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Comparative Performance of Granulated Carbon and Commercial Resin as Adsorbents for the Extraction of Phytohormones from Coconut Water in Batch Adsorption Experiments

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ABSTRACT

The study evaluated coconut-shell derived granulated carbon as a cost-effective, sustainable alternative to commercial synthetic resin for extracting phytohormones from waste coconut water via batch adsorption experiments. The adsorption performances were compared in terms of the following parameters: isotherm type, adsorption capacity, effective loading, equilibration time, separation factor, and extracted phytohormones. Although commercial resin provides higher adsorption capacity of 61.8 mL/g (versus 14.6 mL waste coconut water/g adsorbent) and desirably lower effective loading of 20 g/L (versus 200 g adsorbent/L waste coconut water), granulated carbon offers faster equilibration time of 1 h (versus 3 h) and more favorable separation factor of 0.008 (versus 0.040) at a lower adsorbent cost of PHP 7/L (versus PHP 300/L waste coconut water). Both adsorbents follow Freundlich isotherm of reversible, physical adsorption, for which all obtained extracts contain gibberellic acid 3, indole-3-acetic acid, with kinetin as the most dominant phytohormone. Despite its lesser capacity, granulated carbon could potentially deliver lower processing costs for phytohormone extraction from waste coconut water especially if considering an upscale production.

Keywords: *adsorption, phytohormones, waste coconut water, granulated carbon, polymeric resin*

INTRODUCTION

Waste coconut water is a by-product of the coconut processing industry. As differentiated from tender coconut water from young coconuts often used as beverage, waste coconut water originates from the mature coconuts used in desiccated coconut and virgin coconut oil industries (Mu et al., 2024). Large volumes of these waste coconut water effluents are potential environmental hazards and require proper treatment, which can be very costly (Kumar et al., 2003). As an alternative to treatment, waste coconut water can instead undergo novel value-adding procedures such as recovery of high-value phytohormones.

Phytohormones are naturally-occurring plant growth regulators (PGR) which exert beneficial effects on seed germination, tissue development, and flowering in plants (Checker et al., 2018; Dilworth et al., 2017; Santner et al., 2009; Zhao et al., 2021). Phytohormone application even at low concentrations have been correlated with improved harvest (Fu et al., 2011). Auxins such as indole-3-acetic acid (IAA), gibberellins such as gibberellic acid 3 (GA3), and cytokinins such as kinetin are the common phytohormones in waste coconut water, and these can be further concentrated for potential commercial use (Jayawardena et al., 2021; Yong et al., 2009).

Researchers led by Dr. Juanita Mamaril from the National Institute of Molecular Biology and Biotechnology in the University of the Philippines Los Baños developed crude extraction methods for phytohormones in waste coconut water and generated a more stable concentration which worked as soaking medium for germinating seeds, growth hormone substitute for tissue culture, and foliar application for flowering (Mamaril et al., 1988; Mamaril et al., n.d.). However, the halt in research in 1970 left the production technology at the laboratory level amidst continuing demand for local, cheaper, and organic alternatives to the imported and synthetic PGRs. Thus an upscale study was considered to open up opportunities for better commercial production at a larger scale.

Column adsorption is considered to have a good scale up potential for phytohormone extraction from waste coconut water, with its capacity to receive significant volume without compromising selectivity. Adsorption as a separation process has already been demonstrated to allow selective phytohormone extraction from waste coconut water using various agricultural biochar (Banzuela et al., 2024; Castillo et al., 2024; Paranaque et al., 2024). As an initial step for column adsorption scale up, batch adsorption studies for the extraction of phytohormones from waste coconut water was conducted using two promising adsorbents – a commercially available polymeric resin, and coconut-shell-derived granulated carbon. The study aimed to assess the better adsorbent in terms of phytohormone adsorption performance in terms of isotherm type, adsorption capacity, effective loading, equilibration time, separation factor, and extracted phytohormones. Additionally, an efficient, low-cost analytical method for determining crude phytohormone concentration was also explored.

