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Potential use of feedlot cattle manure for bioethanol production

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HIGHLIGHTS

- Anaerobic digestion of cattle manure reduces glucan and xylan content in fibers.
- AD fibres are poor substrates for cellulase saccharification (12% sugars/ODM).
- Pretreatment of feedlot cattle manure with dilute sulphuric acid was investigated.
- Enzymatic saccharification of pretreated manures yield up to 79% sugars/ODM.
- Industrial yeast rapidly and efficiently fermented recovered glucose.

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ABSTRACT

This paper reports on processing options for the conversion of feedlot cattle manures into composite sugars for ethanol fermentation. Small-scale anaerobic digestion trials revealed that the process significantly reduces the content of glucan and xylan (ca. 70%) without effecting lignin. Moreover, anaerobic digestate (AD) fibres were poor substrates for cellulase (Cellic® CTec 2) saccharification, generating a maximum combined sugar yield of ca. 12% per original dry weight. Dilute acid pretreatment and enzyme saccharification of raw manures significantly improved total sugar recoveries, totalling 264 mg/g (79% theoretical). This was attained when manures were pretreated with 2.5% H_2SO_4 for 90 min at 121 °C and saccharified with 50 FPU CTec 2/g glucan. *Saccharomyces cerevisiae* efficiently fermented crude hydrolysates within 6 h, yielding 7.3 g/L ethanol, representing glucose to ethanol conversion rate of 70%. With further developments (i.e., fermentation of xylose), this process could deliver greater yields, reinforcing its potential as a biofuel feedstock.

1. Introduction

Approximately 330 ML (megalitres) of grain-based ethanol was produced in the Australia in 2009 (ABARE, 2010). Although reducing Australia's dependency on foreign oil importation, grain-based ethanol clashes with food/feed markets and does not significantly diminish greenhouse gas (GHG) emissions (de Vries et al., 2010). These shortcomings can be addressed by producing ethanol from low cost lignocellulosic material such as agricultural and forest waste residues. Manure falls into this category owing to its relatively high (up to 50%) fibre content (Chen et al., 2005). Moreover, unlike other lignocellulosic feedstocks, livestock manure is concentrated at or near farms and is thus inexpensive to collect and transport. Intensive livestock production systems in Australia produce more than three million tonnes of manure each year (Tromp, 2012). The feedlot cattle industry accounts for nearly a third of the total, with over 870,000 head producing about 1 million tonnes per annum (ABARE, 2010; Davis et al., 2012). Although there are no reported estimates of Australian dairy cattle manure production at present, it is assumed that the amounts are similar given that the population of dairy cows approaches 1.4 million (ABARE, 2010).

Conventional on farm management practises for feedlot cattle manures primarily consists of stockpiling (dry pad and lagoon) and application to soils, either directly and/or following composting. However, issues associated with microbial/nutrient runoff and contamination of surface and groundwater (Klein et al., 2010; Miller et al., 2011), high nitrogen and phosphorous soil loads, odours and generation of GHG such as methane and nitrous oxide (Davis et al., 2012), diminish their environmental, health and economic appeal. Anaerobic co-digestion of livestock manures

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with agricultural resides is fast becoming an attractive alternative disposal option.

Michigan State University researchers have recently reported the merits of co-digesting swine manure with corn stover residues for biogas production (MacLellan et al., 2013). In fact, the researchers advocate the use of anaerobic digestate (AD) fibre as substrate feedstock for bioethanol fermentation in an integrated bioenergy process. Under these circumstances, 1 kg of dry mixed feed was converted into 152 g of methane and 50 g of ethanol (MacLellan et al., 2013). This concept was formulated on the basis of previous findings which revealed that (i) dairy AD fibre contains higher cellulose content (24%) than its raw manure counterpart (17%), (ii) AD fibre was more amenable to hydrolysis than raw dairy manure, thereby leading to greater monomeric hexose (C6) sugar yields, and (iii) comparable glucose conversion of dairy AD relative to switchgrass and corn stover (71.4%, 70.6% and 66.6% respectively) following pretreatment with sodium hydroxide and enzyme saccharification (Teater et al., 2011; Yue et al., 2010). AD fibre was also reported to contain less pentose (C5) sugars and reduced particle size (Yue et al., 2011), the former impacting on ethanol titres whilst the latter potentially leading to saving in ethanol production. However, given the additional costs associated with anaerobic digester and methane storage construction and the challenging operational demands in maintaining process stability over long retention times (das Neves et al., 2009), the appeal of an integrated biogas and ethanol approach in treating livestock manures may diminish as technological advances in lignocellulosic biofuels are implemented. Moreover, sugar losses (particularly C5) incurred during anaerobic digestion is counterintuitive for commercial and economic success of large-scale lignocellulosic ethanol production, which depends on maximal extraction and fermentation of all sugars.

