

**CHARACTERIZATION OF FREEZE DRIED AND OVEN DRIED NITROHUMIC
ACIDS DERIVED FROM THE MUKAH COAL, SARAWAK.**

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DECLARATION

No portion of the work referred to in this description has been submitted in support of an application for another degree of qualification of this and any other university or institution of higher learning.

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Characterization of the freeze dried and oven dried nitrohumic acids derived from the Mukah coal, Sarawak.

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ABSTRACT

Alkaline extraction was used to extract humic acids from Mukah coal. The alkaline solution used to perform this extraction is potassium hydroxide, KOH. Humic acids were extracted via oven-drying and freeze-drying approach. The characterization of oven-dried humic acids and freeze-dried humic acids was conducted by measuring moisture content, ash content, carboxyl group (-COOH), total acidity (COOH phenolic- and/or enolic -OH) and phenolic (-OH). The spectroscopic characterization for both humic acids was done by using FTIR spectroscopy, Scanning Electron Microscopy (SEM) and UV/VIS spectrophotometer. Factorial design was also conducted to determine the factors (temperature, time and concentration) that will affect the yield of humic acids. The study showed that high concentration of alkaline solution (KOH) will give rise to high yield of humic acids. The results of statistical analysis (Paired t-test) show that there was no significant difference in the characteristics between them except for E_4/E_6 ratio.

Keywords: humic acids, alkaline extraction, FTIR.

ABSTRAK

Pengekstrakan beralkali telah digunakan untuk mengekstrak asid humik daripada arang batu yang berasal daripada Mukah. Larutan beralkali yang digunakan dalam pengekstrakan ini ialah kalium hidroksida, KOH. Asid humik diekstrak melalui kaedah pengeringan secara oven dan pengeringan beku. Ciri-ciri bagi asid humik yang dikering secara oven dan asid humik yang dikering beku dilakukan dengan mengukur kandungan kelembapan, kandungan abu, kumpulan karboksil (COOH), jumlah keasidan (COOH fenolik dan/atau enolik -OH) dan fenolik (-OH). Ciri-ciri spektroskopik bagi kedua-dua asid humik dilakukan dengan menggunakan spektroskopi FTIR, Imbasan Elektron Mikroskopi (SEM) dan UV-VIS spektrofotometer. Rekabentuk faktorial juga dilakukan untuk mengenalpasti faktor-faktor (suhu, masa dan kepekatan) yang akan mempengaruhi hasil asid humik. Kepekatan KOH yang tinggi akan membawa kepada hasil asid humik yang banyak. Keputusan analisa secara statistik (Paired t-test) menunjukkan tiada perbezaan ciri yang ketara di antara kedua-dua asid humik tersebut kecuali untuk nisbah E_4/E_6 .

Kata kunci: asid humik, pengekstrakan beralkali, FTIR.

CHAPTER 1

INTRODUCTION

Humic substances are natural materials in the environment and very important in agriculture especially in soil science. They consist of about 95% of dissolve organic matter in aquatic system. In addition, it contains chains of aromatics and hydroaromatic units that are joined by covalent cross-links, hydrogen bonds and Van der Waals interactions (Galambus *et al.*, 2004).

As a whole, humic substances are naturally occurring biogenic, heterogeneous organic substances that can be characterized as being yellow to black in colour, have high molecular weight and refractory (Muhammad, 2005). They are formed in the process of humification from different structural precursors of biotic origin and have irregular structure with varying composition (Zavarzina *et al.*, 2002). They can be divided into three components that are humic acids, fulvic acids and humin according to their solubility (Peña-Méndez *et al.*, 2005).

Humic acids are defined as the fraction that are not soluble at pH below 2 but are soluble under alkaline condition. Fulvic acids are the fraction of humic substances that are soluble at all pH while humin are the fraction of humic substances that are insoluble at any pH (Magdaleno and Coichev, 2005). The macromolecular structure of humic substances is highly porous (Önal and Akol, 2003) and contains high molecular weight organic molecules. Therefore, it is a major organic constituents actively participating in the global carbon cycle and controlling the fate of organic and inorganic pollutants in surface aquatic environments (Li *et al.*, 2006).