MATERIALS AND METHODS

Adsorbent preparation

Both polymeric resin and granulated carbon were commercially obtained. The specific type of polymeric resin was selected based on the nature of the solvent (coconut water) and the chemistry of phytohormones IAA, GA3, and kinetin using a four-stage decision diagram. The polymeric resin has a harmonic mean particle size of 0.49 to 0.69 mm, a specific gravity of 1.01 to 1.03, a porosity of 0.5 at most, and a maximum specific surface area of 750 mm^2/g. On the other hand, the granulated carbon was obtained from coconut shell biomass, and did not undergo activation based on the supplier's information. The granulated carbon has a mesh size of 6 × 12, an average particle size of 2.55 mm, a specific gravity of 1.022, a porosity of 0.55, and a sphericity of 0.8. The adsorbents were kept in dry storage until needed.

Waster coconut water preparation

Fresh waste coconut water samples from a local virgin coconut oil production plant were collected, then rapidly cooled, initially clarified via cloth filter, and then suction-filtered using Whatman #1 filter paper. The filtered samples were pooled, transferred to small containers, and then frozen to 4°C or lower. When needed, the frozen samples were thawed at room temperature and prepared for the batch adsorption experiments.

UV-Vis spectrophotometry

Thawed waste coconut water samples were subjected to absorbance measurements in a quartz cuvette by UV-Vis spectrophotometry (Shimadzu, UV-1601, Japan). A wave scan between 190 nm to 300 nm was done in order to obtain the wavelength of maximum absorbance. The wavelength values for the wave scan were considered to be the range where phytohormones exhibit maximum absorbance. Thus, absorbance measurements at the wavelength of maximum absorbance of the phytohormones was considered to be a quick and convenient method for estimating phytohormone concentration.

Single flask adsorption experiment

The adsorption time for each adsorbent was determined via interval sampling in single batch equilibration done in duplicate. A 100-mL sample of waste coconut water in an Erlenmeyer flask was loaded with specified amount of adsorbent, and then agitated using a tabletop shaker. The amount of adsorbent employed (2.5 g for resin, and 200 g for granulated carbon) were based on preliminary experiment for effective loading. At designated time intervals, 0.5-mL portions were collected via 1-mL plastic syringe. The absorbance of these aliquots was measured using UV-Vis spectrophotometer and then plotted as a function of time. The equilibration time was determined from the graph at the point where the absorbance became relatively constant as verified via two-tailed t-test at 95% confidence level.

Single flask adsorption experiment

Using the obtained equilibration time, the effective loading for each adsorbent was determined via batch adsorption equilibrations at different levels of adsorbent loading: (1) between 1 g/L and 50 g/L for resin, and (2) between 5 g/L and 300 g/L for granulated carbon. After reaching equilibrium at room temperature, waste coconut water was separated via gravity filtration using Whatman #1 filter paper. The absorbance (A) of each equilibrated waste coconut water sample was measured using UV -Vis spectrophotometer and plotted as a function of adsorbent loading. The adsorbent from each sample was brought into contact with 50-mL of ethanol for a minimum of 10 hours and agitated to ensure complete desorption. Similarly, the absorbance of each ethanol extract was measured using UV-Vis spectrophotometer and plotted as a function of adsorbent loading. The effective adsorbent loading was then determined from the two plots at the point where the absorbance readings were not significantly different from each other as verified by analysis of variance (ANOVA) at 95% confidence level.

Isotherm fitting

The absorbance data from the previous batch equilibrations at different loadings were fitted into the linearized form of Langmuir and Freundlich equations, respectively (**Equations 1 and 2**). The isotherm which has a value of linearity coefficient (R^2) closer to unity was considered to better describe the corresponding phytohormone – adsorbent adsorption system.

$$\frac{C_e{}'}{q_e{}'} = \left(\frac{C_0}{q_{max}}\right)\frac{A_e}{A_0} + \frac{K}{q_{max}}$$ ***Equation 1***

$$\ln\left(\frac{q_e{}'}{C_0}\right) = (1/n)\ln\frac{A_e}{A_0} + \ln\left(K_f C_0{}^{1/n-1}\right)$$

Equation 2

Repeated flask adsorption experiment

Using the obtained effective loading, the actual maximum adsorption capacity (q_{max}) of each adsorbent was determined via repeated batch equilibrations following the earlier procedure. After the equilibration time, the adsorbent was filtered out using Whatman #1 filter paper. The absorbance of the equilibrated waste coconut water was measured using UV-Vis spectrophotometer and then compared to its original absorbance.

