ASEAN Journal of Chemical Engineering 2023, Vol. 23, No. 3, 360 – 369

Application of Nanocrystal Cellulose Based on Empty Palm Oil Fruit Bunch as Glucose Biosensing

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Submitted 27 March 2023 *Revised* 9 December 2023 *Accepted* 14 December 2023

Abstract. Biosensors for glucose sensing purposes are important since diabetes is a worldwide disease. One of the components of glucose biosensors is cellulose nanocrystals (CNCs). CNCs are cellulose derivatives that could be extracted from oil palm empty fruit bunch (OPEFB). Indonesia has a high potential for OPEFB due to its abundance of resources. CNCs have poor conductivity as biosensors, so adding supporting electro-conductor components such as graphene and carbon nanotubes (G-CNT) is necessary. In this research, the amount of bleaching agent of H_2O_2 in CNCs extraction varies between 1.5% and 10%, and the portion of CNCs in the composite varies between 5%, 15%, and 30%. The purpose of this research is to create an optimum biosensor composite based on its CNCs quality through particle size analysis (PSA) and X-ray diffraction (XRD) tests followed by cyclic voltammetry to determine biosensor's impedance, limit of detection (LOD), and performance stability. Fourier transform infra red (FTIR) tests are also conducted as process control. The research shows the success of delignification in CNC extraction based on FTIR. Crystallinity enhancement up to 51% as delignification using 1.5% and 10% H_2O_2 yields CNC with a crystallinity index of 87.1% and 94.0%. The average size of CNCs with delignification by 1.5% and 10% H_2O_2 are 640.0 nm and 579.8 nm, respectively. Results of testing the biosensor glucose G-CNT/CNC showed the best composition is 5% CNCs that using 10% H_2O_2 which the highest oxidation peak is 0.00205 A and reduction peak is -0.00223 A. Data of variance composition show the difference of the data is significant by using ANOVA SPSS Test. The biosensor has an accuracy of 83.2% in a test for diabetic urine.

Keywords: Biosensor, Empty Palm Fruit Bunch, Glucose, Nanocrystal Cellulose

INTRODUCTION

Indonesia has an area of 14,858 kilo hectares of oil palm plantations in 22 provinces, with palm oil production of 46,223 kilo tons per year based on Indonesia Central Bureau of Statistics (BPS) data in 2021. The oil palm empty fruit bunches (OPEFB) are the largest waste from oil palm plantations where every 1 ton of oil palm yields OPEFB 23% or 230 kg (Mandiri, 2012). Only 10% of the OPEFB has been used for boiler fuel and compost, and the rest is still waste (Ngadi, 2014). OPEFB has not been utilized according

DOI: 10.22146/ajche.83422

to its chemical content. One of the OPEFB contents is cellulose. The cellulose content in OPEFB was 30-40% by weight, according to Isroi *et al.* (2017) of 40.37%, according to Sudiyani (2009) of 33.25%. Cellulose is a renewable and biocompatible resource with unique mechanical, optical, and electrical properties that make it suitable for materials applications, actuators/sensors, drug delivery systems, and biomedical science (Thielemans, 2009). The potential of cellulose can be maximized by changing its structure into cellulose nanocrystal (CNC). CNC was used for various applications such as color pigments and NMR biomolecular comparison agents by Fleming (2001) and Peng (2011), and glucose detection biosensors from polypyrrole (PPy)/CNC from microcrystalline cellulose (MCC) using acid hydrolysis nanocomposites in GOx adsorption by Esmaeili *et al.*, (2015). The nanocrystalline, porous, and high surface area of the cellulose nanocrystals reflex that the penetration of the target molecules into the substrate is relatively faster, the sensitivity is higher, and the response time is faster so that the CNC has the potential to be a biosensor. One of the most important applications of biosensors is to detect glucose. To detect glucose, glucose oxidase and CNCs are used as the stability membrane.