Thus, the initial aim of this work was to assess and validate anaerobic digestion as a means of producing cellulose enriched material from cattle feedlot manure. Specifically, sugar profiles pre- and post anaerobic digestion using different retentions times and yields following cellulase saccharification were examined. The remainder of the paper reports on the use of dilute acid and cellulase saccharification as pretreatment options for the production of sugars from raw cattle feedlot manure. Previous studies have shown that pentose and glucose sugars can be recovered at satisfactory levels (ca. 96–111% and 40–52%, respectively) from raw dairy manure using dilute acid pretreatment and enzyme hydrolysis (Liao et al., 2004, 2005; Wen et al., 2004). Within this context, the impacts of varying dilute acid concentrations, pretreatment time and cellulase dosage for maximising cellulose hydrolysis of raw cattle feedlot manure are examined detailed. Pentose sugars and potential inhibitory compounds released or generated during pretreatment and hydrolysis are also described, as well as demonstration of the fermentation potential of manure derived sugar hydrolysates for bioethanol production.

2. Methods

2.1. Materials

Feedlot cattle manure used in this investigation was sourced from Rangers Valley Cattle Station, Glen Innes, NSW, Australia. Cattle were fed with a mixture of grain (70% mainly wheat and barley), silage (10%), cotton seed and roughage (10%) and molasses. Manure samples were dried in an oven at 50 °C, ground in a rotary mill (Gelder & Co., NSW, Australia) and sieved to select for a particle size of $\leqslant$1.4 mm (ASTM No. 14 sieve) and $\geqslant$1.0 mm (ASTM No. 18 sieve). Milled material was stored at room temperature in airtight containers and dried in an oven at 50 °C for 24 h prior to use.

All chemicals used were of reagent grade or higher and purchased from Sigma Chemical Co. (St. Louis, MO). Cellulase (Cellic® CTec 2) was kindly supplied by Novozymes (Bagsværd, Denmark).

2.2. Anaerobic digestion

Laboratory scale anaerobic digestions were conducted in 80 mL Schott Duran® bottles with a working volume of 70 mL. Each bottle contained 7 g of dried feedlot cattle manure plus 60 mL of ultrapure water and 10 mL of inoculum. Inoculum was provided by Lismore Sewerage Treatment Plant (STP), NSW, Australia. Bottles with slightly loosen tops were placed in 3.5 L anaerobic jars (Oxoid) at 30 °C. Pressure was maintained at 0.3 bar with periodic release of biogases via out-put Schrader valve. This method of generating anaerobic digestate was selected because of its technical simplicity. Anaerobic digestions proceeded for 15, 20 and 25 days. After specified period, digestates (solids) were filtered by vacuum with glass microfiber (1.2 μm, 90 mm/Filtech) and washed with water until pH 7. The solids were dried overnight (~8–10 h) at 70 °C. Recovered solid residues were stored in airtight containers until further analysis.

Large scale anaerobic digestions were conducted in 30 L Brigalow plastic fermenter drum fitted with a water lock (Brigalow Brewing, Qld Australia). The digester was set up with a working volume of 20 L containing 2 kg of feedlot cattle manure and 1 L of inoculum (from Lismore STP), maintained in a temperature controlled glass house (35 °C) and was intermittently mixed manually (vigorous rotation of the drum). After 45 days, solids were separated by hand, washed with water until neutral pH, air-dried and stored in airtight containers until further analysis.

Biogas samples were qualitatively assessed for presence of methane at the completion of anaerobic digestion. Headspace gas was sampled directly into pre-evacuated 12-mL blue-cap Exetainer® vials with grey silicon septa (Labco, UK). Samples were then analysed through a flame ionisation detector (FID) on an Agilent 7890A gas chromatography with nitrogen as carrier gas and temperature set at 250 °C. Total inlet flow was set at a constant 23 mL/min.

2.3. Pretreatment

A 2^3 factorial design was employed to evaluate the effect of variations in acid strength and time during pretreatment. Milled manure samples at a solid loading of 10% (w/v) were treated in sulphuric acid (H_2SO_4) solution at 0.5%, 1.5% and 2.5% (v/v), for 30, 60 and 90 min. Pretreatments were performed in triplicate at 121 °C using a Labec AA20 autoclave (Labec, Australia). Pretreated material was separated into solid and liquid hydrolysate fractions using a Buchner funnel fitted with fibre glass filter (GF-A, Whatman®). Liquid prehydrolysates were stored at −20 °C and retained for further analysis. Pretreated solids were washed with water until the filtrate registered neutral pH, then air dried and stored at room temperature in air tight containers until required.

2.4. Enzyme saccharifications

Prior to enzyme saccharification, raw cattle feedlot manure and anaerobic digestates were sterilized in autoclave for 30 min at 121 °C. Enzymatic hydrolysis of all solid substrates (loading of 5% w/v) were performed in 50 mM citrate buffer pH 5.0 containing 0.02% sodium azide and incubated for up to 72 h at 50 °C. Cellic® CTec 2 cellulase blend was used for all enzymatic saccharification studies and loads are quoted as FPU (filter paper units)/g dry material (DM) for anaerobic digestate and FPU/g glucan for acid pretreated manures. Controlled isodosing conditions using a fixed cellulase loading are as specified throughout the text. Enzymatic