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Improved production of ethanol using bagasse from different sorghum cultivars

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ABSTRACT

For improved production of ethanol from whole sorghum residues, physico-chemical compositions and fermentation characteristics of the substrates are important factors to consider. In the present study, Nigerian sorghum cultivars SSV2, KSV8 and KSV3 were grown under rain-fed conditions without chemical fertilization in Kano state, Nigeria. On harvest, the whole sorghum residues (bagasse) comprising crushed stalks, leaves, panicles and peduncles were collected for further processing. Bagasse samples, which had different macromolecular composition and carbohydrate pasting properties, were pre-treated with dilute sulphuric acid at 75 °C followed by enzymatic hydrolysis and sequential detoxification by $Ca(OH)_2$ over-liming and charcoal filtration. Hydrolysate samples were subsequently fermented with the yeasts, *Saccharomyces cerevisiae* and *Pachysolen tannophilus*. Sugar consumption, carbon dioxide evolution and ethanol production were shown to vary depending on the sorghum cultivar type. While KSV3 yielded most favourable biomass of 37 t ha^{-1} (dry basis), bagasse from cultivar SSV2 yielded the most favourable level of sugars (69 g/100 g) after enzymatic hydrolysis, and also consistently exhibited improved fermentation performance. Detoxification of pre-treated sorghum bagasse to remove potential yeast inhibitors resulted in improvement in ethanol yield, with 23 g L^{-1} ethanol (representing 72% of theoretical yield) being achieved from SSV2 bagasse following fermentation with *P. tannophilus* without exogenous nutrient supplementation. Our findings reveal that the choice of sorghum cultivar is important when converting bagasse to ethanol, and further that pretreatment with dilute acid at moderate temperature followed by detoxification improves fermentation kinetics and ethanol yield.

1. Introduction

Plant biomass is the conventional sugar source for bioconversion to ethanol by yeast [1]. Stalk juice from sugarcane, starch from grains/tubers and lignocellulose from crop residues represent valuable fermentable sugar sources for bioethanol destined for use as a liquid transportation fuel [2,3].

Sorghum is a high biomass yielding cereal which is a water efficient crop that can be grown in 2–3 crop cycles per annum [4]. Typical lignocellulose residues from sorghum harvest

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comprise crushed stalks (after juice extraction), panicles, peduncles and leaves. The sorghum lignocellulosic biomass usually comprises 25–27% hemicellulose, 34–44% cellulose and 18–21% lignin [5,6]. Cellulose and hemicellulose are polysaccharide polymers intertwined by tough lignin fibre [7]. Lignin acts as a barrier to efficient enzymatic hydrolysis of the cellulose and hemicellulose polysaccharides [8]. Previous studies [9–14] have investigated various pre-treatment methods designed to facilitate enzymatic hydrolysis of sorghum cellulose and hemicellulose polymers to fermentable sugars [6,15,16]. For example, sulphuric acidic pre-treatment appears economically attractive as a low cost pre-treatment option for commercial scale cellulosic bioethanol production [5,17]. However, the method typically requires high temperatures and/or extreme pH levels for effective degradation of lignin [15,18]. Consequently, a range of lignocellulose degradation by-products are generated including phenolic compounds from lignin and acetic acid derived from deacetylation of hemicellulose xylose side chains. In addition, formic acid is generated from the degradation of furfural or 5-hydroxymethyl furfural [19]. The individual or synergistic effects of these compounds on yeast include extended fermentation lag time and inefficient yeast metabolism resulting in reduced ethanol yield [19,20]. Dilute acid pre-treatment has the benefit of being less corrosive to handle and moderate hydrolysis temperatures will minimise cost of process energy requirement, in addition to preserving the substrate's nitrogenous content.