Cellulose nanocrystals (CNCs) are suitable for membrane stability for glucose oxidase enzymes (Ren *et al.*, 2009). Still, cellulose has poor electrical conductivity and hydrophilic properties, - incompatible with organic compounds. Therefore, these biodegradable materials must be modified with materials that have conductivity properties and are not easily biodegradable to be used as support materials in biosensor applications. Graphene-carbon nanotube (G/CNT) is a suitable material to use because it has high conductivity and biodegradation properties, and graphene and CNT can be used together for various applications such as electronics, batteries, and sensors (Info, 2019). Analytical detection techniques, immobilization methods, and enzyme activity and stability are important parameters that affect biosensor performance (You and Pak, 2014). There is a different type of glucose meter on the market called home blood glucose monitoring (HBGM) in a test strip kit, a quick and easy-to-use blood glucose test. However, HBGM shows lower accuracy compared to glucose measurement in the laboratory. Therefore, research on glucose detectors is still being conducted to increase user trust and achieve standardization as a commercial device. In this study, glucose detectors were made by utilizing cellulose nanocrystal from oil palm empty fruit bunch combined with graphene-carbon nanotubes (CNC OPEFB/G-CNT), which would later be used to bind glucose oxidase as a glucose biosensor.

The novelty of this research is the production of cellulose nanocrystals from waste, especially empty oil palm fruit bunch waste, which will be used for the first time as an immobilization agent for the enzyme glucose oxidase to become a glucose biosensor with a combination of graphene and carbon nanotubes which has never been studied before.

MATERIALS AND METHODS

Materials

OPEFB was obtained from an oil palm farm in Pangkalanbun, Central Kalimantan. CNTs (60-100 nm) were bought from Jiangsu XFNANO Materials, China. Graphene (10-50 μm) and GOx (0.15-0.17 mm) were bought from Xi'an Asclepius Bio-Tech, China. APS,

PBS, DMF, sodium hydroxide, hydrogen peroxide, chloric acid, and distilled water were bought in Surakarta.

As we know, OPEFB possesses high potential as a platform membrane of GOx based on its characteristics, which is used for biosensor purposes. However, OPEFB is a poor conductor, so to overcome its disadvantage, the addition of conductive material such as graphene or CNTs is necessary. To fabricate the composite and preserve its stability, the utilization of polymer or resin such as paraffin is needed. This research aims to observe the effectiveness and efficiency of biosensors produced based on composition variations and methods conducted in fabricating composites.

Cellulose Extraction

Cellulose was extracted from OPEFB. OPEFB was rinsed using tap water. OPEFB dried within the furnace at 50°C to remove its water content for 24 hours. The dried OPEFB was cut into 2–3 cm pieces. For further drying, those pieces were stored in an enclosed system to equalize the moisture content of the pieces. The pretreated OPEFB was then delignificated using an autoclave digester to lessen the lignin content within OPEFB. 50grams of OPEFB were soaked in 750mL sodium hydroxide solution (12% w/v). The suspension was then digested inside the autoclave (Dewanti, 2018). Digestion was run at 120°C and 1 barg for 90 minutes. Digestion yields were filtered so that the cake could be obtained.

Furthermore, the cake was bleached by using hydrogen peroxide 1.5% and 10% v/v over 3 times process at 80–90°C for 2 × 45 minutes so that the cake's lignin and hemicellulose content discharged, dissolved within the bleaching agent (Suriyatem *et al.*, 2020). The bleaching process produced cellulose, which then was dried in a furnace at 70°C for over 24 hours.

Hydrolysis of Nanocrystal Cellulose

Cellulose was powdered using a miller to increase reaction area effectivity in the acid hydrolysis so CNCs yield would be maximized. Cellulose was added to 1 M APS solution by cellulose–APS ratio 10:1 g/L at 75°C for 16 hours in an enclosed–aluminum foil–continuous stirred system. The soaked suspension was rinsed by using centrifuge processes for 4 cycles at a duration of 15 minutes per cycle. The obtained cake was then reserved inside a freeze dryer at -18°C to remove its water content. The dried cake was then powdered, so CNCs were produced.

Biosensor fabrication

Five mg of MWCNT were immersed into 0.5 mg/mL graphene oxide solution. The suspension was then dispersed by a mixer for 2 hours. Centrifugation was run for 2 × 30 minutes at 2500rpm to separate unstable graphene–MWCNT with the suspension. Drying by the furnace was conducted to eliminate water content in the suspension. The dried cake was then powdered using mortar and pestle, as it became G–CNTs powder. The powder then was dissolved in 2,5mg/mL DMF (Mani *et al.*, 2013). CNCs were added into the suspension as much as 5%, 10%, 20% (w/w total mass), and then homogenized, such a composite paste was formed. As the process ran, paraffin was heated and molten at 70ºC. Molten paraffin and composite paste were fused in a ratio of 3:7 at the paraffin's melting point.