Batch equilibration using the obtained adsorbent and fresh waste coconut water were made until no significant change in the final absorbance was observed, or as ratio of original and final absorbance values approaches unity. The equilibrium adsorption capacity was approximated (**Equation 3**).

$$\left(\frac{q_{max}}{C_0}\right) = \frac{\sum_{i=1}^{n}\left(1 - \frac{A_{e,i}}{A_{0,i}}\right)}{W_{ads}/V} \quad \textit{Equation 3}$$

After the repeated equilibrations, the adsorbent was collected and brought into contact with 50-mL of ethanol for a minimum of 10 hours and agitated to ensure complete desorption. The absorbance of obtained ethanol extract was then measured using UV-Vis spectrophotometer. The absorbance data from isotherm testing and repeated batch equilibration were employed to determine the adsorption favorability. The constructed Y-versus-X isotherm plot and the calculated separation factor (*La*) from the linearized alternative Langmuir equation were then evaluated.

Thin layer chromatography

To confirm the presence of phytohormones in the ethanol extracts, thin layer chromatography (TLC) analyses using petroleum ether – diethyl ether – glacial acetic acid (12:6:1 v/v) solvent system were performed. The standard IAA, GA3, and kinetin solutions, and phytohormone extracts (pre-concentrated using rotary evaporator) were spotted onto commercially available silica gel plates using 10-µL syringe. The plates were dried, placed into developing chambers, and then air dried upon development of chromatogram with an identified solvent front.

The dried plates were placed in an iodine chamber to visualize the spots. The distances of the spot and the solvent front were measured to determine the retention factor (*Rf*) of each spots. The presence of phytohormones in the extract was confirmed via two -tailed t-test at 95% confidence level on the calculated *Rf* values of the spot and of the matching phytohormone standard.

RESULTS AND DISCUSSION

Wavelength of maximum absorbance for phytohormones

In determining the concentration of phytohormones, techniques such as gas and liquid chromatography coupled with mass spectrometry, immunoassays, and radioimmunoassays are typically employed. However, since these methods are time-consuming as they require sample purification prior to analysis, not to mention the high cost of these instruments (Shcherbatiuk et al., 2020).

Thus, a more convenient method (given the resource limitations) to estimate the concentration of phytohormones from waste coconut water was considered via UV-Vis spectrophotometry. In this method, the wavelength of maximum absorbance must be first determined since here is where an analyte exhibits greatest sensitivity and therefore, attaining a more accurate determination of crude phytohormone concentration. The obtained wave scan showed two peaks: first at 206.4 nm wavelength and second at 267.5 nm wavelength. Despite having smaller absorbance measurement, the second peak was selected standard as it aligned with the literature value conventionally established at 265 nm (Ma et al., 2008).

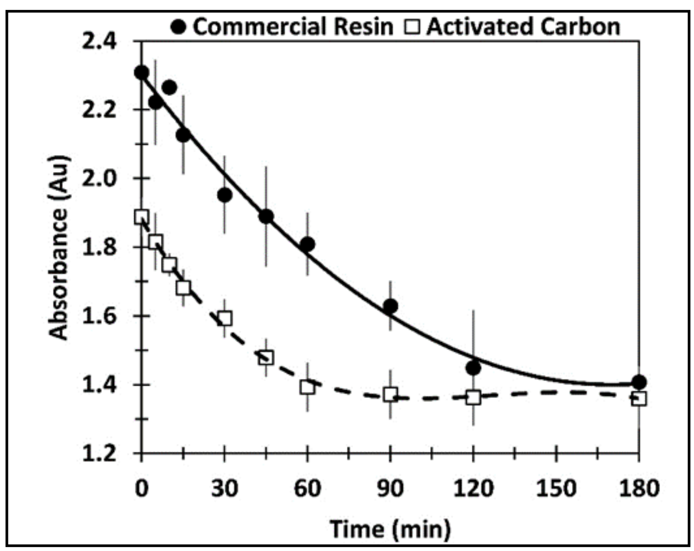

Figure 1. Time course graph of absorbance for determination of equilibration time of phytohormone – adsorbent system.

Faster equilibration time for granular carbon

One aspect in adsorbent selection is the required contact time. Equilibration time, also known as optimum contact time, is the minimum amount of time sufficient to reach equilibrium as verified via two-tailed t-test at 95% confidence level. At equilibrium, the rate of adsorption is equal to the rate of desorption. As shown in **Figure 1**, the commercial resin exhibited longer equilibration time of 180 minutes (3 hours) as compared to that of granulated carbon with 60 minutes (1 hour), which is shorter by three-folds.