According to Hayes and Swift (1978), humic substances play important roles in the formation and maintenance of good soil structure. They are also essential for improvement of water entry in soil and for retention of plant nutrients by cation-exchange processes. Flaig (1990) revealed that the transport of metals into plant roots, and the possible stimulatory effects on plants growth are affected by the presence of humic substances. In addition, they can immobilize some anthropogenic chemicals that influence crop growth, improve the buffering capacities of soils, and raise the soil temperature as a result of increased solar radiation absorption.

Taking into consideration of the diverse roles of humic acids in soils, they are often used as soil conditioner to promote the aggregation of soil particles for holding micronutrients for plant growth (Lawson and Stewart, 1989). The beneficial effects of humic substances on soil properties are likely attributable to the presence of vast carboxyl and phenolic groups (MacCarthy, 2001).

Humic acids are extracted with typical acid base fractionation method with the extracts dried with freeze drying approach. Freeze drying may not be feasible for mass production of humic acids as soil conditioner. Sim *et al.* (2006) employed oven dried approach for the production of nitrohumic acids. The characteristics of the humic acids generated with oven drying approach may not be comparable to the humic acids extracted with the reference method (freeze drying). Limited work has been published reporting the characteristics of humic acids produced with both drying approaches.

The objectives of the study are:

- a) to extract the humic acids via oven-drying and freeze-drying approach.
- b) to characterize the oven-dried and freeze-dried humic acids.
- c) to study the effects of extraction time, temperature and solution concentration on the yield.

CHAPTER 2

LITERATURE REVIEW

2.1 Definition of humic substances

Humic substances are amorphous mixture of refractory molecules and consist of high molecular structure and highly polydisperse molecules (Stevenson, 1994). They are ubiquitous in the environment and they are formed via processes that involve chemical reaction of species randomly chosen from a pool of diverse molecules and through random chemical alteration of precursor molecules. It also exhibits both hydrophobic and hydrophilic characteristic and can bind to mineral surfaces (MacCarthy, 2001).

Molecular weight of humic acids is greater than fulvic acids, besides, they are also less highly charged, less polar and more aromatics (Hayes *et al.*, 1989). Humic acids are fraction of soil organic matter which can be precipitated at pH 2 from aqueous alkaline extracts of soils. Humic acids constitute the higher molecular weight fraction from 1500 to 5000 and 50,000 to 500,000 Da in streams and soils. Coal humic acids are dark coloured substances derived from coals (Magdaleno and Coichev, 2005). They occurred naturally in some lignites and brown coals (Lawson and Stewart, 1989).

Fulvic acids can be studied in aqueous solution under acidic or alkaline condition while, humic acids can be investigated in aqueous solution only under alkaline conditions or when the alkaline solutions are adjusted to pH close to neutrality. Fulvic acids have moderate

molecular weight substances from 600 to 1000 and 1000 to 5000 Da in streams and soils, respectively (Magdaleno and Coichev, 2005). Humic is generally studied in the solid phase because humic does not dissolve in all pH (Hayes *et al.*, 1989). Figure 1 and Figure 2 illustrate the model structure proposed for humic and fulvic acids.