Composite had to be made to conduct cyclic voltammetry. The fusion was compacted into an anode testing tube and left to dwell for 3 nights to stabilize the

composite. The composite was then immersed in PBS with a composite ratio of PBS as 2mg/2mL for 24 hours. The anode was then dried and tested using cyclic voltammetry.

Variances were created to determine biosensor performance's effect based on its composition. Namely, NKSA is a composite sample made of bleached CNC by 1.5% H_2O_2, while NKSB is made of CNC bleached by 10% H_2O_2. Then, the following code is CNC/CNT ratio.

Table 1. Variation of biosensors composition (weight ratio CNT:G = 1:1)

Sample	CNC, %	CNT, %
NKSA5	3.5	33.25
NKSA10	7.0	31.50
NKSA20	10.5	29.75
NKSB5	3.5	33.25
NKSB10	7.0	31.50
NKSB20	10.5	29.75

CNCs Characterization

Lignin–Hemicellulose delignification

Lignin–hemicellulose content was measured by Fourier transform infrared spectroscopy (Shimadzu, using KBr pellets), which showed subtraction progress between raw, delignificated, bleached, and isolated cellulose.

Crystallinity Index (CI)

The crystallinity Index was determined by X-ray diffraction (MQ-MD-10 Precision Mini-X-ray diffractometer with a range of 14-78°). Method for calculating the crystallinity index using Segal equation using crystal peak in 16,2-16.3° and 21.9-22.5° also amorph peak in 18.3-18.4°. Pure cellulose was used as a reference to determine CNCs crystallinity between bleaching treatments based on the bleaching agent concentration.

Particle Size Distribution

The particle size of CNCs was measured by a particle size analyzer (DynaPro Nanostar SLS 0.2 – 2500 nm). Measurement was conducted to confirm that CNCs reached crystal objective particle size and compare different bleaching treatments.

Biosensor characterization Sensitivity

The sensitivity of the biosensor was measured by cyclic voltammetry (Metrohm Autolab, PGSTAT 100 N 100 V/250mA) at a scan rate of 100 mV/s, with Pt anode and Ag/AgCl as reference cathode. Standard glucose solution at 140 mg/dL was made using 0.1 M HCl as the solvent, which is based on the common human urine volume at 30 mL.

Accuracy

The accuracy of the biosensor was measured by comparing electrolytes used between glucose solution and urine. C1 was a mixture with a glucose concentration of 136 mg/mL and 0.1 M HCl as the electrolyte in urine 30 mL. C2 was a mixture of 0.1 M HCl as the electrolyte in the diabetic's urine within concentration 136 mg/dL as much as 30 mL.

$$Accuracy = (1 - \frac{C_1 - C_2}{C_2}) \times 100\% \qquad (1)$$

C1 was current on the glucose solution sample (A), and C2 was current on the diabetic' urine sample (A).

RESULTS AND DISCUSSION

IR-Spectroscopy

The performance of the test is shown in Figure 1. The result was then compared using Sigma Aldrich Standard Spectroscopy (I.R. Spectrum Table and Chart).

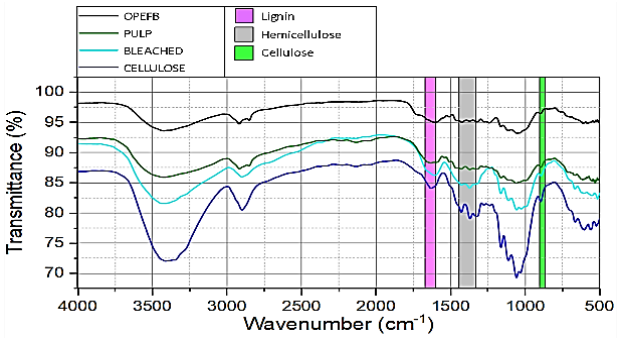

Fig. 1: FTIR spectrum of OPEFB (black), pulped (green), bleached (cyan), cellulose (blue).

