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Biocatalytic conversion of syngas to liquid biofuels using *Clostridium acetobutylicum*

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Abstract

This study aimed to develop a pilot-scale bioreactor for ethanol production from syngas produced from biomass gasification and evaluate its performance. Gasification of characterized feedstock, namely *Casuarina* wood and coconut shell, is performed in a 2 kg hr^{-1} downdraft gasifier and the produced gas is cooled in a heat exchanger and scrubbed in a scrubbing bed to produce syngas free of moisture, tar and particulates. The syngas are fermented to produce biofuels in a 5 L capacity bioreactor with *Clostridium acetobutylicum* strains. The percentage yield of acetone, butanol and ethanol was verified with the standard concentration and found to be 12%, 5% and 7% from casuarina wood and 13%, 7% and 6% from coconut shell respectively. The syngas components *viz.,* carbon monoxide (CO), carbon dioxide (CO_2) and hydrogen (H), are fermented to produce acetone, butanol and ethanol along with larger proportions of acids. These acids can be converted into bio-alcohol if the fermentation period or gas-liquid transfer is enhanced. The pretreatment of feedstock is not required in syngas fermentation as it is a major process in conventional bio-alcohol production methods. This pays a way to superior technology in terms of cost and time.

Keywords

acetone; biomass; butanol; ethanol; gasification; syngas

Introduction

Currently, biofuels are predominantly produced from feedstocks, namely sugar, starch and oil seeds, using well-established methods. Even though it occupies a smaller fraction of the global energy market due to limited scalability due to competition in food resource availability, To address these issues, it is necessary to employ non-food feedstock for sustainable biofuel production. An agricultural waste/residue will be a promising renewable feedstock for producing biofuel. It also paves the way for reducing the effects of climate change by reducing greenhouse gas emissions (1). For biofuel production to be sustainable, it must demonstrate profitability by leveraging low-cost, non-food feedstocks (2). It ensures economic viability to attract investment in industrial investment strategies and the impact of micro-structural shocks on private funding behaviour (3). The primary bottleneck of most renewable energy generation methods is cost competitiveness, as the average production cost of biodiesel is ₹90-₹120/L compared to ₹33-₹42/L for fossil diesel (4). Biodiesel or algal biofuel production methods emphasize the importance of novel cost-reduction techniques and optimized protocols to enhance economic feasibility and market viability (5). Two methods to produce liquid biofuels are biomass gasification to produce synthetic gas/syngas (6) and syngas fermentation with microbial catalysts for conversion into liquid biofuels (7). To increase the generation of alcohol during fermentation, biomass must first undergo pre-treatment. The pre-treatment process is evicted by using unprocessed biomass to create syngas by gasification (8) and this syngas is fermented to produce liquid biofuel (9).

Syngas fermentation is an emerging concept as the information regarding syngas properties is meagre. It is a favorable alternative for Carbon cycling and valorization of lingo-cellulosic biomass (10). Syngas composition utilized in fermentation procedures is a major factor in selecting microbes (11). Syngas was produced by upgrading the producer gas that was produced by the gasification of biomass. Biomass gasification generates producer gas, comprising CO, CO_2, H, hydrocarbons, nitrogen (N), moisture and tar, at temperatures ranging from 800 to 1200 °C under limited oxygen (O) conditions. The syngas is produced by upgrading producer gas to remove tar, solid particles and other impurities. It primarily comprises H and CO. The varying syngas composition affects the quantity and quality of liquid biofuels (12). The syngas was converted into liquid biofuels through fermentation using *Clostridium acetobutylicum* strains as microbial catalysts. Syngas can also be produced by utilizing biochar as feedstock. Biochar production aligns with the broader goal of using non-food feedstocks to enhance the sustainability and profitability of biofuel production (13).

The liquid fuels constitute biobutanol, bioethanol and by-products, namely fatty acids (14). The selection of microbial catalysts results in highly enriched desired products and eases the process of downstream results with low product recovery costs (15). Reactor design is given importance for low-cost substrate, better gas-liquid mass transfer and productivity to produce high-value-added chemicals by reducing production cost and paving the path for economic and technical feasibility (16).

