Biotechnological application of sustainable biogas production through dry anaerobic digestion of Napier grass

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Abstract Napier grass (*Pennisetum purpureum*), represents an interesting substrate for biogas production. The research project evaluated biogas potential production from dry anaerobic digestion of Napier grass using batch experiment. To enhance the biogas production from ensiled Napier grass, thermal and alkaline pre-treatments were performed in batch mode. Alkali hydrolysis of Napier grass was performed prior to batch dry anaerobic digestion at three different mild concentrations of sodium hydroxide (NaOH). The study results confirmed that NaOH pretreated sample produced high yield of biogas than untreated (raw) and hot water pretreated samples. Napier grass was used as the mono-substrate. The biogas composition of carbon dioxide (30.10%), methane (63.50%) and 5 ppm of H₂S was estimated from the biogas. Therefore, fast-growing, high-yielding and organic matter-enriched of Napier grass was promising energy crop for biogas production.

Keywords Biogas · Dry anaerobic digestion · LBR · Napier grass · Pretreatment

Introduction

Biomass feedstocks, such as leaf litter, weeds, crop residues including corn stover and sugarcane bagasse, grass crops, and woody plants and agro-residues, have been

considered as alternative feedstocks to meet rural energy needs in Thailand. Thailand is an agricultural area suitable for growing of many plants, especially annual crops that can be used as an energy crop or raw material of agricultural biogas plant (Bussabong et al. 2013).

Grassland biomass is suitable in numerous ways for producing energy. Using grassland biomass for producing energy, especially biogas production currently, is the most common (Dussadee et al. 2014). There are so many types of grasses that are popularly grown in Thailand. Grass is converted to silage to be used as feedstock for anaerobic digestion (Rösch et al. 2013). Furthermore, grass, due to its high digestible organic matter content, is also an excellent feedstock for anaerobic digestion. Napier grass has high potential dry matter yield (Yang et al. 2004).

Furthermore, grass, due to its high digestible organic matter content, is also an excellent feedstock for anaerobic digestion. Napier grass is one of the most promising grasses available for large production in tropics and sub-tropics (Johnson et al. 1986). Furthermore, Napier grass is the latest energy crop being promoted by the Thai government. The Ministry of Energy's Department of Alternative Energy Development and Efficiency has run projects promoting bio gas production from Napier grass for the purpose of generating electricity, cooking gas, and bio gas for vehicles. However, as lignocellulosic substrates, the methane production of grass straw mainly depends on their complex structure, which limits their biodegradability. In fact, the structure of lignocellulosic materials is mainly composed of cellulose, hemicelluloses and lignin, strongly linked to each other. Cellulose and hemicelluloses are quite easily degraded by anaerobic micro-organisms and can be converted into bio-methane. Moreover, as for complex substrates, the hydrolysis of lignocellulosic substrates is

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considered the rate limiting step during anaerobic digestion (Tong et al. 1990).

The physical structure and chemical composition of lignocellulosic materials can be altered through various methods of pre-treatment, breaking down the linkage between polysaccharides and lignin, thus making cellulose and hemicelluloses more accessible to hydrolytic enzymes (Hendriks and Zeeman 2009). Therefore, pre-treatments could accelerate the hydrolysis process and improve the final methane production.

A wide number of anaerobic digester designs have been used for treating various solid organic substrates including energy crops, e.g. continuously stirred tank reactor systems and plug-flow digesters, sludge bed anaerobic reactor, etc. Currently, leach-bed reactor (LBR) system has been developed mainly to treat the high-solids organic waste and to recover biogas at high rates (Dogan et al. 2008). Jagadabhi et al. (2010) stated LBR systems have been successfully used for treating various feedstocks such as organic fraction of municipal solid waste, vegetable wastes, food wastes, animal manure and energy crops. In an LBR system, solids are subjected to hydrolysis by re-circulating the leachate collected at the bottom of the reactor continuously over the substrate bed. Depending upon the feed characteristics and scale of operation, LBR systems can be operated either in a one-stage or two-stage system. Consequently, the main purpose of this research was to produce biogas yield from Napier grass. Therefore, the objective of this study was to examine (1) the effects of alkali pretreatment (NaOH loading rates of 1, 2 and 3%)

with different reaction times (24, 48 and 72 h), (2) the effects of thermal pretreatment (i.e. 100 °C) with different reaction times (including 0.5, 1, 1.5 and 2 h), and after pretreatment results the suitable method is selected for scale up study and future applications.

