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ORIGINAL ARTICLE

Conversion of oil palm empty fruit bunch into bioethanol through pretreatment with CO_2 as impregnating agent in alkali explosion

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Abstract

Pretreatment is an important step in lignocellulosic bioethanol production which aims to reduce lignin content and break down lignocellulosic structure thereby increasing the accessibility of enzymes in hydrolysis. Therefore, this research explored the pretreatment process on oil palm empty fruit bunch (EFB) with CO_2 impregnation followed by alkali explosion. EFB was impregnated with CO_2 at 5 °C for 12 h. After impregnation, EFB was mixed with 2.5 M of NaOH solution (1:5 of S/L ratio) in an alkali explosion reactor. Alkali explosion was conducted at 150 °C, 4 kg/cm^2 of pressure with the variation of reaction time for 15, 30, and 45 min. The parameters analyzed in this study include EFB recovery, EFB composition and characteristics, glucose yields, and ethanol yields. EFB composition was analyzed as cellulose, hemicellulose, and lignin while the characteristics of the EFB were examined in functional groups. The results indicated that combined pretreatment using CO_2 impregnation followed by 15 min of alkali explosion obtained higher delignification of EFB, glucose, and ethanol yield than only alkali explosion for 30 min. The highest glucose and ethanol yields were 99.33 and 83.80 w/w% of glucose mass in EFB, respectively. These results prove that CO_2 can be used as an impregnation agent in lignocellulosic pretreatment and could be combined with alkali explosion.

Keywords Alkali explosion · Bioethanol · CO_2-impregnation · Oil palm empty fruit bunch · Pretreatment

Statement of novelty Indonesia as one of the largest oil palm producers in the world not only produces oil palm but also generates huge amounts of lignocellulosic biomass such as empty fruit bunch (EFB). This biomass can be converted into energy, for example, bioethanol. Pretreatment is an important stage in lignocellulosic bioethanol production. This study inquires about the application of CO_2 as an impregnating agent in the alkali explosion of EFB for bioethanol production. This research reports for the first time that adding CO_2 as an impregnating agent before alkali explosion could enhance the percentage of delignification, hydrolysis, and fermentation yield in bioethanol production from EFB. This report was assessed in terms of the percentage of EFB solid recovery after pretreatment, the percentage of the chemical composition of EFB, the characteristics of EFB, the yield of glucose, and ethanol yield after separate hydrolysis and fermentation (SHF).

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1 Introduction

Fossil fuel combustion releases emissions that negatively impact the environment and humans [1]. Therefore, many researchers have carried out research to substitute fossil fuels with new and renewable energy (NRE). One of the alternative raw materials for renewable energy that is currently being developed is biomass. Biomass has been the most energy source in the world for heating application [2]. However, modernization of bioenergy production systems is needed so that biomass can be optimally applied as feedstock. Bioethanol is one of the bioenergies that can be produced from biomass, such as starch, sugar, lignocellulosic biomass, and algae [3–5]. An alternative non-food material that has the potential to be developed as a source of bioethanol production is oil palm empty fruit bunches (EFB). EFB is the largest waste in the palm oil industry, reaching 22 to 25% of the weight of fresh fruit [6]. EFB has cellulose (20–50%), hemicellulose (23–36%), lignin, and other derivatives (22–51%) [7, 8]. The significant variations of the constituents of EFB due to differences in the sample's origin

such as size, age, growth phase, soil condition, geographic location, and climate leverage related to the oil palm tree, as well as different analytical methods and sample preparation before analysis of EFB [9, 10].

Lignocellulosic bioethanol production mainly has four primary processes: pretreatment, hydrolysis, fermentation, and purification [11]. In the process, the constraint faced is lignin content in lignocellulose, which inhibits the conversion process, and causes a low hydrolysis rate resulting in a low ethanol concentration production [12]. In addition, the structure of lignin molecules is a physical barrier in hydrolysis due to preventing the accessibility of enzymes to cellulose and hemicellulose [13, 14]. Therefore, the pretreatment of lignocellulose is an important step in bioethanol production.

Previous research has been done with various pretreatment methods, such as biological, chemical, mechanical, and thermal processes, as well as a combination of each method to accelerate the hydrolysis of lignocellulose. Alkali explosion is one chemical pretreatment that can provide a high yield of delignification. However, this method requires high temperature and pressure as well as takes a long time to process [15].

