UNIVERSITI PUTRA MALAYSIA

ADSORPTION STUDIES OF I-NAPHTHALENEACETIC ACID AND 2,4-DICHLOROPHENOXYACETIC ACID ON ACTIVATED CARBON IN AQUEOUS SOLUTION AND OIL PALM TISSUE CULTURE MEDIA

NORHAFIZAH BINTI SARMIJAN

FSAS 2002 37
ADSORPTION STUDIES OF 1-NAPHTHALENEACETIC ACID AND 2,4-DICHLOROPHENOXYACETIC ACID ON ACTIVATED CARBON IN AQUEOUS SOLUTION AND OIL PALM TISSUE CULTURE MEDIA

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

March 2002
A study on the adsorption of activated carbon on oil palm culture media was carried out in two main parts, the adsorption of plant growth regulators 1-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) and root initiation of oil palm tissue culture. Four commercially available activated carbons, namely AC1, AC2, AC3 and AC4 were used in this study.

The adsorption of NAA and 2,4-D by activated carbons were determined using UV/Vis spectrophotometer. The adsorption of NAA and 2,4-D at different concentrations increased rapidly at the initial stage and then followed by a slower uptake until the equilibrium is established in 40 and 30 minutes, respectively. The maximum uptake was achieved at higher concentration for both of the plant growth regulators. The higher maximum uptake was obtained for higher surface area of the activated carbons.
The adsorption isotherms have been obtained at various concentrations. The adsorption isotherm for NAA on AC3 was better fitted to Langmuir equation compared to other activated carbons. On the other hand, Freundlich equation was slightly better fitted to the adsorption of NAA for other activated carbon samples. It was found that the adsorption capacity of NAA on AC3 was higher compared to the other activated carbons. Adsorption isotherm of 2,4-D showed that the Langmuir equation isotherm was better fitted to the experimental data for AC1 and AC3, but AC2 and AC4 are better fitted to the Freundlich equation. It was observed that AC4 showed the highest adsorption capacity. The n value of AC4 indicated that AC4 was the effective adsorbent for 2,4-D.

The ability for root initiation of shoots from the oil palm explants was studied on different treatments. The results indicate that there is no significant difference between the clones but the different samples of activated carbon added into the rooting medium showed a highly significant difference. Addition of activated carbon in the rooting medium improved the overall rooting capacity.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains.

KAJIAN JERAPAN ASID 1-NAFTALENASETIK DAN ASID 2,4-DIKLOROFENOKSIASETIK KE ATAS KARBON TERAKTIF DI DALAM LARUTAN AKUAS DAN MEDIA TISU KULTURA KELAPA SAWIT

Oleh

NORHAFIZAH BINTI SARMIJAN

Mac 2002

Pengerusi : Profesor Madya Zulkarnain Zainal, Ph.D.
Fakulti : Sains dan Pengajian Alam Sekitar

Kajian jerapan karbon teraktif ke atas media kultura kelapa sawit telah dijalankan di dalam dua bahagian utama: kajian jerapan penggalak tumbesaran tumbuhan asid 1-naftalenasetik (NAA) dan asid 2,4-diklorofenoksiasetik (2,4-D) dan penghasilan akar kelapa sawit di dalam kultura tisu. Empat karbon teraktif komersil, iaitu AC1, AC2, AC3 dan AC4 telah digunakan di dalam kajian ini.

Jerapan NAA dan 2,4-D oleh karbon teraktif telah ditentukan dengan spektrofotometer UV tampak. Jerapan NAA dan 2,4-D pada kepekatan yang berlainan meningkat secara mendadak pada peringkat awal dan kemudian diikuti oleh proses yang perlahan sehingga mencapai keselimbahan pada masing-masing 40 dan 30 minit. Jerapan maksimum boleh dicapai pada kepekatan yang tinggi bagi kedua-dua penggalak tumbesaran tumbuhan. Nilai jerapan maksimum yang tertinggi boleh diperolehi dengan luas permukaan karbon teraktif yang tinggi berbanding luas permukaan yang rendah.
Isoterma jerapan telah diperolehi pada kepekatan yang berlainan. Isoterma Jerapan bagi NAA ke atas AC3 adalah lebih mematuhi persamaan Langmuir berbanding karbon teraktif yang lain. Manakala persamaan Freundlich memberikan keputusan yang lebih baik bagi sampel karbon teraktif yang lain. Didapati bahawa kapasiti jerapan NAA ke atas AC3 adalah lebih tinggi dari karbon teraktif yang lain. Isoterma jerapan 2,4-D pula menunjukkan persamaan isoterma Langmuir lebih mematuhi data-data eksperimen bagi AC1 dan AC3. Manakala, AC2 dan AC4 lebih mematuhi persamaan Freundlich. Didapati AC4 menunjukkan nilai kapasiti jerapan yang tertinggi. Nilai n bagi AC4 menunjukkan bahawa AC4 merupakan penjerap yang berkesan bagi 2,4-D.