Materials and methods

Preparation of grass

Napier grass was obtained from the agriculture farm which was located around Mae Taeng district, Chiang Mai, Thailand. The grass was a first cut (cut at 45 days old mature stage). Napier grass was crushed by machine into small particles. Stored grass was pulverized into small particles (1.0 mm) before use. The grass collecting and silage preparations are shown in (Fig. 1). The experiment was carried out in the Energy Research Center, School of Renewable Energy, Majeo University, Thailand. For all experiments, Napier grass (*Pennisetum purpureum*) was used as a mono-substrate.

Pretreatment

The alkaline and thermal pre-treatments were performed in batch mode. The pre-treatment experiment was carried out in 20 L dry anaerobic batch digestion tanks, so called leach-bed reactor (LBR). Each pretreatment experiment was conducted with 3 kg of grass biomass and 5 L of

Fig. 1 Grass collection and silage preparations
a Cultivation, **b** transportation of grass, **c** grass crushing machine and **d** small particle of grass

Fig. 2 Schematic diagram and experimental set-up of leachate recirculation digester: digester 1 and 2; gas storage tank 3

wastewater. Wastewater was obtained from continuous stirred tank reactor (CSTR).

For alkali pre-treatment, 1, 2 and 3% sodium hydroxide (NaOH) solution was prepared to soak 3 kg of biomass. This reaction mixture was subjected to alkali hydrolysis at different reaction times of 24, 48 and 72 h. For thermal pretreatment, steam or hot water is also effective in hydrolysis of crops and crop by-products; the boiled water 100 °C was used at different reaction times of 30 min to 2 h reaction time (i.e. 0.5, 1, 1.5 and 2 h). Thermal and chemical pretreatments were compared for potential biogas production. In addition, untreated Napier grass biomass experiment was conducted for effective comparison of pretreatment biogas production.

Leachate recirculation digester

After the pretreatment method selection, further scale up study was conducted. A prototype 100 L dry anaerobic batch digester was employed so called LBR system, sometimes called percolating anaerobic or dry anaerobic digester (Cysneiros et al. 2008), and experimental set up is shown in (Fig. 2). Specification of experimental parameters and biogas measurements are listed in Table 1. In this design, LBR was sequentially loaded with grass biomass and mixed with residual digested solids and leachate from an LBR that has completed its digestion cycle.

For all experiments, prepared grass was used as a mono-substrate. Biogas production was achieved through improvements in the fermentation process using Napier

grass and water. Thirty kilograms of grass substrates were introduced in a leachate recirculation digester. The reactor working volume was 60 L.

Theoretical biochemical methane potential (BMP) tests

The methods described below are designed to easily determine the methane productivity of a specific substrate from its elemental composition to obtain reliable results quickly and get an economic advantage. These methods are applied considering that all the organic material is degraded; therefore, a proper adjustment of this value is necessary, using the biodegradability obtained from the experimental BMP tests. The stoichiometric equation based on the atomic composition of the waste material is also used to calculate the theoretical methane composition by taking into account the elements C, O, H and N (Table 2). From the elemental composition of plants can be calculated the amount of methane and carbon dioxide; calculation process is shown in Eq. 1 and 2 (adopted from Pavlostathis and Giraldogomez 1991).

Table 1 Specification of experimental parameters and biogas measurements

Parameter	Equipment or method
Nepiar grass particle size	1.00 mm
Grass substrate	30 kg
Reactor type	Leachate recirculation digester
Digesting system	Dry anaerobic digester
Volume of reactor	100 L
Used volume of reactor	60 L
Methane	ASTM D 1945 and theoretical calculation
Carbon dioxide	ASTM D 1945-03 and theoretical calculation
Hydrogen	ASTM D 1945-03
Hydrogen sulfide	ASTM D 5504-01
Oxygen	ASTM D1945
Sulfur	ASTM D 6667-04
Theoretical biogas production	Adopted from Pavlostathis and Giraldo Gomez (1991)