To optimize the pretreatment process, several studies reported that the addition of impregnating agents such as H_2SO_4, SO_2, or CO_2 could decrease inhibitor production and improve the enzymatic hydrolysis of biomass [16]. Therefore, this study applied CO_2 as an impregnating agent before alkali explosion for EFB in bioethanol production. Impregnation refers to the imbuing or saturating process with something [17]. Impregnation of biomass could change the morphology by enhancing cellulose's permeability and increasing biomass's surface area [18]. The use of CO_2 as impregnating agent is considered to provide benefits such as strong solubility, low toxicity, weak corrosivity, and low occupational risk [19, 20]. Moreover, CO_2 is also a by-product of ethanol fermentation; thus, it is enormously available in bioethanol plants [21].

The variation of reaction time during alkali explosion was also explored in this study. According to previous studies, the reaction time for the alkaline explosion of EFB ranged between 15 and 45 min [22, 23], and the optimal delignification process was 30 min [24, 25]. Therefore, optimization of reaction time in combined pretreatment with CO_2 impregnation followed by alkali explosion be necessary to explore.

2 Materials and methods

2.1 Materials

This study obtained EFB from oil palm plantation in Sumatra Island, Indonesia. EFB was chopped and milled to obtain in fiber form with the size ± 3 mm. Furthermore, EFB was dried to attain 10% of moisture content. CO_2 gas was provided by PT WAP Andalan Indonesia. The enzymes used were Cellic® Ctec2 and Cellic® Htec2 from Novozymes Korea Ltd. Commercial instant dry yeast *Saccharomyces cerevisiae* was applied in fermentation. All chemicals used were analytical grade.

2.2 Pretreatment process

The pretreatment was performed in a 5-L reactor that was manufactured by Changhae Ethanol Co., Ltd. In the experiments with CO_2 as catalyst impregnation, the EFB fiber was placed in a plastic bag, and CO_2 was supplied from a gas cylinder at atmospheric pressure. CO_2 gas was added as much as 3% w/w based on the moisture content of EFB where the water content in the EFB used in this study was 10% w/w. The weight of CO_2 was determined by weighing the bag before and after adding the gas. The sample in a plastic bag was stored at 5 °C for 12 h to complete impregnation [19]. Furthermore, the impregnated EFB was treated in the reactor using 2.5 M NaOH solution with 1:5 of the solid–liquid (S/L) ratio. Alkali explosion was carried out at 150 °C of temperature and 4 kg/cm^2 of pressure [15]. The variation of alkali explosion time was 15, 30, and 45 min. Subsequently, the pretreated-EFB are neutralized with water until pH of 6–8 at room temperature and dried for 24 h at 50 °C in the oven. The alkali explosion without CO_2 impregnation was also conducted as a blank sample with the process at 150 °C, 4 kg/cm^2 for a 30-min process.

2.3 Separate hydrolysis and fermentation (SHF) process

The ethanol was produced by separate hydrolysis and fermentation (SHF) method.

2.3.1 Enzymatic hydrolysis process

The substrate concentration used in the hydrolysis process was 10 w/v% of EFB-treated and, then, was mixed with a citrate buffer and enzymes until 100 mL of total volume. The enzymes used were 30 FPU/g substrate of Cellic®Ctec2. Next, Cellic®Htec2 is added with a volume ratio of 1:5 of Cellic®Ctec2. The hydrolysis process was carried out at a temperature of 50 °C with the agitation of 150 rpm in the shaking incubator for 72 h.

2.3.2 Fermentation Process

After 72 h of enzymatic hydrolysis, 1 w/v% of dried *S. cerevisiae* was added into hydrolysate for the fermentation

process. The process was carried out at 32 °C of temperature, 150 rpm for 72 h.

2.4 Analytical methods

The chemical component of EFB (cellulose, hemicellulose, lignin, and ash) was analyzed using standard biomass analytical procedures from National Renewable Energy Laboratory (NREL) [26]. The structural changes of EFB were evaluated by a Shimadzu FTIR spectrometer. The glucose and ethanol concentration after SHF was determined using high-performance liquid chromatography (HPLC) equipped with HPX-87P (Bio-RAD, CA, USA) column and analyzed with a RID detector. The eluent used as a mobile phase was 5 mM H_2SO_4 solution at a flow rate of 0.6 mL/min [21].