Based on the above, this study investigates the integration of gasification and biocatalytic fermentation processes for high-value liquid biofuel production from the selected feedstock, *Casuarina* wood and coconut shell. Utilizing this waste in biofuel production results in effective waste management, greenhouse emission reduction and promotion of Carbon cycling. The main objective is to develop a biocatalytic syngas fermentation system via gasification, gas conditioning and fermenter to produce liquid biofuels from biomass feedstock and evaluate the developed system's performance. This environmental-economic nexus is perilous to biofuel technologies scale up by promoting a transition to a sustainable energy future.

Materials and Methods

The biomass feedstock, namely *Casuarina* wood and coconut shell, was collected from the Tamil Nadu Agricultural University premises. Proximate composition, such as moisture, volatile matter, ash and fixed Carbon content of the biomass feedstock, was determined using ASTM standards D3172 - 75 (ASTM, 1977). Elemental compositions such as Carbon, hydrogen, N and O content of the biomass feedstock were assessed by following ASTM standards E777-778. A Bomb calorimeter determined the heat content of biomass feedstock. The chemical constituents such as cellulose, hemicellulose and lignin were determined using the National Renewable Energy Laboratory (NREL) procedure.

The process flow diagram of the developed syngas fermentation system was drawn using AUTOCAD software and shown in Fig. 1. The selected biomass feedstock was gasified in a 2 kg hr^{-1} TNAU downdraught gasifier. The blower provided the air to initiate and sustain gasification in a gasifier. The conditions in the gasifier were 800 to 1000 °C and 10% excess of stoichiometric O (17). The producer gas is comprised of CO_2, CO, hydrocarbons, N and impurities (18). Producer gas was conditioned to yield syngas through a gas conditioning unit. The gas conditioning unit comprised of a heat exchanger and a scrubbing unit to cool and scrub the gas to yield syngas (19). Bioreactor, namely CSTR of capacity 5 L, was developed to undergo syngas fermentation following the acetyl-CoA pathway (20). It consisted of a reactor vessel and an agitator. The agitator powered by motor ensured the effective gas-liquid mass transfer by proper mixing and the inoculum port introduced the culture strain into the fermenter. To optimize the fermentation process, the control system monitored and regulated the parameters: pH, temperature, syngas flow rate and agitation steep. The gas

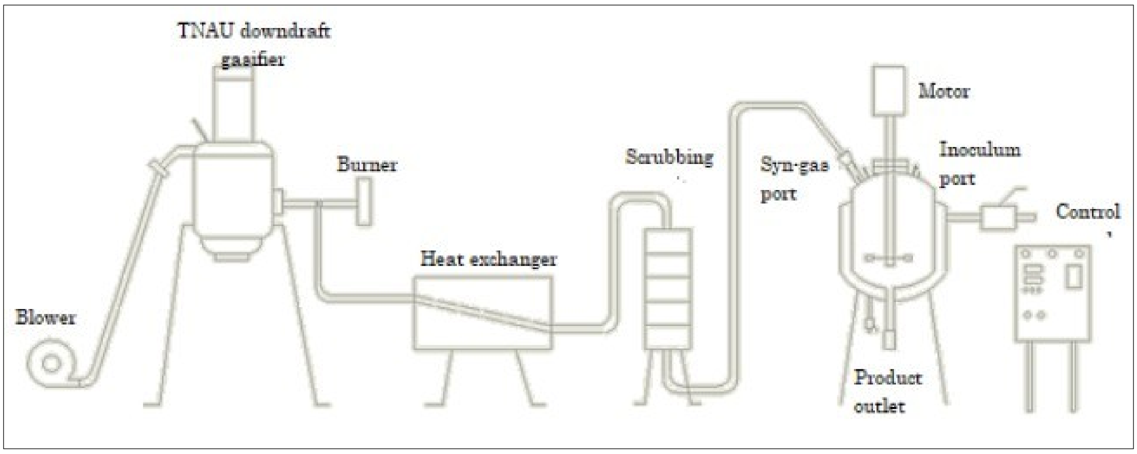

Fig. 1. Process flow diagram of Syngas fermentation experimental set up.

composition was measured using a Testo flue gas analyzer and bio-alcohol quality was analyzed using gas chromatography-mass spectrometry (GC-MS) (Agilent) (21). The liquid biofuels can be processed downstream and recovered to separate the acetone, butanol, ethanol and fatty acids. The overall system performance, including syngas production, fermentation efficiency and biofuel yield, was evaluated to assess its technical and economic feasibility.