Table 2 Proximate, ultimate, chemical composition of Napier grass

Property	Biomass
pH ^a	4.85
Proximate analysis (wt.%)	
Moisture ^a	77.74
Ash	3.18
Ultimate analysis (wt.%) ^b	
Carbon (C)	44.19
Hydrogen (H)	6.00
Nitrogen (N)	2.00
Oxygen (O)	43.80
Sulphur (S)	0.06

^a As received at harvest; ^b Dry basis; unit % by weight

Analytical methods

The solid contents, including total solids (TS), volatile solids (VS), total suspended solids (TSS), and volatile suspended solids (VSS) were characterized using the Standard Methods for the Examination of Water and Wastewater (method# 2540) (APHA-AWWA-WEF 2005). Metrohm 774 pH-meter was used in all pH measurements.

A titration method with sulfuric acid was used to determine total alkalinity (TA) at pH 4.3 as CaCO₃ (Jenkins et al. 2004). The total chemical oxygen demand (COD) and soluble COD (sCOD) were determined by standard methods# 5220 (APHA-AWWA-WEF 2005). The nitrogen concentration, as total Kjeldahl nitrogen (TKN), was determined using the Nessler method# 8075 (HACH 2003). Total fat, ash, moisture, fiber contents and volatile fatty acids (VFA) were determined using AOAC official method (AOAC 2012). The composition of biogas (CH₄, CO₂, H₂S, H₂ and O₂) was measured using a biogas analyzer (GFM 416 series, UK).

Results and discussion

Chemical composition of raw substrates

Results of physical and chemical composition of the Napier grass after drying and milling are summarized in (Table 1). Biogas production of a specific crop is affected by the chemical composition of the plant which changes as the plant matures (Gunaseelan 1997), and timing and frequency of harvest are thus critical in order to optimize the biomass yield and feedstock quality. Amon et al. (2007), reporting on a multifaceted crop rotation to increase the yield of methane per hectare, found that the first cut at vegetation stage was selected as the optimum option for harvesting. Napier grass harvested stage (45 days) was reported has high biomass (Butt et al. 1993). Napier grass cultivation had high biogas yield, as reported in literature (Cirne et al. 2007; Demirel et al. 2012). In this study, the particle size of pulverized Napier grass was 1.0 mm.

Table 2 shows the moisture, ash, pH, carbon, hydrogen, nitrogen and oxygen content tested in this study. The grass showed distinct differences in their chemical composition. The moisture, ash, pH, carbon, hydrogen, oxygen and nitrogen contents were in grass, 77.74, 3.18, 4.85, 44.19, 6.00, 43.80 and 2.00%, respectively (Table 2). Consequently Napier grass has plenty of nutrients for biogas production process; grass is suitable to be used as energy crop for biogas production.

Mechanism and theoretical estimation of biogas from Napier grass

The anaerobic fermentation process has achieved growing importance in practice in recent years. Anaerobic fermentation is especially valuable because its end product is methane, a renewable energy source. Biogas is created in a multistage process in which different microorganisms use the energy stored in carbohydrates, fats and proteins for

their metabolism. In order to produce biogas, any organic substrate that is microbiologically accessible can be used (Thiele 2009). Anaerobic digestion is a synergistic process of a consortium of microbes which can be classified along with a series of metabolic pathways (Pavlostathis and Giraldogomez 1991).

Anaerobic degradation of organic matter is a complex series of metabolic interactions among different anaerobic microorganisms and is classified into four main stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The first step involves the enzyme-mediated transformation of insoluble organic material and higher molecular mass compounds such as lipids, polysaccharides, proteins, fats, nucleic acids, etc. into soluble organic materials. This step is called the hydrolysis and is carried out by strict anaerobes such as *Bactericides*, *Clostridia* and facultative bacteria such as *Streptococci*, etc. In the second step, acidogenesis, another group of microorganisms ferments the break-down products to acetic acid, hydrogen, carbon dioxide and other lower weight simple volatile organic acids like propionic acid and butyric acid which are in turn converted to acetic acid. In the third step, these acetic acid, hydrogen and carbon dioxide are converted into a mixture of methane and carbon dioxide by the methanogenic bacteria. The final stage is called as methanogenesis. Acetate is converted into methane, also carbon dioxide converts organic matter into methane. According to Pavlostathis and Giraldogomez (1991), the elemental composition of plants can be used to calculate the amount of methane and carbon dioxide; calculation process is shown in Eqs. 1 and 2. Calculated from Napier grass are composed of methane had high percentage which means that organic matter in the grass. It was decomposed and converted into methane by 48.38% and biogas was 190.27 L/kg VS, respectively, as shown in (Table 3), which was consistent with available literature; biomass are composed of higher methane than carbon dioxide.