In this study, we investigated bioconversion of residues from different Nigerian sorghum cultivars for bioethanol production. SSV2, KSV8 and KSV3 are relatively high grain yielding sorghum cultivars that have similar numbers of crop cycles per year. Previous work has investigated bioconversion of crushed stalks and/or leaves of sorghum to ethanol under various pre-treatments and fermentation conditions [21,22]. However, the current study focused on the fermentation characteristics of whole sorghum residue (bagasse) after dilute acid pre-treatment at moderate temperature and enzymatic hydrolysis. We also investigated the effects of detoxifying the resultant bagasse hydrolysates on the fermentation performance with the yeast species *Saccharomyces cerevisiae* and *Pachysolen tannophilus*.

2. Materials and methods

2.1. Sorghum crop cultivation and harvest

SSV2, KSV8 and KSV3 sorghum cultivars were cultivated in Kano (Nigeria) under rain-fed conditions and with only cow dung application as a fertilizer. For maximum extractible stalk juice yield, crops were harvested before grains reached physiological maturation (i.e. when grains were at soft-dough stage). Thus, SSV2 cultivar was harvested 11 weeks after the planting date, when the grains were observed to have reached soft-dough maturation. However, KSV8 and KSV3 cultivars were harvested 16 weeks after planting date, because that was when their grains reached soft-dough maturation. The fresh bagasse (comprising crushed stalks, leaves, peduncles and panicles) was sun-dried for 2 days followed by oven drying at 60 °C for 72 h. The dried samples were hammer milled and sieved through 4 mm screen (Retsch, Germany). Moisture and total lignin contents of samples were determined according to National Renewable Energy Laboratory standard analytical procedure [23]. Proteins were determined by adding 2 g bagasse (dry wt.) into conical flasks containing 2 M NaOH solution (50 mL). The mixtures were stirred at room temperature for 2 min followed by incubation in a rotary shaker at 120 rpm and 60 °C for 2 h. The final mixtures were centrifuged at 3800 rpm for 10 min. The supernatant (containing solubilised proteins) was filtered, diluted (1:10) and 1 mL of the solution transferred into 2 mL cuvettes. The protein concentrations were determined using Bradford™ reagent (Sigma–Aldrich, UK) according to manufacturer's standard protocol. Bagasse samples total starch content was determined using K-TSTA total starch kits (Megazymes®, Northern Ireland) according to manufacturer's standard procedure. Furthermore, the bagasse samples pasting properties were determined courtesy Scotch Whisky Research Institute Edinburgh (SWRI). A Rapid Visco-Analyser equipment (Newport Scientific, Australia) was employed for the analysis.

Hammer milled sample of KSV8 bagasse (2.91 g) was added into canister containing distilled water (25.09 g). The suspension was homogenised using the canister paddle [24]. The paddle was placed into the canister and then inserted into the Rapid Visco-Analyser for analysis. The typical RVA cycle profile is summarised in Table 1, while Fig. 1 shows the typical pasting profile for un-malted cereals. Important features of the RVA pasting profile shown in Fig. 1 include the peak viscosity which indicates the water binding capacity of the mixture being analysed and it correlates with final product quality. It is also indicative of the viscous load to be encountered by a mixing cooker. Also, at the hold temperature (95 °C), the ability of a sample to withstand the heating and shear stress of the RVA run is an important factor for many processes. It has been shown that RVA peak and final viscosities are highly correlated to ethanol yield [24,25]. The implications of these are discussed later in this paper.

2.2. Bagasse pre-treatment and saccharification

Bagasse (20 g dry wt.) was added into a conical flask containing 2% v/v dilute H_2SO_4 acid (80 mL). The mixture was incubated at 75 °C for 3 h with 150 rpm orbital shaking. This was followed by the addition of distilled water (30 mL) to the slurry and afterwards autoclaved at 121 °C for 15 min. Samples were

Table 1 – The Rapid Visco Analyser (RVA) run cycle profile.

Cycle time profile	Parameter	Value
00:00:00	Temperature	50 °C
00:00:00	Speed	960 rpm
00:00:10	Speed	160 rpm
00:00:30	Temperature	50 °C
00:04:30	Temperature	98 °C
00:09:00	Temperature	98 °C
00:11:00	Temperature	65 °C
00:15:00	Temperature	65 °C

Note: Idle temp. = 50 °C, total cycle time = 15 min, readings interval = 4 s.

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