Results

The properties of selected biomass (bulk density, moisture, volatile, ash, fixed carbon, Carbon, hydrogen, N, O, heating value, cellulose, hemicellulose and lignin) were analyzed as per the protocol mentioned in the materials and methods section and the results were presented in Table 1.

The bulk density of selected feedstock varied from 108 to 125 kg m^{-3}. The feedstock's moisture and ash content were approximately 10% and below 5% respectively, which is desirable for gasification. The C/O ratio was approximately one, which also proves to be a good feedstock for the gasification process. Liquid is the major drawback in the biomass fermentation process and it can be overcomed by biomass gasification. High volatile matter content with low ash content is the main criterion for thermochemical conversion (22). Biomass majorly consists of cellulose, hemicelluloses and lignin, among which microbial catalysts utilize cellulose and hemicellulose in the form of depolymerized sugars as a Carbon source to produce xylitol, ethanol, organic acids, industrial enzymes, etc (23). Hence the selected biomass can be studied for bioalcohol production in the developed system (Fig. 2). The design details of the developed syngas fermentation system are tabulated in Table 2. The selected feedstock in this integrated process will provide valuable foundational insights; they offer a promising starting point for scaling up to commercial applications with further optimization. It will pave the way for broader application in highly efficient biofuel production systems.

Fig. 2. Developed syngas fermentation system.

Table 1. Properties of biomass

Properties	*Casuarina* wood	Coconut shell
Bulk density (kg m^{-3})	108	125
Moisture content (%)	9.87	9.33
Volatile matter (%)	66.30	78.00
Ash content (%)	4.72	0.70
Fixed carbon (%)	28.91	21.30
Carbon (%)	48.58	49.0
Hydrogen (%)	5.61	5.36
Nitrogen (%)	0.12	0.57
Oxygen (%)	40.35	43.60
Heating value (MJ kg^{-1})	21.23	20.28
Cellulose (%)	48.70	19.8
Hemicellulose (%)	27.13	68.7
Lignin (%)	26.64	30.1

Table 2. Design details syngas fermentation system

	Specification	*Dimension*
Downdraft gasifier- 2 kg h^{-1} capacity	Throat diameter (mm)	5
	Hopper diameter (mm)	152
	Hopper height (mm)	63.3
	Height of reactor (mm)	910
	Length of burner (mm)	650
	Diameter of burner (mm)	162
Heat Exchanger	Height of heat exchanger (mm)	400
	Length of heat exchanger (mm)	600
	Breadth of heat exchanger (mm)	300
Scrubbing unit	Height of the cylinder (mm)	800
	Diameter of the cylinder (mm)	300
	Thickness of the porous plate (mm)	2 mm
Bioreactor	H/D ratio of the reactor vessel	1.00
	Volume of the reactor vessel (m^3)	0.0096
	Impeller diameter (D_i) (mm)	70
	Disc diameter (D_d) (mm)	50
	Clearance (impeller and tank bottom) (mm)	80
	Length of the blade (L) (mm)	20
	Width of the blade (W) (mm)	20

Gas generated from gasifiers results in impurities, *viz.,* moisture, nitric oxide, ammonia, tar and particulates, which negatively impact fermentation without clean-up (24). The solubility of impurities in the fermentation process varies significantly, which creates a change in the metabolic pathway. Scrubbing of producer gas is required for the syngas fermentation process (25). In the scrubbing process, the layered beds can be used with adsorbents such as silica gel (26), activated Carbon and zeolite (27). The selection of proper adsorbent is based on its equilibrium and kinetic characteristics. Adsorption materials required for 1 m^3 of producer gas are silica gel, activated Carbon and zeolite 5A, occupying 0.005 m^3 of the scrubbing unit (Table 3).

A bioreactor was designed and developed to carry out the study on the production of bioalcohol from the selected feedstock. The system consists of a reactor vessel, agitator and control panel. Biomass conversion to biofuel by gasification was suitable for gas engines and fit for both developed and developing countries (28). The selected biomass feedstock was gasified in a downdraft gasifier. The producer gas from the gasifier was cooled with a heat exchanger and made to pass through the scrubbing unit. It is then introduced into the bioreactor gas inlet. The performance of the down draft gasifier and gas conditioning unit were tabulated in Table 4.