Effect of pretreatments on solubilization and biogas yield

Beside mechanical pretreatment by chopping, milling or shredding various other forms of physical and chemical or biological pre-treatment are well known (Hendriks and Zeeman 2009), and leading techniques not only to particle size reduction, but also helping in anaerobic digestion of solid materials such as energy crops and crop residues is hydrolysis of complex polymeric substances (Mata-Alvarez et al. 2000). Since, Napier grass containing cellulose, hemi-cellulose and lignin comprise up to 75% of dry matter of grass (van der Weijde et al. 2013). Grass was lignocellulose rich material and lignocellulose is in most cases

extremely resistant to anaerobic digestion (de Araújo et al. 2013). Napier grass contains 30.40% lignin, 36.34% cellulose, and 34.12% hemicelluloses (de Araújo et al. 2013). Hence, pretreatment can enhance the bio-digestibility of the wastes for biogas production and increase accessibility of the enzymes to the materials. It results in enrichment of the difficult biodegradable materials and improves the yield of biogas from the biomass. Therefore, pretreatment process was needed to be able to get a high biogas yield.

Two different pretreatment methods for Napier grass were investigated in order to determine how each method affects the composition of the grass and the digestibility of the grass in biogas production. In addition, pretreatment methods also compared with untreated grass (i.e. control); the effect of pre-treatment characteristics and biogas yield. The pre-treatments were performed in batch mode with TS, VS, COD and pH, and all pre-treatment conditions are presented in the Table 3. The biogas yield was calculated from VS and composition of biogas was measured using a biogas analyzer (GFM 416 series, UK).

The study results clearly exhibited that NaOH pretreated sample produced high yield of biogas than untreated (raw) and hot water pretreated samples. Furthermore, the results show that alkali pre-treatment resulted higher at using 1% of NaOH reacted with 24 h biogas result was 179.38 L/kg VS. Also 1% of NaOH and 24 h reaction time could be better for cost and time saving. The VS/TS ratio is very important for biogas production. The range of VS/TS was 0.6–0.8 was produced higher amount methane (Lübken et al. 2010; Deublein and Steinhauser 2008). This study results also demonstrated similar range with literature. Also, alkali pretreatment opened the bonds between cellulose, hemicelluloses and lignin. Pretreatment most likely dissolved a portion of the lignin and hemicelluloses components, producing a soluble substance and allowing more access for the hydrolysis process. The substrate porosity for pretreated material increases after alkali pretreatment, which could improve contact between microorganism, and thus facilitate the hydrolysis which is the first phase of biogas fermentation. Ward et al. (2008) declared that a higher concentration of alkali might result in a decline in biogas yield. Extra induction of sodium (Na^+) would inhibit the activity of anaerobic microbes and thus cause a decline in biogas yield. Na^+ could be increased with increasing of NaOH concentration; the reason may be that 2 and 3% of NaOH inhibited the microbial function in the system and 1% of NaOH concentration on pretreatment process was suitable for biogas production of Napier grass as monosubstrate. Consequently, this study has selected 1% of NaOH with 24 h reaction time for further scale up study.