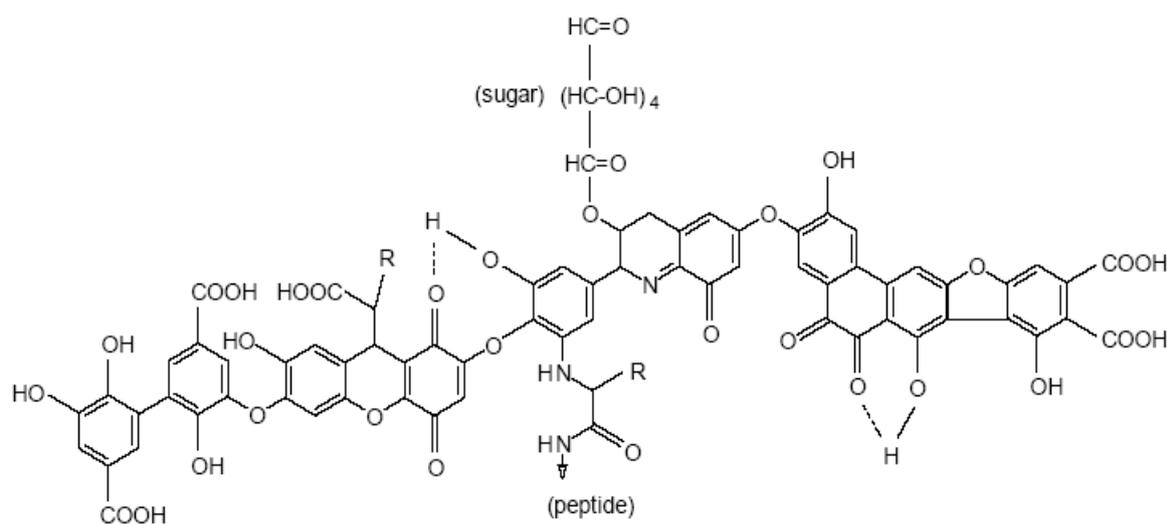


Figure 1: Model structure of humic acids (Stevenson, 1982); R can be alkyl, aryl or arakyl.

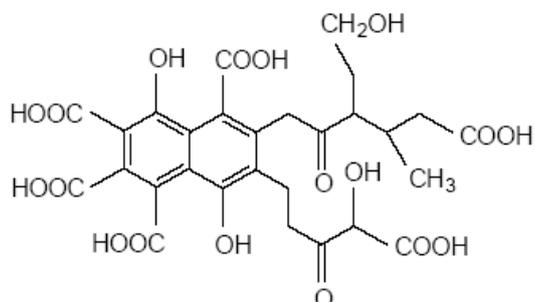


Figure 2: Model structure of fulvic acids (Buffle *et al.*, 1977).

2.2 Formation of humic substances

Humic substances are ubiquitous and can be found wherever organic matter is being decomposed, or has been transported as in the case of sediments as they are also present in sediments and waters (Hayes *et al.*, 1989). There are also aquatic humic substances that are the most abundant in effluents from watershed which drain acid peat, forest litter deposits, and sodic soils rich in organic matter. They are the least abundant in hard freshwaters and in the waters of deep oceans and polar ice caps.

The formation of humic substances incorporates an initial degradative step involving microbial decomposition of the biomatter into small molecules. Biomatter refers to biologically synthesized matter that is no longer living or part of living cells. After decomposition, the small molecules are then consumed as an energy and carbon source by microbes with the release of other small molecules.

The steps involve initial degradation of biomatter into diverse small molecules follow by condensation reactions to generate substances that resist breakdown by organisms, unlike the initial biological matter. These refractory materials are called humic substances. Another formation process of humic substances was suggested involving chemical alteration at sites along polymer chains of biomolecules such as lignin or partially degraded lignin. The chemical alterations include condensation, hydrolytic or oxidative processes (MacCarthy, 2001).

2.3 Importance of humic substances

Humic substances serves as a major reservoir of organic carbon in soil and marine sediments and play a key role in a number of geochemical cycles, including transport of contaminant (Kramer *et al.*, 2001). Other applications are for example as filler in ebonite, as additives in drilling muds, in leads accumulators and in pollution control in synthetic fuel manufacture (Lawson and Stewart, 1989).

Chen and Aviad (1990) stated that humic substances produce various morphology, physiology, biochemical and genetic effects on higher plants. Therefore, humic substances are known as the major constituent of soil organic matter that is important for crop production in providing nutrients and to improve physical properties of soils (Seyedbagheri and Torrell, 2001). Humic substances play an important role in agronomical by influence significantly the quality and productivity of the soil such as moisture condition. It also important to soil fertility because it has high base exchanged capacity. Humic substances currently used as additives in fertilizer. As the indirect effects, humic substances can integrates iron to the chelates that make it available to the plants (Peña-Méndez *et al.*, 2005). Humic acids are important in agricultural because they slowly release macronutrients to plants, enhance microbial growth, have high water holding and buffering capacity, reduce soil erosion and stimulate plant growth (Fataftah *et al.*, 2001). Humic acids contribute to mineralization and immobilization of nitrogen in soil. As the complexes formed between ammonia and humic acids, nitrogen is released slowly into soil (Beames, 1986). Ammonium humate is a salt of humic substances that have a significant growth-stimulating effect. Other salts of humic

substances such as calcium humate were used to increase soil fertility while sodium humate increase the fertilizing effects (Peña-Méndez *et al.*, 2005).