It is inferred that an increase in CO content and a decrease in CO$_2$ content, is due to the adsorption by the scrubbing material and no change was found in the hydrogen and N content of syngas. Around 65 % of cooling is achieved

Table 3. Details of adsorbent material

Adsorbents	Silica gel	Activated carbon	Zeolite 5A
Size (mm)	2-5	2.5	1.7-2.5
Porosity	0.34	0.37	0.35
Density (kg/m^3)	748	400	650
Mass (kg)	4.1	2.0	3.5
Height in the scrubber (mm)	300	150	200

Table 4. Experimental results

Biomass		CO	CO_2	H_2	O_2	CH_4	C_nH_m	N_2	Temperature (°C)
Casuarina wood	Before scrubbing	14.98	13.50	4.43	5.80	5.20	0.00	56.09	120
	After scrubbing	28.10	10.25	4.25	0.00	0.28	0.00	57.12	41
Coconut shell	Before scrubbing	12.45	11.75	5.61	6.45	7.56	0.29	55.89	116
	After scrubbing	28.99	8.67	5.17	0.00	0.25	0.00	56.92	39

CO: Carbon Monoxide; CO_2: Carbon Dioxide; H_2: Hydrogen; O_2: Oxygen; CH_4: Methane; C_nH_m:Hycrocarbons; N_2: Nitrogen.

through gas conditioning unit. A study emphasized ethanol production from syngas composition of 23% CO, 13% hydrogen, 8% CO_2, 1% methane and 46% N produced from plant materials with freshly cultured *Clostridium butyricum* for syngas fermentation (29). The inoculum *Clostridium acetobutylicum* strains, such as NCIM RCM 1710, MR 1011(3) and NCIM RCM 8728, were introduced into the fermenter. The inoculum gas mixture was mixed using an agitator driven by a motor. The bacterium facilitated the fermentation of syngas to produce liquid biofuels. For syngas fermentation, treated syngas with higher CO content was preferred, as most of the *Clostridium* species were grown by utilizing all the available hydrogen (H) for reducing equivalents on best fermentation conditions (30). Thus, scrubbed gas can be effectively used for syngas fermentation.

The samples were drawn continuously at 12, 24, 36, 48, 60, 72, 84 and 96 hr. The sample was tested to find out the presence of bio-alcohol in a GC-MS detector. GC-MS analysis of 96 hr drawn sample using *Casuarina* wood and coconut shell showed final confirmation of syngas into liquid fuels (Fig. 3 and 4).

The percentage yield of acetone, butanol and ethanol was verified with the standard concentration and found to be 12%, 5% and 7% from *Casuarina* wood and 13%, 7% and 6% from coconut shell, respectively, at 96 hr fermentation period. The other compounds present are 2-butanone, 2-isopropoxyethylamine, acetic acid, formic acid 2-methyl propyl ester, methoxy-ethanol, 2-nitro-, propionate, propanoic acid, anhydride with formic acid Hydroperoxide, 1-methyl pentyl, butyrolactone, butanoic acid, 4-hydroxy-butane, 2,3-dimethyl-N, N-di methylethane sulfonamide, butanal, 3-hydroxy-dimefox. Apart from acetone, butanol and ethanol concentration, acids were present in a larger proportion and were found to decrease as the fermentation period increased. For scale-up, pilot scale studies are often required for designing large-scale production systems (31). Acetone, butanol and ethanol productivities were enhanced with *Clostridium acetobutylicum* strain, which exhibited a total percentage solvent productivity of 30% in a membrane cell-recycle bioreactor (32). The bioalcohol yield may be increased by immobilizing the fermentation culture in CTSR through a packed bed by utilizing the dissolved syngas constituents from the liquid as it flows (33). The immobilization of cells increases the acetogen reusability and makes cells and product separation easier with the subsequent treatment process.