Results indicate that overall isolation treatment proceeded properly, from the pretreated sample to the final CNCs. Peak reduction in the processes conducted indicates the effectiveness of sodium hydroxyde in delignificated cellulose fiber (Saleh *et al.*, 2009). Those results also indicate effectiveness of potassium hydroxide in the hemicellulose segregation by breaking its amorphous structure.

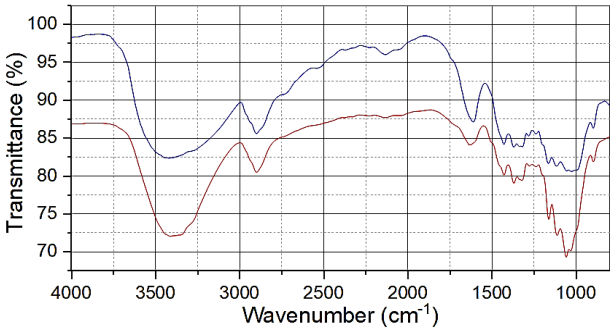

Fig. 2: FTIR of CNCs (above) and Cellulose (below)

Figure 2 shows FTIR results of pure cellulose and produced CNCs. The graph shows that both pretreated samples and produced CNCs share a similar pattern of peaks. CNCs' peak spectrums showed higher areas of absorbance and intensity than pure cellulose. This difference proves polymers disjoint into shorter-chain polymers (Nisak, 2018). Also, in the O-H bond at 3550-3200cm^{-1}, there is the distribution of absorbance because hydrogen bonds involve hydroxyl groups, which form crystalline structures (Lamaming *et al.*, 2016). The amount of crystal in cellulose fiber may be observed at 1280 cm^{-1} based on the C-H bond, which indicates CNCs existence (Ilharco *et al.*, 1997). As shown in Figure 2, the level of spectrum vibrance is increased, which means the total amount of CNCs is also increased. Thus, FTIR results signify that CNCs were successfully produced.

Crystallinity

Cellulose and CNCs share the same structure. However, CNCs have a higher crystallinity index than cellulose (Börjesson and Westman, 2015). The XRD results, such as in Figure 3, may determine the crystallinity index.

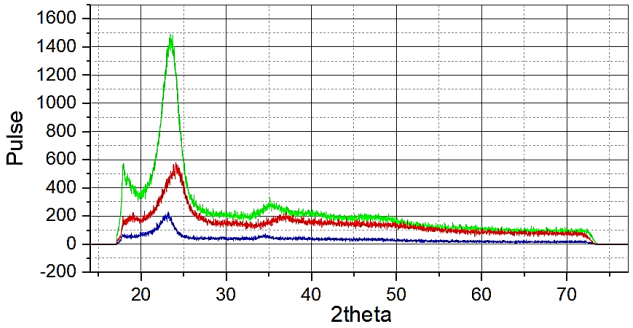

Fig. 3: Diffraction spectrum of NKSA (red), NKSB (green), and cellulose (blue)

Based on the Figure, Peaks can be observed at 16.2˚, 18.22˚, and 23.46˚. Those peaks correspond to Zianor's (Zianor Azrina *et al.*, 2017) diffraction spectrum that mentions pure cellulose peaked at 16.2˚-16.3˚ with an area of the crystal, 18.30˚-18.40˚ with an area of amorphous phase, and 21.9˚-22.5˚ which an area of cellulose crystal (Azrina *et al.*, 2017).

Segal's method was followed to define the crystallinity index based on maximum

intensity and intensity peak at the amorphous phase (Segal *et al.*, 1959). Using Segal's equation, the crystallinity index of cellulose is 51.6%, CNCs bleached by 1.5%, H_2O_2 is 87.1%, and CNCs bleached by 10%, H_2O_2 is 93%. These differences occurred due to the concentration of bleaching agents used in delignification (Riama *et al.*, 2011). Results are relatively higher than raw OPEFB, with its crystallinity index of 75% (Lamaming *et al.*, 2016), 77% (Aprilia and Arahman, 2020), and 77.4 (Anjana, 2016). Theoretically, the crystallinity index of CNCs shall be 100% (Lin and Dufresne, 2014). The higher concentration of bleaching agents is equivalent to the crystallinity index of CNCs produced. This result went exactly according to the reaction's kinetic, which explains that the reaction's efficiency will be increased on par with the reactants' concentration. The efficiency of the reaction in acid hydrolysis affects CNCs yield.