2.5 Delignification and yield calculation

Percent of delignification, glucose, and ethanol yields were calculated using the equations below:

1) Percentage of delignification [27]

$$\%\text{delignification} = \frac{\text{lignin content of EFB}_i - \text{lignin content of EFB}_f(\%\text{w/w})}{\text{lignin content of EFB}_i(\%\text{w/w})} \times 100\% \quad (1)$$

where EFB_i = EFB before pretreatment and EFB_f = EFB after pretreatment.

2) Glucose yield calculation [21]

a) Glucose yield calculation based on theoretical glucose from cellulose in the substrate

$$\%\text{Ygc} = \frac{\text{glucose in hydrolysate(g)}}{\text{theoretical glucose in substrate(g)}} \times 100\% \quad (2)$$

b) Glucose yield calculation based on the substrate

$$\%\text{Ygs} = \frac{\text{glucose in hydrolysate (g)}}{\text{weight of substrate (g)}} \times 100\% \quad (3)$$

3) Ethanol yield calculation [21]

a) Ethanol yield calculation based on theoretical ethanol from cellulose in the substrate

$$\%\text{Yec} = \frac{\text{ethanol in broth fermentation (g)}}{\text{theoretical ethanol in cellulose (g)}} \times 100\% \quad (4)$$

b) Ethanol yield calculation based on the substrate

$$\%\text{Yes} = \frac{\text{ethanol in broth fermentation(g)}}{\text{weight of substrate(g)}} \times 100\% \quad (5)$$

3 Results and discussion

3.1 Application of CO_2 as an impregnating agent in alkali explosion for EFB

Table 1 shows the recovery percentage of solids generated after pretreatment and washing. The alkali explosion pretreatment with prior CO_2 impregnation was about one-third of the initial substrate. The value was almost similar to the percent recovery of the blank sample. The weight losses are considered to be related to the lignin content lost due to the process. Moreover, the decomposition of the polysaccharide compound, silica content removal, and evaporation that occurs in several components (CO, CO_2, CH_4, and other hydrocarbons) have a major role in sample weight losses [28]. Evaporation could occur during an alkali explosion, which uses high temperatures and pressures. Carbon cellulose chains, hemicellulose, and lignin are degraded due to the high temperature in the pretreatment process [29], such as in an alkali explosion.

The chemical component of EFB before pretreatment as an untreated sample, EFB after alkali explosion as a blank, and EFB after combined pretreatment of CO_2 impregnation followed by alkali explosion can be seen in Table 2. The results show that lignin and hemicellulose were degraded after pretreatment. The percent delignification of EFB after combined pretreatment of CO_2 impregnation followed by

Table 1 Recovery weight of EFB solids left after pretreatment and washing

Sample code	CO_2	Alkali explosion time (min)	Recovery weight of solids (w/w%)
Blank	-	30	31.19
A	+	15	33.25
B	+	30	29.00
C	+	45	31.30

Table 2 Chemical composition of EFB before and after pretreatment

Sample code	Pretreatment condition		Chemical composition (w/w%)				Delignification (%)
	CO_2-added	Time of alkali explosion (min)	Cellulose	Hemicellulose	Lignin	Ash	
Untreated	-	-	35.24	22.84	37.16	2.51	
Blank	-	30	63.12	9.88	11.72	0.69	68.46
A	+	15	76.19	5.96	9.79	0.57	73.65
B	+	30	77.88	6.45	7.42	0.34	80.03
C	+	45	79.92	5.40	7.23	0.59	80.54

alkali explosion was higher than the blank sample. From the results of combined pretreatment, delignification increased alongside the increasing explosion time. The percentage of delignification of samples A, B, and C was 73.65, 80.03, and 80.54%, respectively. These findings indicate that the percent delignification of sample B at 30 min of explosion time was higher than sample A at 15 min of reaction time but almost similar to sample C at 45 min. Several studies also showed that the optimum reaction time of alkaline pretreatment for biomass was 30 min [25, 30, 31]. According to Monte et al., alkaline pretreatment using NaOH in rice husk could significantly remove silica and hemicellulose as well as partially removed lignin during 30 min of reaction time. After that, the non-cellulosic biomass extraction's reaction rate weakened and almost stopped after 60 min of reaction time [30].