Table 3 The effect of pretreatment and biogas yield

Pre-treatment	Parameters					
	TS (mg/L)	VS (mg/L)	VS/TS	COD (mg/L)	pH	Biogas yield (L/kg VS)
No treatment	121,900	81,675	0.67	15,608	6.40	164.06
NaOH 1% 24 h	99,867	81,700	0.82	21,087	6.73	179.38
NaOH 1% 48 h	107,467	87,200	0.81	19,520	6.90	175.43
NaOH 1% 72 h	96,133	85,500	0.89	20,304	6.73	176.61
NaOH 2% 24 h	144,333	102,500	0.71	25,804	7.35	138.54
NaOH 2% 48 h	122,133	96,800	0.79	24,236	7.35	147.02
NaOH 2% 72 h	142,867	106,400	0.74	25,785	7.27	145.51
NaOH 3% 24 h	177,467	124,900	0.70	30,487	7.90	111.29
NaOH 3% 48 h	157,667	105,700	0.67	28,136	7.93	138.13
NaOH 3% 72 h	177,166	114,100	0.64	26,593	7.88	133.22
Boiled 100 °C 0.5 h	83,350	68,350	0.82	13,670	6.67	150.69
Boiled 100 °C 1 h	79,983	69,917	0.87	13,983	6.67	155.91
Boiled 100 °C 1.5 h	80,017	70,000	0.87	14,000	6.50	155.71
Boiled 100 °C 2 h	83,317	68,317	0.82	13,663	6.60	152.23

Anaerobic digestion process of alkalinity, pH, VFA and COD in the fermenter

The temporal variation of alkalinity, pH, VFA and COD in the fermenter results were presented in (Fig. 3). The experiments were carrying out 90 days retention time operated of the dry anaerobic digestion in the LBR system. The pressure during operation was 10 cm H₂O. The experiments are being carried out under mesophilic conditions. The mesophilic temperature ranged from 32 to 42 °C. The performance of the biogas digester in terms of alkalinity was shown in (Fig. 3a). Alkalinity is a measure of the amount of basic compounds in the reactor. The higher the alkalinity, the higher the buffer capacity and the more possible it is to achieve stable pH. The Alkalinity in digester operation ranged between 1350 and 2760 mgL⁻¹, with an average value of 2246 mgL⁻¹. Alkalinity had a very high variation throughout operation time. However, this amount of buffering capacity should enough for preventing pH drop due to increasing amount of VFA in the system (Metcalf and Eddy 2004). Total alkalinity (TA) measured by titration of the sample to pH 4.3 was proved to be insensitive since the combination of VFA and bicarbonate results in a stable TA level (Björnsson et al. 2001). In the form of CaCO₃, the ability to resist changes in pH can be confirmed by the ratio of volatile fatty acids in alkaline conditions.

Figure 3b showed that pH conditions in the range of 5.20–7.40 with an average value of 6.13. pH is an important factor for keeping functional anaerobic digestion. The

accumulation of intermediate acids leads to pH changes during fermentation. The microorganisms in the anaerobic digester are sensitive to the pH and have different pH optima. The methanogens have a pH optima of between 6.5 and 8.0, while the acetogens work with a pH between 5.0 and 8.5.

Volatile fatty acids are the intermediary compounds produced during anaerobic conversion of organic materials (Møller et al. 2004). In the reactor, concentration of volatile fatty acids ranged between 510 and 768 mgL⁻¹, with an average value of 639 mgL⁻¹. VFA was lower in the reactor because VFA was produced from fresh substrate in the batch reactor. As shown in (Fig. 3c), the start of the experiment was higher. After a period of time, the system goes into steady state levels of volatile fatty acids are low and relatively constant. However, the final amount of volatile fatty acids in the biogas tank was in the proper range in this study, because cannot exceed 2000 mgL⁻¹ generally. And microbes were generated methane in the reactor can operate in a balanced and effective.

Figure 3d showed that COD conditions in the range of 7636–30,688 with an average value of 14,881 mg/L. The results demonstrated that increased performance of anaerobic digestion was attributed to an increased soluble substrate which increased anaerobic biodegradability concurrently, and significantly improved COD removal efficiency and biogas production in dry fermentation digester system. The decomposition of organic matter transformed into organic acids process was usually express with COD. The COD decreased until the end of the

Fig. 3 a–d Experiment results of alkalinity, pH, VFA and COD

experiment and COD removal efficiency was 75.12%. The potential for COD reduction was estimated and the associated methane production.