Keupayaan untuk menggalakkan pertumbuhan akar dari eksplan kelapa sawit dikaji dengan pelbagai rawatan. Keputusan telah menunjukkan bahawa tiada perbezaan yang nyata di antara klon tetapi rawatan dengan menggunakan karbon teraktif yang berlainan menunjukkan perbezaan yang nyata. Penambahan karbon teraktif ke dalam media pengakaran memberikan kesan yang baik secara keseluruhannya.
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Alhamdulillah is the first phrase that comes out of my lips when I am typing this thesis, all praises should go to ALLAH for giving me strength and patience to the completion of this thesis. Many people also have played an important role in the completion of this thesis. But let my thanks go to a few people that can apply equally well for the others.

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My deepest gratitude dedicates to my husband, Noor Hamka Abdul Halim who had held my hands through this journey. Although chemistry is such an alien subject to him and he could not stand the idea that scientist are more important than engineers in this world. Nonetheless his loving support has no boundary and kept me going at the roughest times and my precious baby, Nurhamizah Aida for all the hugs and kisses that let my heart at rest. Last but not least, my greatest debt extended to all my family for their endless encouragement and love, which had made my life more meaningful.

Finally, I would like to acknowledge the financial support of this research by the Ministry of Science, Technology and Environment through IRPA mechanism, which enabled this work to be carried out.
I certify that an Examination Committee met on 19th March 2002 to conduct the final examination of Norhafizah Binti Sarmijan on her Master of Degree thesis entitled "Adsorption Studies of 1-naphthaleneacetic Acid and 2,4-dichlorophenoxyacetic Acid on Activated Carbon in Aqueous Solution and Oil Palm Tissue Culture Media" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

NORHAFIZAH SARMIJAN

Date: 14 JUN 2002
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LIST OF ABBREVIATIONS

NAA  1-naphthaleneacetic acid
2,4-D  2,4-dichlorophenoxyacetic acid
IUPAC  International Union of Pure and Applied Chemistry
BET  Brunauer, Emmet and Teller
FTIR  Fourier Transform Infrared Analysis
UV/Vis  Ultra-violet/visible
ICP  Inductively Coupled Plasma
MS  Murashige and Skoog's
SOA  MS liquid medium with NAA added with unsaturated AC4
SOB  MS liquid medium without NAA added with unsaturated AC4
SOD  Distilled water added with unsaturated AC4
SNB  MS liquid medium without NAA added with AC4 saturated with NAA
SND  Distilled water added with AC4 saturated with NAA
CHAPTER 1
INTRODUCTION

Activated carbon has been used in plant tissue culture media to improve culture growth and/or promote morphogenesis in a wide variety of species. Generally, better growth responses of the plant tissues are associated with the addition of activated carbon to the medium (Zaghmout & Torello, 1988). The beneficial effects of activated carbon are attributed to the removal of inhibitory substances from the media, produced either on autoclaving (Weatherhead et al., 1978) or by the tissue itself (Fridborg et al., 1978). Through its adsorptive capacity, activated carbon could reduce the toxic effects of high level of 2,4-dichlorophenoxyacetic acid (2,4-D) (Zaghmout & Torello, 1988) and indolebutyric acid (IBA) (Constantin et al., 1977) in culture media. Detrimentally the adsorption of auxins and cytokinins by activated carbon might render these essential substances inactive for tissue culture purposes (Weatherhead et al., 1978). Although beneficial effect of activated carbons have been documented, it is a complex substance and the entire range of its effects on tissue culture media and the subsequent growth and morphogenesis of tissue culture is unknown. Activated carbon is not a growth regulator but it modifies the medium by absorbing a wide range of compounds and can therefore have an unpredictable effect.

The absence of precise information on the concentrations of growth regulators in tissue culture media in the presence of activated carbon is a major obstacle to determining the ideal levels of growth regulators to be added. Plant growth regulators such as 1-naphthaleneacetic acid (NAA) and 2,4-
dichlorophenoxyacetic acid (2,4-D) which included in a culture media are usually bind to activated carbon and the adsorption behavior of the hormone were studied. For this reason, these studies were conducted to have a more defining understanding of the effect of activated carbon in culture media and the adsorbability of NAA and 2,4-D on the activated carbon.

Activated Carbon

Charcoal has been used as a purifying and decolorizing compound for liquids since the 18th Century. Charcoal used prior to the present Century was produced by pyrolysis only. They were not subsequently oxidized and then purified to produce the activated charcoal (activated carbon), which is widely used at present. Activation of carbon by treatment of the pyrolysis with a stream of carbon dioxide to produce an activated carbon of superior adsorbing properties is covered in patents dated 1900 till 1901 by the Russian inventor Ostrejko (Yam et al., 1990).

Activated carbon is the generic term used to describe a family of carbonaceous adsorbents with a highly crystalline form and extensively developed internal pore structure. A wide variety of activated carbon products are available exhibiting markedly different characteristics depending upon the raw material and activation technique used in their production. In selecting an activated carbon, it is important to have a clear understanding of both the adsorptive and physical characteristics of the material in order to optimize the performance capabilities.
Manufacturing of Activated Carbon

Different kinds of activated carbons are prepared for different purposes. Activated carbons that are prepared for the adsorption of gases are harder and denser than those used for purification of liquids. Activated carbon that produced from wood, wood waste, paper-mill waste and peat are generally used in culture media (Pan & Van Staden, 1998). Activated carbon can also be produced from petroleum coke and coals.