Particle Size Distribution

Size measurement of CNCs followed by PSA to observe comprehensive particle surface area capability. PSA results are shown in Figure 4.

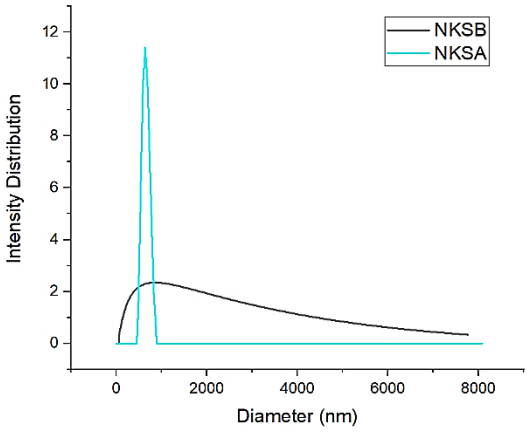

Fig. 4: PSA of CNCs sample, NKSA (blue), NKSB (black)

CNCs bleached by 1.5% H_2O_2 have an average particle diameter of 640 nm. However, CNCs bleached by 10% H_2O_2 have an average particle diameter of 579.8 nm. This result shows that a delignification concentration of plays a major role in increasing the percentage of polymer breakage during hydrolysis. Segregation of lignin affects reaction area expansion, increasing kinetic reaction in the breakage process. Based on Calvo (Calvo *et al.*, 1997), nanoparticle size is around 10-1000nm, meaning CNCs produced are categorized as nanoparticles.

Sensitivity

Biosensing tests have been performed based on the electrochemical cell scheme. Oxidation-reduction occurs in electrochemical cells and is a primary indicator for measuring biosensing capability. Hence, the peak formed on the cathode and the anode is the main parameter in Cyclic Voltammetry as it indicates a reduction-oxidation reaction (Pratiwi, 2018). For further explanation, the reduction reaction is the electron being captured by the medium on an electrochemical cell. The oxidation reaction is the electron released by the medium on an electrochemical cell (Keenan *et al.*, 1980).

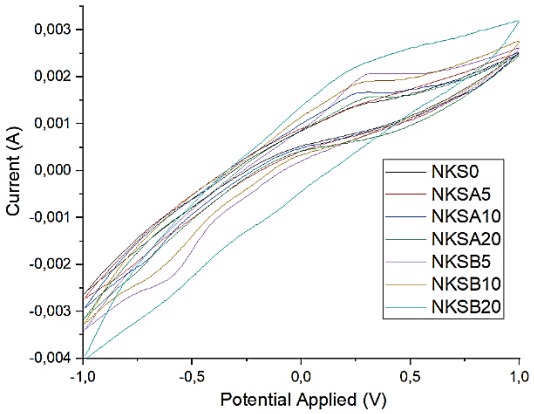

Fig. 5: Cyclic voltammetry (CV) spectrums result

Figure 5 signifies intensity escalation is equivalent to a concentration of CNCs in the composite. The quality of bleaching in CNCs hydrolysis also affects biosensing capability, which is pointed out by the fact that NKSB (higher concentration of bleaching agent) performs better than NKSA (less concentration of bleaching agent). Yet, the soaring intensity of the spectrum does not determine the peaks being formed.

Table 2. Cyclic voltammetry comparison result

Variation		Oxidation(A)	Reduction(A)
Control	0	-	-
NKSA	NKSA5	-	-
(1,5%	NKSA10	0.00116	-0.00118
H_2O_2)	NKSA20	0.00157	-
NKSB	NKSB5	0.00205	-0.00223
(10%	NKSB10	0.00187	-0.00205
H_2O_2)	NKSB20	-	-

Based on Table 2, both three variants, NKSA10, NKSB5, and NKSB10, could be observed. The optimum composite G-CNT/CNC variant is NKSB5, which has the highest oxidation-reduction intensity. To determine the significance of CNCs effect on biosensing capability, a test with One-Way ANOVA was conducted. In the manner of utilization of IBM SPSS Statistic 26 standard below 0.05, results of analysis are presented in Table 3 and Table 4.