The percentage of hemicellulose compound in EFB-treated decreased from hemicellulose in EFB-untreated. Pretreatment using an alkali solution such as NaOH, ammonia, or $Ca(OH)_2$ can remove lignin and hemicelluloses [32, 33]. After pretreatment, these components will dissolve in an alkali solution known as black liquor. The ability of NaOH to dissolve lignin is due to the opening of aromatic rings of lignin caused by the use of high temperatures and high pressure and then, the resulting explosive effects can dissolve these components [34]. The explosive effect resulting from the reactor due to rapid temperature drop will help in the delignification process due to an increase in the surface area of the pretreatment sample [35]. Adding CO_2 before alkali pretreatment is also believed to increase the pores of lignocellulosic biomass surfaces; thus, the percent delignification of EFB treated using alkali explosion with prior CO_2 impregnation was higher than the blank sample. Therefore, decreasing the percent content of lignin and hemicellulose enhanced cellulose percentage in EFB-treated. Cellulose is the main compound that can be converted into glucose using enzymes and subsequently fermented by yeast to produce ethanol.

Figure 1 shows The FTIR spectra from EFB before and after pretreatment. The wavenumber of EFB-treated (blank, A, B, C samples) at around 3279 – 3552 cm^{-1} appeared

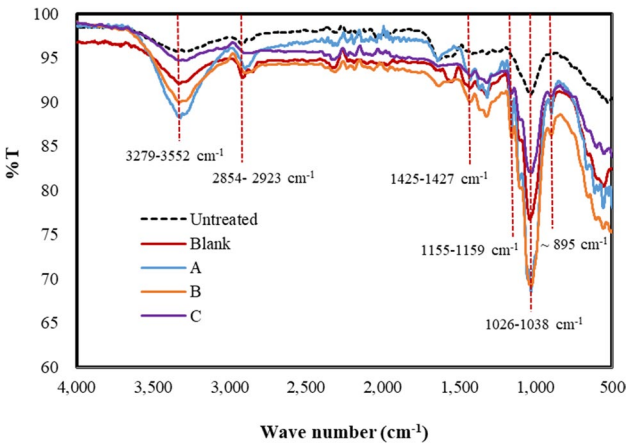

Fig. 1 FTIR spectra of EFB before and after pretreatment

clearer than EFB-untreated. The wavenumber indicated hydrogen-bonded (O–H) stretching absorption. O–H stretching region at a peak of 3000–3600 cm^{-1} of EFB spectra was more assigned to the O–H stretching region from cellulose [36]. From the results, the clear peak of O–H stretching from the cellulose of EFB treated indicated that the cellulose content in EFB treated was higher as compared to EFB-untreated. The CH stretching mode at around 2923 cm^{-1} was shifted to a lower wavenumber at around 2854 cm^{-1} after pretreatment. Then, the wavenumbers between 1425 and 1427 cm^{-1}, 1155 and 1159 cm^{-1}, and around 895 cm^{-1} were found in the EFB after pretreatment. The wavenumber around 1429 cm^{-1} and 1157 cm^{-1} indicates CH_2 bending and C–O–C asymmetric stretching known as cellulose-related bands [37]. Their appearance wavenumbers in EFB after pretreatment indicate an increase in cellulose amount. Moreover, the C–H–O stretching of the β-(1–4)-glycosidic linkage bands at around 895 cm^{-1} appear stable enough in EFB-treated [36]. The changes in intensity were also found in the band at wavenumber 1026–1038 cm^{-1}. The band at wavenumber around 1032 cm^{-1} was indicated to the C–O stretch in cellulose and hemicellulose [36]. The intensity of this band was increased after pretreatment.