The feedstock properties, gasification efficiency and fermentation efficacy affect the liquid biofuel yield. These findings provide insights into optimizing biomass-to-bio alcohol pathways for enhanced scalability and economic feasibility. The solid pyrolysis residue, particularly biochar, holds promise for syngas production. It reduces the concentration of nitrous oxide (N_2O) emissions in syngas with an economical and eco-friendly approach to biofuel production. This dual benefit underscores the value of integrating pyrolysis residues into the commercial gasification process, contributing to a circular economy (34).

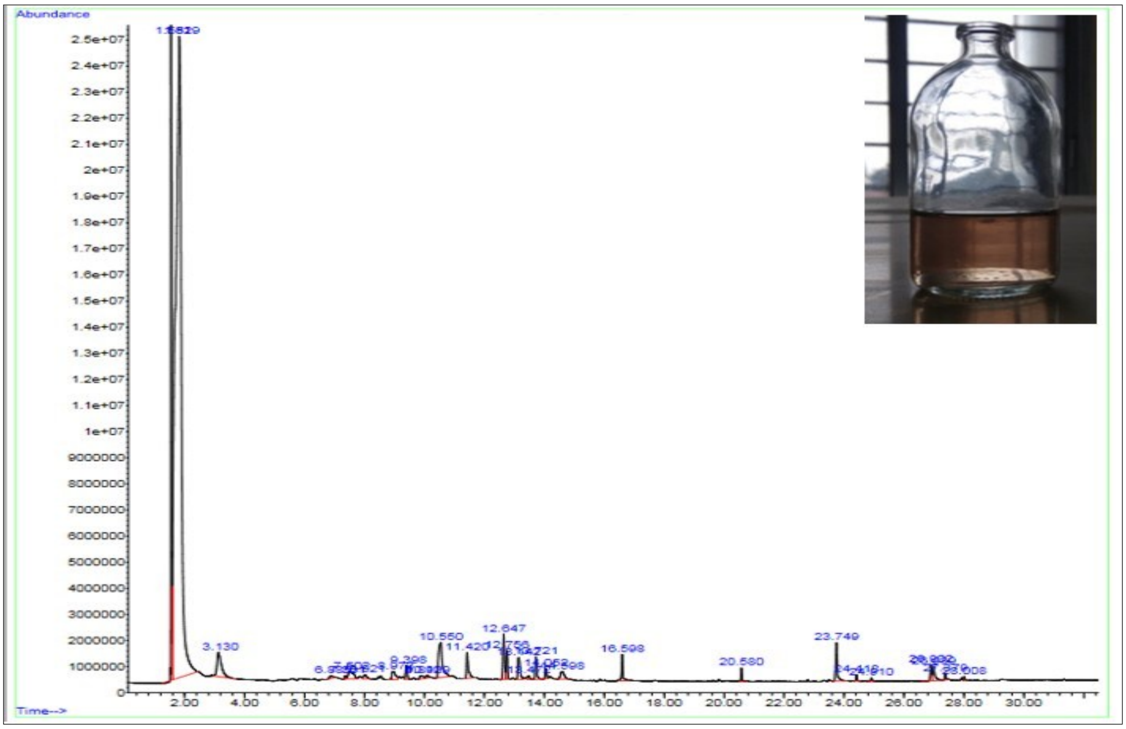

Fig. 3. GC-MS graph of liquid biofuel produced from fermentation of syngas generated from casuarina wood.

