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# Methanolysis of duckweed and azolla: A comparative analysis

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**Abstract:** This paper compares the bio-oil and chemical compositions produced from methanolysis of duckweed and Azolla. The methanolysis was carried out at 230°C, 250°C and 300°C for 30 minutes using the CJF-0.1L reactor. Oil yield produced from azolla was 34% and this is 3% higher as compared to the bio-oil yield produced by duckweed at 250°C. The Gas Chromatography Mass Spectrometry (GCMS) analysis showed that alcohol, ketone, amine, ether and ester compositions in the bio-oil produced from duckweed were slightly higher than azolla. However, carboxylic acids, amide and phenol compounds in the bio-oil produced from azolla are different and higher than duckweed. The results suggest that duckweed and azolla are the potential feedstocks from aquatic biomass to be further investigated for bio oil use.

## 1. Introduction

Biomass is devised as one of the most encouraging options to fossil fuels for the production of energy, bio-fuel, and chemical. The scientific community have received a challenge to find new techniques to accomplishment renewable energy in order to meet the world's demand for energy as well as reducing greenhouse gases as the increasing of CO<sub>2</sub>. Biomass is one of the largest sources of energy in the world. There is a developing consideration to the advancement of technologies to convert biomass into more valuable fuels in addition to the direct energy production. In this situation, liquefaction can be a fascinating technology to produce a biofuel from a wet feedstock without the need of energy engrossing drying process.

A conversion of aquatic biomass to liquid fuel by pyrolysis have been studied previously to produce bio-oil, gas, and bio-char [1]. The bio-oil from the aquatic biomass contained a huge fraction of aliphatic hydrocarbon likely from lipids, together with important proportions of aromatic nitrogen and oxygen compounds, likely derived from proteins [2]. The use of aquatic biomass to generate bio fuel gained



attention as it did not challenge with food for land usage, high photosynthetic efficiency, and fast growth rate.

Duckweed, which is an aquatic plant that floats on the surface of ponds, is the biomass selected for our study. This aquatic biomass has drawn enormous consideration from researchers due to its fascinating properties such as rapid multiplication, strong adaptability and low processing costs [3-6]. According to Catallo et al.[7], aquatic plants are considered as an ideal feedstock for production of biofuel due to its high photosynthetic efficiency, high area specific yield and fast growth rate. Recently, duckweed has gained many interest by researchers for conversion to biofuels. As it usually contains high moisture content after its harvesting, it can be considered for conversion of biofuel [7]. Duckweed can be recovered from the cultivation medium using simple mechanical separation, which is in sharp contrast to microalgae [4]. In addition, the ability to accumulate starch, which is required for creating biofuels, makes duckweed a promising candidate for biofuel feedstock [5-6].

Meanwhile, previous studies on Azolla showed that it is a useful feedstock for biofuel production. For example, Muradov et al.[8] investigated the possibility of Azolla plants for wastewater treatment and generation of renewable fuels. They found that Azolla contains several varieties of petrochemicals including straight chain C<sub>10</sub>-C<sub>21</sub> alkanes, which can be an alternative for direct use of biodiesel fuel or component of bio-diesel [8]. Miranda et al. [9] investigated the capability of Azolla for production of hydrocarbons through liquefaction. They revealed that the amount of produced ethanol of Azolla was greater than woody plants and Miscanthus. Furthermore, the high C/N ratio content in the composition of Azolla can generate high amount of hydrogen gas, that makes it a capable feedstock for hydrogen generation [9]. In addition, previous studied by Biswas et al. [10] also found that high percentage of aliphatic functional groups in azolla bio-oil from the pyrolysis of azolla, *sargassum tenerrimum* and water hyacinth, making it an advantageous feedstock to generate bio-fuel and bio-chemicals [10]

The most promising thermochemical conversion for aquatic biomass is through liquefaction. Liquefaction can be conducted at low temperature and high pressure using wet/dry samples in which the biomass is converted into three products; bio-oil fraction, a gas fraction and a solid residue fraction, in water or another suitable solvent [11]. There are many aquatic liquefaction studies from previous researchers, however, none of the studies focuses on the comparison of bio-fuel production from several types of aquatic biomass. In this study, the bio-oil from different aquatic biomass were compared for its yield and chemical compositions. To date, there is no study comparing the products from liquefaction of Azolla and Duckweed, and hopefully this study will give a quick insight on the potential of azolla and duckweed.

## 2. Research Methodology

### 2.1 Materials

Azolla and duckweed were collected from the Normala Kamis Herbs nursery in Panggau, Perlis (Malaysia) and air-dried for 24 hours. Samples were dried in an oven at temperature of 105°C for 24 hours. The samples were grounded into small particles size (<0.06mm) by mortar and pestle and then kept in the air-tight container for further use.

### 2.2 Proximate analysis

Proximate analysis was performed using the thermogravimetric analysis (TGA) DTA/DSC TA Model SDT Q600 according to ASTM D2974. The proximate analysis was performed in order to determine the moisture, volatile matter, fixed carbon and ash in biomass. It was performed under inert nitrogen gas and purified air with a constant flow rate of 100 ml/min and heating rate of 20 °C/min. 6mg of sample was weighed and put into the alumina crucible and the temperature was kept isothermal for 0.5 min until a steady condition was obtained before ramping to the desired temperature [12-13].