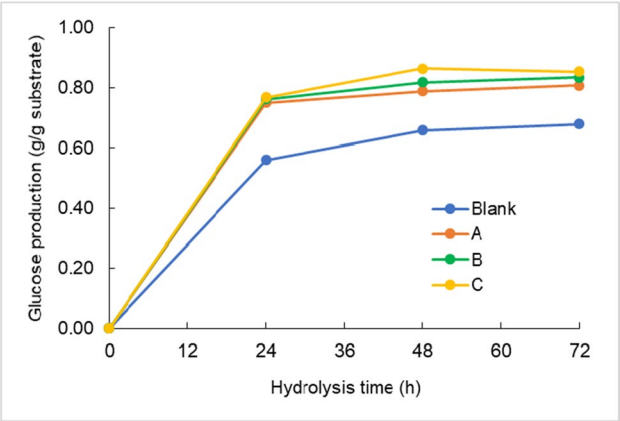

Fig. 2 Change in glucose production from the blank, A, B, and C samples

3.2 Separate hydrolysis and fermentation (SHF) of EFB

Figure 2 shows the changes in glucose production during the hydrolysis of EFB. It was observed that glucose production of EFB after combined pretreatment using CO_2 impregnation followed by alkali explosion was higher than EFB after alkali explosion (blank sample). The tendency of glucose production in the A, B, and C samples was similar. After 72 h of hydrolysis, 0.81–0.85 g glucose/g substrate was obtained from the A, B, and C samples, while a blank sample provided 0.68 g glucose/g substrate. Moreover, the glucose production of sample A with 15 min of reaction alkali explosion process was higher than glucose of blank sample with 30 min of alkali explosion process. Therefore, the utilization of CO_2 as an impregnating agent could reduce the time of alkali explosion. CO_2 impregnation allows the biomass's pores to expand, improving the result of alkali explosion and enhancing enzyme accessibility during the hydrolysis step [21, 38]. An accomplishment rate of the hydrolysis process is influenced by the reduction of lignin levels in biomass, the disruption of component structure of lignocellulose, and the breakdown of the crystallinity of cellulose [39].

According to Table 3, hydrolysis of EFB after combined pretreatment using CO_2 impregnation followed by alkali explosion achieved more than 98% of glucose yield (cellulose basis) and 80% of glucose yield (substrate basis). These results were higher than the blank sample, obtaining 97.94% of glucose yield (cellulose basis) and 68.00% of glucose (substrate basis). Therefore, it indicated the significance of utilization of CO_2 as an impregnation agent before alkali explosion to enhance the hydrolysis result.

Table 3 Yield of glucose in hydrolysis process

Sample code	Hydrolysis time (h)	Glucose yield (cellulose basis) (w/w%)	Glucose yield substrate basis) (w/w%)
Blank	72	97.94	68.00
A		98.87	80.82
B		99.08	83.56
C		99.33	85.40

Table 4 shows ethanol production and ethanol yield from the fermentation of EFB. After 72 h of fermentation, the ethanol concentration of blank, A, B, and C samples was 27.29, 32.80, 35.30, and 37.60 g/L broth fermentation, respectively. The ethanol yield of EFB after combined pretreatment using CO_2 impregnation followed by alkali explosion was higher than EFB after alkali explosion (blank sample). Ethanol yield (cellulose basis) of A, B, and C samples was between 76.84 and 83.80 w/w% and a range of 32.80 – 37.60 w/w% (substrate basis), while ethanol yield of the blank sample was 76.22 w/w% (cellulose basis) and 27.29 w/w% (substrate basis). The results indicated that ethanol yield increased in line with increasing explosion time in combined pretreatment. More interesting, after combined pretreatment (A) at 15-min reaction time, the sample obtained higher ethanol production than the sample after only alkali explosion (blank). Adding CO_2 impregnation before alkali pretreatment could reduce reaction time in alkali explosions that use high temperatures and pressure. Thus, energy and cost saving for the pretreatment process could be achieved.

As a comparison with other studies, Table 5 shows several studies that have been reported regarding EFB pretreatment and pretreatment technology using CO_2. Pretreatment of EFB using steam explosion and combined CO_2-added steam explosion [21] obtained lower percent delignification and glucose yield as well as ethanol yield than results from this study. Alkali explosion [31] or superheated steam explosion followed by alkaline autoclaving pretreatment [40] of EFB also provided slightly lower percent delignification and glucose yield

Table 4 Ethanol concentration and yield in fermentation process

Sample code	Fermentation time (h)	Ethanol (g/g cellulose in substrate)	Ethanol yields (cellulose basis) (w/w%)	Ethanol yields (substrate basis) (w/w%)
Blank	72	27.29	76.22	27.29
A	72	32.80	76.84	32.80
B	72	35.30	80.80	35.30
C	72	37.60	83.80	37.60