Freundlich isotherm for both adsorbents

The nature of adsorption system is also studied in adsorbent selection. Adsorption phenomenon is commonly studied thru adsorption isotherm, which illustrates the relationship of the equilibrium amount of adsorbate per mass adsorbent and the equilibrium concentration of the adsorbate in the bulk fluid at a specified temperature (Saleem et al., 2024).

As presented in **Figures 2 and 3**, both the commercial resin and granulated carbon adsorption systems follow the Freundlich isotherm based on the values of linearity coefficients closer to unity with parameter *n* equal to 2.705 and 2.727, respectively.

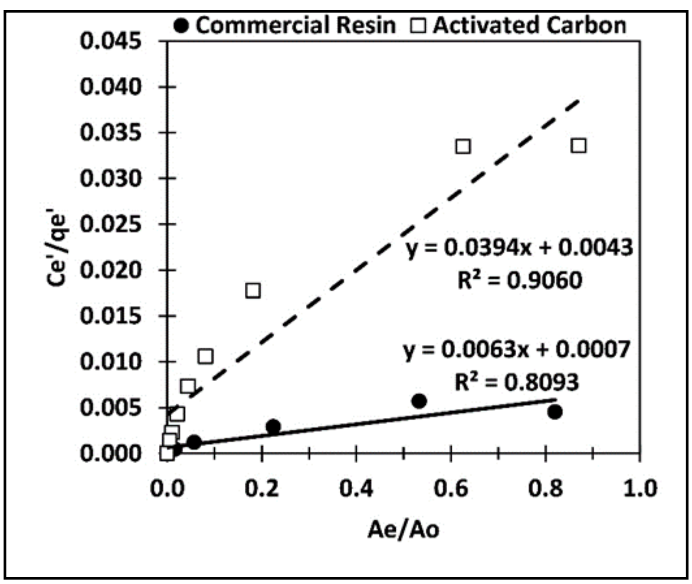

Figure 2. Linearized Langmuir isotherm plot for phytohormone – adsorbent system at 27 °C.

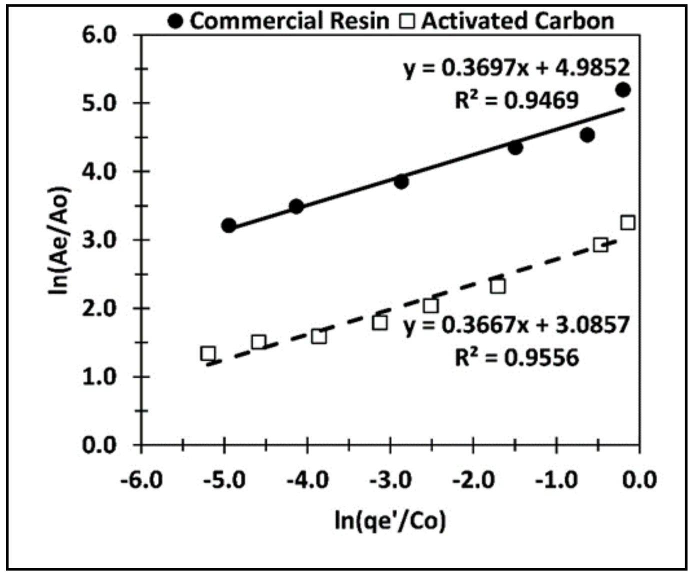

Figure 3. Linearized Freundlich isotherm plot for phytohormone - adsorbent system at 27 °C.

This parameter ($n > 1$) indicates that a physical adsorption occurred (Desta, 2013). It is desirable since physical adsorption is reversible, thus granting the regeneration of adsorbent and the recovery of extracted phytohormones feasible. Freundlich isotherm also indicates that the adsorption proceeds in a multilayer mechanism (versus monolayer mechanism) which may suggest synergy among adsorbed chemistries.

Based on their isotherm parameter q_{max}, the relative adsorption capacities of commercial resin and granulated carbon are obtained at 157.6 mL/g and 25.4 mL/g, respectively. Relative adsorption capacity can be understood as the volume of sample, with an initial concentration (C_0), which provides the maximum adsorbate content that can be adsorbed by a unit mass of adsorbent at its full capacity.