Experimental evaluation of biogas from Napier grass

Daily total biogas production of Napier grass as mono-substrate in the reactor was given in (Fig. 4a). Energy crops and crop residues can be digested either alone or in co-digestion with other materials, employing either wet or dry processes. And after 85 days the rate of biogas production gradually declined. The biogas accumulated throughout the research period, 20.62 L/kg fresh grass or 190.25 L/kg VS is the average total amount of gas 6.87 L/day (=6,870 ml/day), as shown in Fig. 4b. Bussabong et al. (2013) stated the performance of the biogas production of ruzi grass (*Brachiaria ruziziensis*) as the mono-substrate had value of 244 ml/day with CSTR. This study results were demonstrated that biogas yield was 28 times higher than ruzi grass which was performed in CSTR. Since, batch reactors are often leach bed processes where solids are hydrolyzed by circulating leachate over a bed of organic matter. Recirculation of leachate stimulates the overall degradation owing to more efficient dispersion of inoculums, nutrients and degradation products (Lehtomäki et al. 2008). Accordingly, that is main reason this study result was confirmed much higher than CSTR.

Biogas composition results are presented in (Fig. 4b). Biogas composition from experimental measurements starting from 39 days of the experiment showed that the initial composition of the gas as possible. In this stage, methanogenic bacteria having important role to produce methane gas. Theoretical and measured composition of methane and biogas production was presented in Table 4. The biogas composition of carbon dioxide (30.10%), methane (63.50%) and 5 ppm of hydrogen sulfide was estimated from the biogas. Napier grass methane yield was estimated at 48.38% by theoretical calculation of Pavlostathis and Giraldo Gomez (1991), and this study experimental result verified that methane yield was much higher (i.e. 63.50%) than that arrived using theoretical estimation. Because pretreatments are helpful for breaking down the bonds between cellulose, hemicelluloses and lignin, these processes help to increase microbial digestion activity in the fermenter.

H₂S is commonly found in natural gas, biogas, and LPG. It is corrosive, toxic, and odorous; it can significantly damage mechanical and electrical equipment used for process control, energy generation, and heat recovery. Moreover, the combustion of H₂S results in the release of sulfur dioxide, which is a problematic environmental gas emission (Amirfakhri et al. 2006). The usages of biogas with H₂S standard such as steam and fired boilers (<1000 ppmv), fuel engines (<500 ppmv), motor fuels (i.e. CNG and CBG <23 ppmv) and pipe line gas (i.e. gas grid <1 ppmv)

Fig. 4 a Biogas yield (L/day VS) and cumulative biogas yield (L/kg VS), **b** biogas compositions produced from Napier grass

Table 4 Composition of Methane and biogas production from Napier grass

Elements	Napier grass								
	Calculated value						Measured value		
	Percent	Mol ^a	CH ₄ ^b (%)	CO ₂ (%)	NH ₃ (%)	Biogas ^c (L/kg VS)	CH ₄ (%)	CO ₂ (%)	Biogas (L/kg VS)
C	44.19	3.68	48.38	47.72	3.90	190.27	63.50	30.10	190.25
H	6.00	5.22							
O	43.80	2.45							
N	2.00	0.031							

^a Weight is based on kg dry Napier grass

^b Mol g/Mw

^c Volume content of CH₄ calculated from Eq. 1

(Amirfakhri et al. 2006; Maizonnasse et al. 2013). This study verified H₂S was extremely lower (i.e.5 ppm). Therefore, the study approach is certainly applicable for CBG (Compressed Biomethane Gas) engine. Consequently, this study investigated the potential of Napier grass biomass as a feedstock for biogas production. This suggested that it is possible to achieve stable operation using Napier grass, as a substrate for biogas production in pilot or large scale biogas plant in the future. It was concluded that Napier grass as energy crop can be an alternative energy resource.

Conclusions

This study investigated the potential of Napier grass biomass as a feedstock for biogas production. Napier grass is a fast-growing, high-yielding crop and highly nutritious especially, so it is suitable for use as energy crop for biogas production. These results indicated that Napier grass contains rich organic substances and these substances are suitable to use in the anaerobic fermentation process to be used to sustain microbial life and transform nutrients into biogas. Dry anaerobic digestion is a biological method used

to convert organic substances into a stable product for land application without adverse environmental effects. High methane content of (i.e. 63.50%) was found in total biogas from dry anaerobic fermentation in 90-day hydraulic detention time. This suggested that it is possible to achieve stable operation using Napier grass as a substrate for biogas production in pilot or large-scale biogas plant in the future. It was concluded that Napier grass as energy crop can be an alternative energy resource.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

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