In the ceramic industry, humic substances can be used as additives to enhance the mechanical strength of unprocessed ceramics, improve the casting properties of ceramics, coloring the clay tiles and applied in the preparation of earthenware. Furthermore, humic substances can be used to produce human and veterinary medicine. The complexes form by humic acids with heavy metals as cadmium can be used to eliminate heavy metals from living organisms. Medical studies show that humic substances as anticancer, antitumor and resolved the difficult viral respiratory illnesses common in children (Peña-Méndez *et al.*, 2005).

2.4 Properties of humic substances

2.4.1 Studies of the functional groups

Products of oxidative degradation of soil humic substances are generally composed of aliphatic, phenolic and benzenecarboxylic acids that are identified in term of esters and ethers (Griffith and Schnitzer, 1989). Humic substances contain carboxyls group and weakly acidic phenolic groups. These functional groups can contribute to complexation and ion-exchange properties of humic material (MacCarthy, 2001).

The most important functional groups present in humic acids are those containing oxygen such as carboxyl, hydroxyl, carbonyl, methoxy (Lawson and Stewart, 1989). According to MacCarthy (2001), the identification of aromatics and aliphatics component among

degradation products has provided much information about the composition of humic substances in studying the functional groups.

The more polar fraction from the fractionation of humic acids, the more acidic groups can be found (Karavaev and Valibekor, 1962) and this acidity is due mainly to carboxylic acid groups and phenol (Lawson and Stewart, 1989). Humic substances also consist of aromatic rings and aliphatic chains which can carry oxygen, nitrogen and sulfur containing functional groups (Zavarzina *et al.*, 2002).

2.4.2 Elemental analysis

Generally, humic acids contain carbon (56-62%), hydrogen (2-5.5%), oxygen (2-14%), nitrogen, sulfur (0.6-1.1%), and phosphorus (0.2-3.7%). Besides, there are also minerals such as aluminum (5.6%), sodium (up to 0.6%), potassium and magnesium in (Orlov, 1985). Carbon content is increasing with coal rank in the approximate range of 45% to 70%, and with hydrogen between 4% and 6%. Determination of active nitrogen in the product indicates the potential of humic acids as fertilizer (Lawson and Stewart, 1989).

2.5 Charge effect

Humic substances carry an electrostatic charge as they are classified as macro-ions or polyelectrolytes. Soil humic acids have 3-4 charged sites per 1000 daltons. Two types of charge effects in humic substances are intramolecular charge effects and intermolecular charge effects. Intermolecular charge repulsion is effectively suppressed by di- and trivalent

cations at much lower electrolyte concentration. The extraction of humic substances from soil using aqueous extractants are by removing the insolubilizing cations which are complexes such as hydrogen and calcium, then, replacing them with sodium (Swift, 1989).

2.6 Humic substances in the solution

In aqueous system, the balancing cations are largely dissociated and exist as a cloud and there is a net repulsion between the charges of polyelectrolyte, causing molecular expansion. When macromolecule's strand come close together, molecular interaction as hydrogen bonds may form Van der Waals forces come into effect causing the occurring of dipolar interaction (Eisenberg, 1976).

2.7 Extraction of humic acids

The extraction of humic acids is usually done by using alkaline extraction. Humic acids dissolve in the alkali solution and are latter separated from the insoluble residue by filtration or centrifugation. Humic acids are extracted from coal with dilute sodium or potassium hydroxide solutions at concentration of 0.5 to over 10 percent. Weaker base as sodium carbonate and ammonia are less effective. Air may be excluded to avoid oxidation and the temperature employs are usually moderate (Sakagami and Shimizugawa, 1962).

2.8 Regenerated humic acids

Humic acids can be oxidized to water soluble carboxylic acids. The conditions of the oxidation have to be optimized to gain maximum humic acids yields. The oxidizing agents are oxygen (air), nitric acids, alkaline permanganate, chlorine, hydrogen peroxide and performic acids. The use of nitric acids introduces extra nitrogen in the products called nitrohumic acids that contain nitro and nitroso groups (Charmbury *et al.*, 1945). Maximum humic acids are produced by using oxidation method with slow reaction and the requirement of elevated temperature at 150°C. During oxidation, the coals begin to absorb oxygen as soon as they are freed from the seam, the humic acids itself then is attacked readily (Bailey *et al.*, 1954).

2.9 Spectroscopic studies on the characteristic of humic acids

The spectroscopic techniques that are used to investigate humic acids include infrared spectroscopy (IR), ^1H and/or ^{13}C nuclear magnetic resonance spectroscopy (^1H & ^{13}C -NMR.), fluorescence spectroscopy, electron spin resonance spectroscopy (ESR), as well as chemical and thermal degradation techniques (Li *et al.*, 2006).