Table 3. One Way-ANOVA Biosensor sensitivity analysis of NKSB10 and NKSB5

	Σ^2	δf	$\bar{x}^2$	F	Significance
Between Groups	.000	2	.000	48.602	.000
Within Groups	.000	6	.000		
Total	.000	8			

Table 3 points out that NKSB10 and NKSB5 have no significant differences since both variants are treated by a similar bleaching method: bleached by 10% H_2O_2.

Table 4. Homogenous-ANOVA Biosensor Sensitivity test results

Variation	Subset for alpha = 0.05		
	N	1	2
NKSA10	3	.0023	
NKSB10	3		.0039
NKSB5	3		.0043
Significance		1.000	.273

Means for groups in homogeneous subsets are displayed.
Uses Harmonic Mean Sample Size = 3.000

The addition of CNCs is necessary as the composite must have a nano-sized structure, pores, and extensive surface area to increase the efficiency of substrate penetration, which creates composite G-CNT/CNC that has high sensitivity and responsive biosensors (Esmaeili *et al.*, 2015). CNCs also serve as a convenient stability membrane for GOx enzymes on biosensors (Ren *et al.*, 2009). Graphene and CNTs application are also important to increase the conductivity of the composite (Junaidi and Susanti, 2014). Those components are fused on CNC/G-CNTs fabrication. Results of the analysis tell NKSB5 has better performance than NKSA10 due to treatment given. Therefore, bleaching agents are important in biosensor fabrication.

Accuracy

Cyclic Voltammetry deviation between glucose solution and diabetic urine in the same glucose concentration (136 mg/dL) shows biosensor feasibility to detecting diabetes symptoms. Deviation (De) defined by values differs from the diabetic cyclic

voltammetry value. Observation showed that the accuracy of the cyclic voltammetry is 83.2%. This value can be used as a correction coefficient to determine the actual glucose concentration in a diabetic's urine.

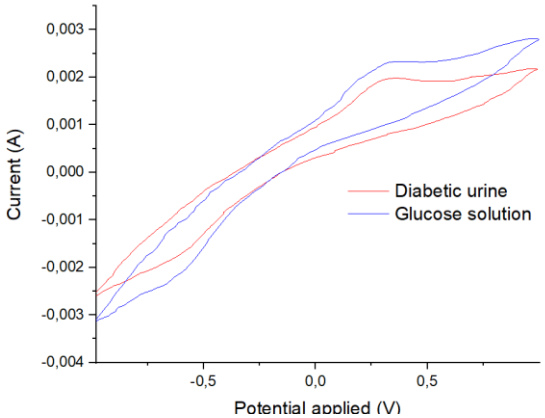

Fig. 6: Different CV results between samples

CONCLUSIONS

Consumption of H_2O_2 10%, as hydrolyzing agent proven, has significantly increased the OPEFB delignification yield rate, which is shown by FTIR spectroscopy noise located at 3550-3200 and 1280 cm^{-1} also CNC that had been produced using H_2O_2 10% as a hydrolyzing agent has a high Crystallinity Index at the value of 93%. In contrast, CNC used H_2O_2 1.5% with Crystallinity Index at 87.1%. Size measurement conducted by PSA shows that CNC with higher and lower hydrolyzing agent concentrations produced CNC particles whose size averaged 578.9 and 600 nm, respectively. Relatively, CNC produced by more concentrated hydrolyzing agents has higher quality CNC compatible as a biosensor since higher concentration means a higher yield rate of a hydrolysis reaction. Followed by cyclic voltammetry, higher quality CNC in G-CNT/CNC has shown better performance, sensitivity, stability, and feasibility on real case usage. Testing between artificial urine and diabetic urine using better biosensors has an accuracy of 83.2 %. Within these data, CNC's quality plays a major role in biosensor fabrication using the component without considering the cellulose source obtained. In a result, OPEFB has great potential as a Celluloses source used as raw material for CNC production, which is of high concern in hydrolysis.

ACKNOWLEDGEMENT

We would like to acknowledge The Indonesia Oil Palm Plantations Fund Management Agency (BPDPKS) for fully funding this research (The selection result of 2020 student palm oil research competition: PENG-3/DPKS/2020). We would also like to express our heartiest gratitude to the Energy Conversion and Chemical Reaction Equilibrium Laboratory in the Study Program of the Chemical Engineering, Faculty of Engineering, Universitas Sebelas Maret for supporting this research.

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