Higher actual maximum adsorption capacity for commercial resin

In adsorbent selection, the capacity is often determined. As illustrated in **Figure 4**, the commercial resin required higher number of equilibrations in comparison with granulated carbon as reflected in their absorbance ratios approaching unity. Translated into actual relative adsorption capacity, commercial resin demonstrated a larger capacity of 61.8 mL/g vis-à-vis granulated carbon's

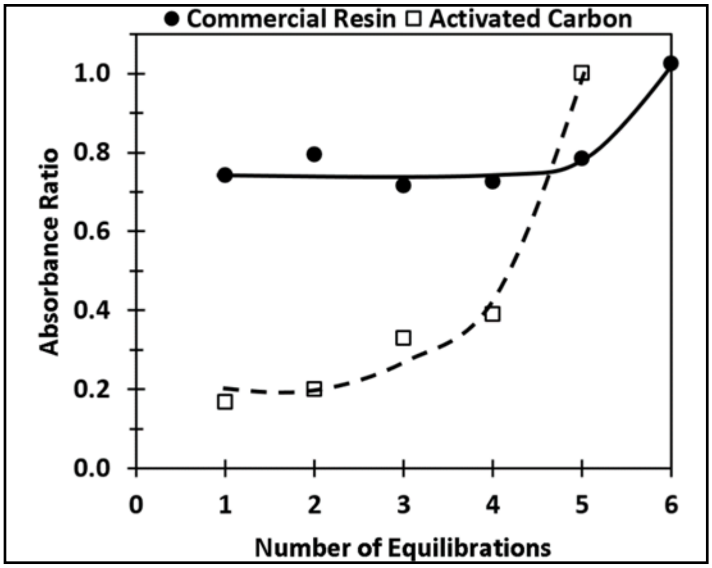

Figure 4. Repeated batch equilibration plot for phytohormone - adsorbent system.

14.6 mL/g. Bigger capacity indicates greater number of sites for adsorption on the adsorbent's surface.

Both of the resulting actual capacities are almost twice lower than that of their corresponding isotherm capacities, which suggest that repeated equilibrations provides a more accurate and realistic value than isotherm testing (de Araujo et al., 2024).

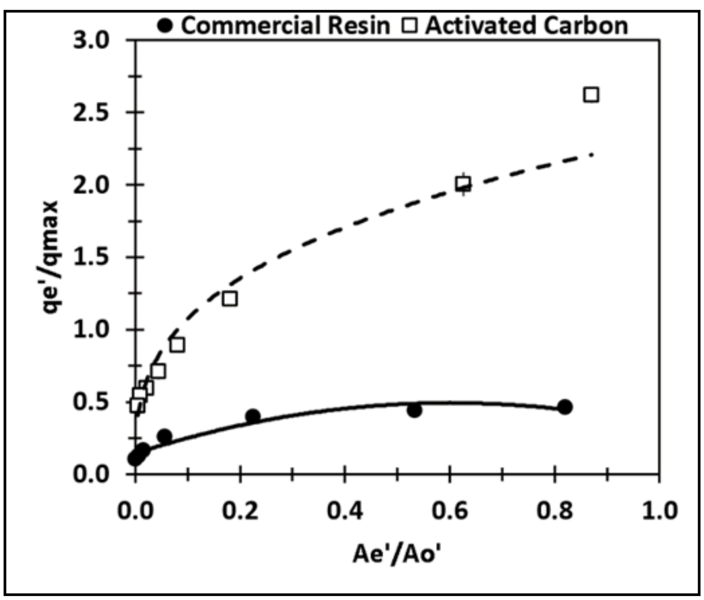

Figure 5. Isotherm plot for phytohormone – adsorbent system.

More favorable adsorption for granular carbon

Another consideration is the adsorption system's favorability. Based on the general isotherm shapes, the concave plots for both system as presented in **Figure 5** indicate favorable adsorption (Vassilis, 2010). Favorable adsorption means that adsorption continues even at very low concentrations of the adsorbate.

In addition, phytohormone adsorption on granulated carbon prove to be more favorable than on commercial resin, as revealed by their separation factor much lower than unity (0.008 vs. 0.040). This also denotes that a slight increase in the amount of adsorbent results to considerable increase in the amount of phytohormones adsorbed.