2.9.1 NMR spectroscopy

The application of this spectroscopy is to investigate the functionality of humic substances (MacCarthy and Rice, 1985). Liquid and solid-state ^{13}C NMR give well resolved spectra for humic substances when the sample is irradiated with the proper pulse sequence. They are the most useful instrumental technique for establishing compositional differences

and similarities among humic substances from various soil, stream, lake and marine environment (Wershaw, 1985). ^{13}C NMR is used to compare coal humic acids with different rank; polar functional groups decrease and aromaticity increases with increasing of coal maturity (Hayes and Swift, 1978).

2.9.2 Infrared spectroscopy

Infrared spectroscopy is the most useful spectroscopic procedures for determining functional groups in humic substances. The identification by infrared spectroscopy of oxygen containing functional groups in humic substances is by derivation, which give rise to a shift in the spectral band. By using derivation techniques, carboxyl, esters, ethers, ketones and hydroxyl functional group can be identified (Hayes *et al.*, 1989). Table 1 summarizes the FTIR absorption bands of the functional groups that are commonly found in humic acids.

Table 1: FTIR absorption bands of the functional group in humic acids (Stevenson, 1994).

Wavelength(cm^{-1})	Assignment (functional groups present)
3400-3300	O-H and N-H stretching
2940-2900	Aliphatic C-H stretching
1725-1720	C=O stretch of COOH group
1650-1630	C=O stretching (amide), aromatic C=C, H-bonded C=C, double bond conjugated with carbonyl and COO-vibrations
1650-1613	COO- symmetrical stretching
1280-1230	Aromatic C-O, phenolic C-O and C-O stretching

2.9.3 UV-Visible spectroscopy

UV-Visible spectroscopy is a technique in studying the absorption bands in the ultraviolet and visible spectra of humic substances. The absorptivity increases at shorter wavelength. The variation of the ultra-violet visible spectra as function of pH is consistent with the occurrence of aromatic carboxylic acids and phenols in humic substances. The absorbance ratio at 465 nm and 665 nm is often used to characterize humic acids. The absorbance ratio which is referred to as the E_4/E_6 is empirically correlated with their degrees of aromaticity. The E_4/E_6 coefficient of humic acids is usually less than 5 and the low ratio indicates high degree of condensation of aromatic constituents (Hayes *et al.*, 1989).

2.9.4 Fluorescence spectroscopy

Study on the variations of the fluorescence spectra as a function of pH or of other variables, and to monitor the ensuing changes but not provide any significant insight into the structure of humic substances. This type of spectroscopy indicates some of the components in the mixture of humic substances fluorescence (Hayes *et al.*, 1989).

2.9.5 X-Ray Photoelectron spectroscopy and Mössbauer spectroscopy

X-Ray spectroscopy provided some compositional information such as the presence of sulfur in humic substances and the studies of metal binding by fulvic acids. Mössbauer spectroscopy this spectroscopy provides information about binding site in humic substances by using Mössbauer-active complexes (Hayes *et al.*, 1989).

2.9.6 Electron spin resonance spectroscopy

Electron spin resonance spectroscopy provides information about the effect of many chemical and physical variables on the free radicals contents of humic substances. Besides, it also studies about the reaction of humic substances involving free radicals processes that may have structural implications (Hayes *et al.*, 1989).

2.10 Aromaticity

The ratio of optical absorbances of humic acids solution at 465 nm and 665 nm (E_4/E_6 ratio) is used to characterize the aromaticity. The E_4/E_6 ratio is governed primarily by its molecular sizes and does not reflect condensed aromatic ring (Chen, 1977). There are relationship between E_4/E_6 ratio and spin content for fulvic acids fractions on Sephadax gel. According to Stuermer *et al.* (1978), ESR spectroscopy is used to measure free radical concentration and aromatic character.

2.11 Effects of drying approach on the structure of humic acids

Soils and clays show considerable shrinkage when dried by using air drying method. In electron micrograph, it is not uncommon for the structure of air-dried sample to appear much denser than that of freeze-dried in frozen sample.

By using freeze drying method, shrinkage and distortion of the sample by surface tension forces are minimized because ice is removed by sublimation (Chen and Schnitzer, 1989).