phytoextraction processes that are typically unutilized or landfilled can be used to produce bioethanol at similar production rates and quality as fresh, unspent VG biomass, which will significantly reduce the cost of bioethanol production. The combination of environmental remediation and bioenergy production can be further explored towards developing a true circular economy model. Different pretreatment methods and different microbial strains for better yields can be further investigated in the future. Strategies to reduce the overall cost of bioethanol production should be explored so it would be competitive with the conventional fuel market.

4. Conclusions

This study explored the generation of bioethanol by vetiver grass from three sources: VG grown in clean soil, VG used for phytoextraction of antibiotics from a constructed

wetland setup, and VG used for phytoextraction of lead from contaminated soils. For all three sources, the lignocellulosic compositions were similar: cellulose, 30–38.51%; hemicellulose, 31.5–42.61%; lignin, 3.67–17.58%; xylose was the dominating monosaccharide (44.21–47.84%). Bioethanol production (142–151 mg/g) was comparable with or superior to other C4 grasses. Their high quality makes them an excellent candidate for blending with fuels. Thus, VG, both fresh and spent, could be a viable candidate for second-generation biofuel feedstock. Future research may further investigate the feasibility of this novel circular economy model.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/en15249597/s1, Figure S1. A representative HPLC chromatograph showing the monosaccharide and bioethanol peaks.

Author Contributions: S.N.: Methodology, Data collection and analysis, Data curation, Visualization, Writing—original draft. D.S.: Conceptualization, Supervision, Funding acquisition, Project management, Writing—review & editing. R.D.: Conceptualization, Writing—review & editing. Z.Z.: Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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