Lower effective adsorbent loading for commercial resin

As a consequence of good adsorption favorability in a concentration-limited system such as the case of phytohormones in waste coconut water, an effective adsorbent loading can be determined. As described in **Figures 6 and 7**, commercial resin obtained a lower effective adsorbent loading of 20 g/L in contrast with granulated carbon's effective loading

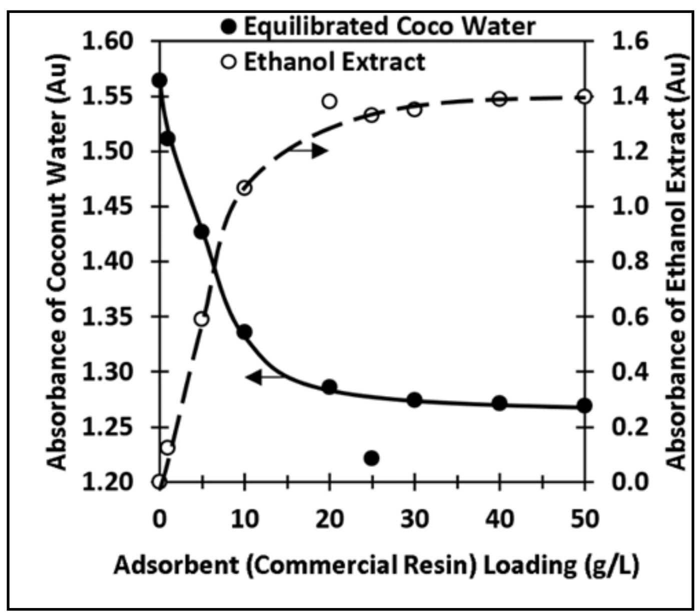

Figure 6. Absorbance of equilibrated waste coconut water and ethanol extract as a function of commercial resin loading.

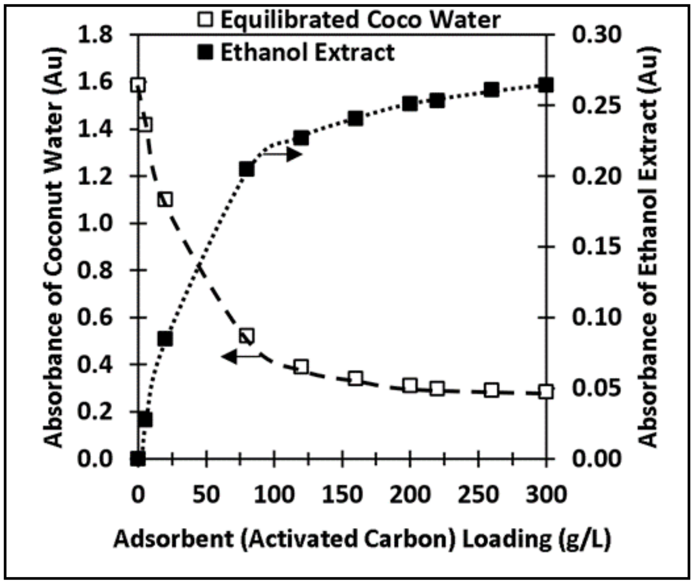

Figure 7. Absorbance of equilibrated waste coconut water and ethanol extract as a function of granulated carbon loading.

of 200 g/L as verified by ANOVA at 95% confidence level. The effective adsorbent loading is the minimum amount of adsorbent that can effectively adsorb the adsorbate content per unit volume of sample. Thus, it is expected that increasing the amount of adsorbent beyond their effective loading will no more extract significant amount of phytohormones from waste coconut water.

Although granulated carbon requires higher loading than commercial resin to extract phytohormones from waste coconut water, the initial estimated adsorbent cost per volume processed is much lower for granulated carbon at PHP 7 per liter than commercial resin with around PHP 300 per liter of waste coconut water. These cost estimates are based on the effective loadings and the current local market prices of these adsorbents.

Presence of phytohormones in extracts for both adsorbents

The presence of kinetin (cytokinin), indole acetic acid (auxin), and gibberellic acid 3 (gibberellins), were confirmed by TLC via two-tailed t-test at 95% confidence level in all ethanol extracts from the batch adsorption experiments, respectively, in decreasing relative amounts. Kinetin was estimated to be the dominant phytohormone found in the extracts via densitometric analysis.