Generally, the preparation of activated carbon from the carbonaceous material may use either a chemical or physical activation agents. In a chemical process, the starting materials are treated with chemicals, to restrict the formation of tars, which are produced after kneading, carbonizing and washing. This method may generate a secondary environmental pollution due to the use of chemicals and so it is not used as commonly as the physical method. The physical process involves two stages; pyrolysis (also called carbonization) and activation. In the first stage, the starting materials are carbonized in an inert atmosphere at a moderate temperature of about 700-900°C to remove the volatile matter and produce chars with rudimentary pore structures. Subsequently, in the second stage, the resulting chars are subjected to a partial and controlled gasification at a higher temperature (above 900°C) with oxidizing gases.

Pyrolysis is an important step in the preparation of activated carbons because the chars property depends not only on the nature of the raw material but also on the history of the pyrolysis (Bansal et al., 1988). The porosity of activated carbon is
developed by oxidation of about 50% of the pyrolized residue during the activation process (Weber et al., 1980). The purpose of activation is to enhance the pore volume created during the pyrolysis process and to create more porosity. In this process, charcoal is heated to a sufficient high temperature to cause an extensive burn off degradation of noncarbon impurities, leaving high surface area and highly porous product (Mattson and Mark, 1971).

Characterization of Activated Carbon

Activated carbons are characterized by very large specific area ranging from 600 to 2000 m²/g and pore distribution ranging from 10 µM to 500 µM (Yam et al., 1990). This extensive surface area is almost exclusively intraparticulate. Structurally, activated carbon can be considered to consist of rigid interlinked clusters of microcrystallite (Snoeyink et al., 1969), each microcrystallite comprising a stack of graphitic planes. The diameter of the planes forming the microcrystallite, as well as the stacking height, has been estimated at 2-5 nm (Snoeyink et al., 1969), indicating that each microcrystallite consists of about 5 – 15 layers of graphitic planes. Each carbon atom within a particular plane is joined to three adjacent carbon atoms by sigma bonds, with the fourth electron participating in a π bond.

The fact that activated carbon has an extremely large surface area per unit weight makes it an extremely efficient adsorptive material. The activation process produces many pores within the particles, and it is the vast areas of the walls within these pores that accounts for most of the total surface area of carbon. The high surface area and complex pore structure of activated carbons resulting from physical
or chemical activation processes. The structure of an activated carbon is composed of pores classified into three groups, namely micropores, mesopores and macropores. A convenient classification of pores according to their average width originally proposed by Dubinin in 1966 and now officially adopted by the International Union of Pure and Applied Chemistry (IUPAC) is summarized in Table 1.

Table 1: Classification of pores according to their width (after IUPAC, 1972)

<table>
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<th>Micropores</th>
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<td>Mesopores</td>
<td>Between 2 and 50 nm</td>
</tr>
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<td>Macropores</td>
<td>More than 50 nm</td>
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The basis of the classification is that the pore size corresponds to their function in adsorption. Micropores usually account for over 95% of the total surface area of the activated carbons (Allen et al., 1995). In micropores, the interaction potential is significantly higher than in wider pores owing to the proximity of the walls and the amount adsorbed is correspondingly enhanced. In mesopores, capillary condensation occurred and it is also perform as the main transport arteries for the adsorbate. In the macropores range the pores are so wide that it is used as the entrance to the activated carbon. Adsorbate molecules penetrate through the macropores and into the micropore structure. Figure 1 illustrates the adsorption process, which involves the pores.
Figure 1: The adsorption process which involves the pores (Cheremisinoff, 1993).
Adsorption

Adsorption, which is often confused with absorption, refers to the adhering of molecules of gases and liquids to the surfaces of porous solids. Adsorption is a surface phenomenon while the absorption is an intermingling or interpenetrating of two substances. The binding to the surface is usually weak and reversible.

Adsorption, the taking up by the surface of a solid or liquid (adsorbent) of the atoms, ions, or molecules of a gas or other liquid (adsorbate). Porous or finely divided solids can hold more adsorbate because of the relatively large surface area exposed. Similarly, the adsorbent surface of a quantity of liquid is increased if the liquid is divided into fine droplets. In some cases, the atoms of the adsorbate share electrons with atoms of the adsorbent surface, forming a thin layer of chemical compound. Adsorption is also an important part of catalysis and other chemical processes.

Activated carbon possesses strong adsorptive properties and is usually used in chemistry to adsorb both gases and dissolved solids. When added to tissue culture media, activated carbon was commonly thought to remove only growth inhibitory substances produced by tissues or present in the ingredients of the medium, but it is still not clear that growth regulators can also be adsorbed and made unavailable to the plant.

The chemical nature of the surface of activated carbon is regarded as the important factor, apart from the porous structure, that determines the adsorption properties. Adsorption was considered as a direct function of the properties of the