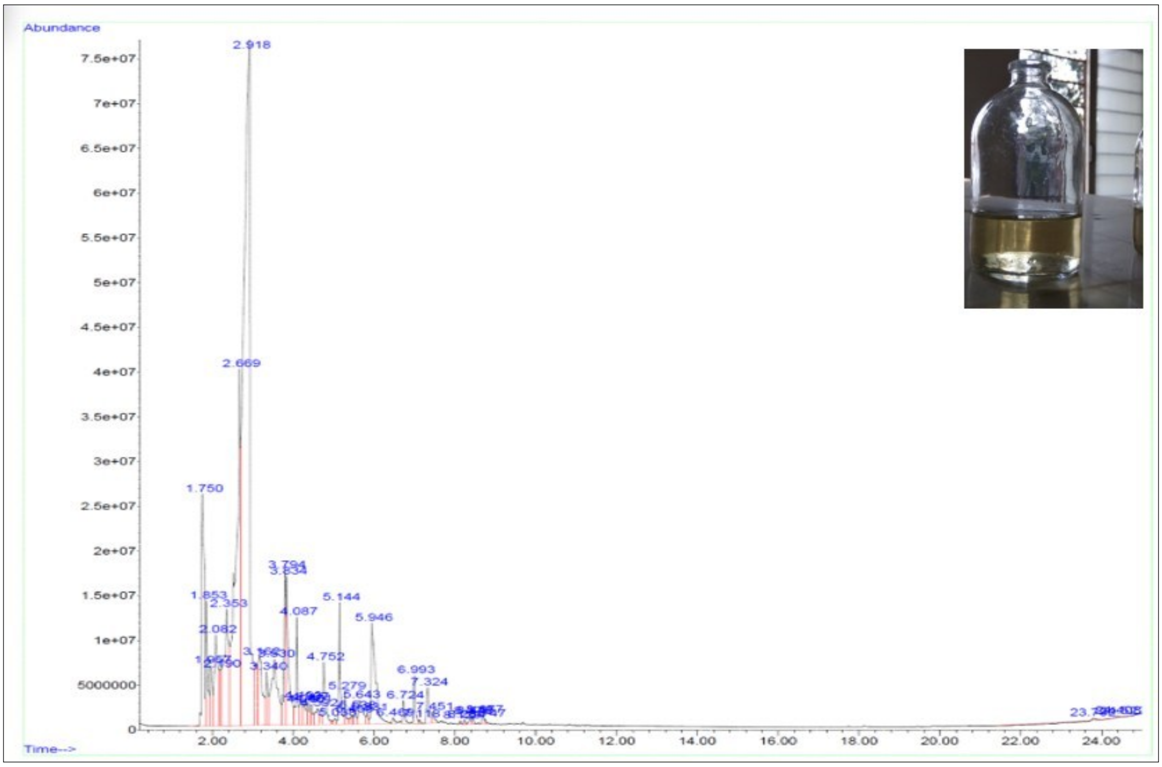

Fig. 4. GC-MS graph of liquid biofuel produced from fermentation of syngas generated from coconut shell.

Impurities may influence the observed lower bio alcohol yields in the syngas gas and the bioreactor design. Further optimization of these factors could enhance scalability and align the results more closely with those seen in larger-scale studies (35). Energy management is deeply intertwined with political priorities, shaping economic growth and social policies over centuries (36). It undergoes persistent challenges of energy poverty and the political will need to address equitable energy access. These insights reinforce that energy strategies are not just technical challenges but pivotal issues in shaping national and global policies (37).

Modern computing methods offer remarkable potential to predict the commercial success of complex technologies. Digital twin simulation modeling and artificial intelligence (AI) - based Internet of Manufacturing Things systems, can be used to simulate real-world scenarios and optimize decision-making processes (38). These advancements enhance the ability to refine processes, assess market viability and ensure the scalability of emerging innovations. Further, AI can significantly assist in addressing multifactorial techno-economic challenges by providing predictive insights, optimizing processes and supporting data-driven decision-making for biofuel production (39). It can improve operational efficiencies, enhance sustainability and promote growth across diverse industrial and business sectors (40).

Conclusion

This study reveals significant and novel findings that expand the scope of sustainable biofuel production. Using *Casuarina* wood and coconut shells is an alternative, cost-effective solution to meet energy demands. Lignocellulosic biomass, *viz.,* casuarina wood and coconut shell, are good feedstock for synthesising gas with predominant CO and H via gasification. The *Clostridium acetobutylicum* strains can be utilized to produce biofuels from bio-syngas and showcase their promise for industrial applications. The integration of gas scrubbing technologies to enhance syngas quality further validates the technical feasibility of the process. However, challenges such as improving gas-liquid mass transfer, optimizing catalyst performance and enhancing product recovery must be addressed to achieve full industrial scalability. Despite these bottlenecks, the concept is economically promising, potentially providing a sustainable alternative to fossil fuels, contributing significantly to the global energy transition.

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Authors' contributions

PV was involved in analysis and writing original draft preparation; GJ was involved in the execution of research in the lab and data analysis; SS was involved in conceptualization, methodology, supervision and fund acquisition; SK formulated research parameters, review and methodology; SP guided with research concept and fund acquisition; DR involved in review, editing and supervision. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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