Faster adsorption of phytohormones at lower cost for granulated carbon

Although both granulated carbon and commercial resin follows reversible, physical adsorption which would allow desorption at ambient conditions, it is the commercial resin that exhibits significantly higher adsorption capacity for phytohormones in its isotherm, actual, and effective loading parameters which may be attributed to its high specific surface area.

However, it is the granulated carbon that provides faster equilibration time and better separation factor while extracting the same set of phytohormones at a considerably lower cost. While capacity is important, the kinetics of a process also factors into the sizing of required equipment as longer processing time would result to bigger equipment for the same volume of waste coconut water. This

Table 1. Summary of performance parameters of adsorbents for phytohormone extraction.

Performance Parameter	Granulated Carbon (GC)	Commercial Resin (CR)	Superior
Adsorption isotherm	Freundlich	Freundlich	GC, CR
Adsorption type	physical	physical	GC, CR
Isotherm capacity (mL/g)	25.4	157.6	CR
Actual capacity (mL/g)	14.6	61.8	CR
Effective loading (g/L)	200	20	CR
Equilibration time (min)	60	180	GC
Separation factor	0.008	0.040	GC
Extracted phytohormones	kinetin, IAA, GA3	kinetin, IAA, GA3	GC, CR
Adsorbent cost (PHP/L)	7	300	GC

consideration plus the synthetic nature of polymeric resin which are typically imported resulting to its expensive cost highlights the economic advantage of granulated carbon. In addition, granulated carbon can possibly still undergo further activation to increase its surface area and improve its affinity for phytohormones to a level at par with commercial resin. Granulated carbon offers a sustainable, locally -derived adsorbent with substantial adsorption performance for the extraction of phytohormones from waste coconut water.

CONCLUSIONS

Batch adsorption studies were conducted in order to assess the adsorption performance of two potential adsorbent for the extraction of phytohormones from waste coconut water: (1) a commercially available resin, and (2) coconut-shell-derived granulated carbon. Initially, UV-Vis spectrophotometry at 267.5 nm wavelength of maximum absorbance was established as a convenient analytical method for estimating phytohormone concentration. Both adsorbents were determined to follow the Freundlich isotherm of reversible, physical adsorption.

However, commercial resin exhibited higher adsorption capacity (61.8 versus 14.6 mL waste coconut water/g adsorbent) and desirably lower effective adsorbent loading (20 versus 200 g adsorbent/L waste coconut water) as compared to granulated carbon. On the other hand, granulated carbon was evaluated with faster equilibration time (1 h versus 3 h) and greater adsorption favorability. The presence of phytohormones (IAA, GA3, kinetin) were confirmed via thin layer chromatography in all obtained ethanol extracts.

Although the commercially available resin presented better adsorption performance, the much cheaper granulated carbon, with its demonstrated performance, can be considered as a more cost-effective alternative. In addition, the results of these batch adsorption studies will also be useful in the process design and scale-up of continuous column adsorption of phytohormones from waste coconut water.

RECOMMENDATIONS

Column adsorption experiments using coconut-shell derived granulated carbon will provide better insights into useful scale up parameters for further feasibility studies. Alongside this, modelling column adsorption process will aid in simulating design and operation of larger-scale extraction of phytohormones from waste coconut water. Additionally, regeneration strategies for adsorbents such as activation of granulated carbon and alternative eluents for desorption can improve the sustainability and economic viability of the process. Finally, more specific and accurate analytical techniques such high-performance liquid chromatography can investigate adsorption behavior of individual phytohormones especially at various physico-chemical conditions of the waste coconut water.

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CONTRIBUTION STATEMENT

Mart Merwin C. Magboo: Conception of the project design, Data collection, Data encoding and processing, Data analysis, Writing of the article, Results and discussion. **Ellison E. Borbo:** Conception of the project design, Data collection, Data encoding and processing, Data analysis, Results and discussion. **Monet Concepcion M. Detras:** Conception of the project design, Data analysis, Writing of the article, Results and discussion. **Jewel A. Capunitan:** Conception of the project design, Data analysis, Writing of the article, Results and discussion. **Catalino G. Alfafara:** Conception of the project design, Data analysis, Writing of the article, Results and discussion. **Veronica P. Migo:** Conception of the project design, Data analysis, Writing of the article, Results and discussion.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY

The data is available upon request to the corresponding author.

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