Table 5 Comparison of several studies of EFB pretreatment and pretreatment technology using CO_2

Technology	Raw material	Reaction condition	Delignification (%)	Yield		Ref
				Glucose	Ethanol	
Steam explosion	EFB	150 °C, 4–7 kg/cm^2, 30 min	10.93	58.1%	41.4%	[21]
CO_2-added steam explosion	EFB	60 min of CO_2 impregnation followed by steam explosion at 150 °C, 4–7 kg/cm^2, 30 min	11.65	84.1%	58.8%	[21]
Alkali explosion	EFB	150 °C, 4–7 kg/cm^2, 30 min with 10% NaOH solution (S/L = 1:5)	> 70	94.88%		[31]
Superheated steam explosion followed by alkaline autoclaving pretreatment	EFB, OPF, and OPT	180 °C, 0.6 MPa, 5 min followed by treatment with 2–20% (w/v) NaOH at 121 °C for 10–60 min in an autoclave		0.90 g/g EFB 0.85 g/g OPF 0.69 g/g OPT		[40]
Steam pretreatment of CO_2-impregnated	Sugar cane bagasse and leaves	205 °C, 15 min (bagasse) 220 °C, 5 min (leaves)		86.6% (bagasse) 97.2% (leaves)		[19]
CO_2-added ammonia explosion	Rice straw	14.3% of ammonia conc., 2.2 MPa of CO_2 loading level, 165.1 °C for 69.8 min		93.6%	97%	[41]
Supercritical CO_2 pretreatment	Rice straw	30 MPa, 110 °C for 30 min at a 1:1 g/g of S/L ratio		32.4 ± 0.5%		[42]
Supercritical CO_2 pretreatment	Sugarcane bagasse	180 min at 40 °C and 300 bar		17.56%		[43]
Supercritical CO_2 combined with ultrasound pretreatment	Corn cob and cornstalk	15–25 MPa and the temperature range of 120–170 °C for 0.5–4 h		75% (corn cob) 13.4% (corn stalk)		[44]
Pretreatment with CO_2 as impregnating agent in alkali explosion	EFB	CO_2 added at 5 °C for 12 h, followed by alkali explosion at 150 °C, 4 kg/cm^2 of pressure for 15–45 min	73.65–80.54%	98.87–99.33%	76.84–83.80%	This study

as compared to results in this study. Moreover, the application of CO_2 in pretreatment was also used for other biomasses such as sugarcane bagasse and leaves, rice straw, corn cob, and corn stalk [19, 41–44]. From their studies, CO_2 was considered to apply in pretreatment as a catalyst or impregnation agent.

4 Conclusion

Pretreatment of EFB using CO_2 impregnation followed by alkali explosion could provide higher percent delignification, glucose, and ethanol yield as compared to pretreatment alkali explosion. EFB from combined pretreatment with CO_2 impregnation followed by 15 min of alkali explosion obtained 73.65% of delignification. This result was higher than the delignification of EFB from 30 min of alkali explosion (blank sample) of 68.46%. Furthermore, in the combined pretreatment, a longer time in alkali explosion leads to an increase in the percent of delignification, yield of glucose, and ethanol. The highest result was 80.54 w/w% of delignification, 99.33 w/w% of glucose yield, and 83.80 w/w% of ethanol yield from EFB after combined pretreatment with 45 min of alkali explosion. Therefore, adding CO_2 as an impregnation agent in alkali pretreatment is believed to enhance the delignification, glucose, and ethanol yield. Further studies are needed to optimize the NaOH concentration and temperature of CO_2 impregnation on combined pretreatment to reduce chemicals and energy uses in the process.

Author contribution ET is the main contributor who conceptualized the study and methodology, collected and analyzed the data, and wrote the original paper. Meanwhile, AKM conducted pretreatment experiments and data collection. MM and RM performed hydrolysis and fermentation processes and analyzed data. YS supervised the study and also the review and editing of the paper.

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Data availability Not applicable.

Declarations

Ethical approval This article does not contain any studies with human or animal participants.

Competing interests The authors declare no competing interests.

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