### 2.3 Methanolysis

For liquefaction process, a 5g of Azolla was loaded into the stainless steel reactor model CJF-0.1L, and followed by 40 ml of methanol. The temperatures were set at 230°C, 250°C and 300°C for 30 min with stirring at 680 rpm to investigate the effect of temperature. Then, reactor was cooled down to ambient temperature. The reacted products, which were solid and liquid were washed with approximately 150 ml DCM and transferred into the conical flask. Heavy oil can be described as the oil product from liquefaction process which has a density of greater than one, while light oil has a density less than one. The solid residue was separated from the liquid product by filtration with filter paper. The filtered liquid product was left dried in the fume cupboard. The obtained crude oil was measured and recorded. The bio-oil yield consists of heavy oil and light oil. Experiments were repeated 4 times for its reproducibility. The yield of each product was calculated as follows [14]:

$$\text{Residue yield} = \frac{\text{mass char (g)}}{\text{initial sample loaded (g)}} \times 100 \quad (1)$$

$$\text{Conversion} = 100 - \text{residue yield} \quad (2)$$

$$\text{Total oil and water yield} = 100 - (\text{residue yield} + \text{gas yield}) \quad (3)$$

$$\text{Heavy oil yield} = \frac{\text{mass heavy oil (g)}}{\text{initial sample load (g)}} \times 100 \quad (4)$$

$$\text{Product loss} = \text{Gas yield} + \text{light oil yield} \quad (5)$$

### 2.4 GCMS analysis

Gas chromatography–mass spectrometry (GC MS – QP2010 Ultra Shimadzu) analyses of the oils (1 µL in DCM) were performed (source temperature 280 °C). Separation was performed on a fused silica capillary column (30 m × 0.25 mm i.d) coated with BPX5 phase (0.25 µm thickness). Helium was used as the carrier gas, with a temperature programme of 35 °C (2 min) to 250 °C at 20 °C/min and hold for 20 min. Injections were performed in full scan mode with split ratio 1:30. Experiments were repeated 3 times for its reproducibility.

## 3. Results and discussion

### 3.1 Proximate analysis of Azolla and duckweed

The properties of Azolla and duckweed are presented in Table 1. The proximate analysis showed that the volatile matter yield for Azolla was higher than the duckweed. A higher yield of volatile matter is expected to produce more liquid product during liquefaction. The high ash content is noteworthy as it leads to reduce liquid yields [15]. Based on the result obtained, azolla contains less ash content than the duckweed and this leads to the production of higher liquid yield as can be seen in Table 2. Previous studies also obtained approximately the same results with this study. For example, Pirbazari et al. [16] reported that the moisture content for Azolla was 6.8%, volatile matter was 75.1% the fixed carbon was 5.7%, and the ash content was 12.4%. The proximate analysis for duckweed obtained from Wang et al. [17] was 13.2% for moisture content, 58.7% of volatile matter, 9.8% of fixed carbon and 18.3% of ash content.

**Table 1.** Proximate analysis of duckweed and azolla

Proximate Analysis	Mass fraction (%)	
	<i>Duckweed</i>	<i>Azolla</i>
Moisture content	13	7
Volatile matter	60	77
Fixed carbon	10	5
Ash content	17	11

### 3.2 Yield of Bio-oil

The percentages of oil yield from duckweed and azolla liquefaction were produced at 230°C, 250°C and 300°C with a reaction time of 30 min. Azolla had the highest bio-oil yield of 35% and it was recorded at 250°C, in agreement with the proximate analysis reported above. Duckweed had the lowest bio-oil yield of 26% and recorded at 230°C. A previous study showed 21.1% of oil yield was obtained from liquefaction of duckweed with temperature of 350°C for 30 min[17].

**Table 2** Oil yield (%) from duckweed and Azolla.

Types of biomass	Oil yield (%)		
	230°C	250°C	300°C
<b>Duckweed</b>	26	32	30
<b>Azolla</b>	28	35	33

### 3.3 Analysis of Bio-Oil

The GCMS was conducted for the samples of bio-oil from methanolysis of azolla and duckweed using 40 ml of methanol solvent at temperature of 250°C for 30 minutes. Those tested parameters were similar as reported by Wang et al.[17]. The GCMS results were shown in Table 3. The major compounds that were found in the bio-oil products were esters, phenols derivatives, ketone, alcohols, and aromatic. Based on the result below, oil yield from duckweed contains a higher ester yield than oil yield from azolla. The comparison of azolla and duckweed bio-oil can be seen in Figure 1.

A lower yield of acidic content for sample of duckweed (i.e 2%) was seen as compared to azolla (i.e 15%). The composition of oil produced from duckweed is explained in previous research by Peigao Duan et al.[18]. The high-yield compounds that were presented in the bio-oil at a reaction temperature of 350°C, with reaction time of 30 min and 2.5 g duckweed were ketones and acids, while in this study ester was the highest yield found. Methanolysis had increased the ester content in the produced bio-oil. The high content of carboxylic acids in azolla is likely due to the decomposition of protein [19].

Although acid content in azolla's bio-oil was higher than duckweed's bio-oil, the percentage of acid content from liquefaction of azolla were lower than the one from pyrolysis process. Acid compound yield from pyrolysis of Azolla by Pirbazari et al. [16] was about 25%, as compared to only 15% of acid content obtained in this study. The results of liquefaction of Azolla have not been reported in any other studies yet. The hydrocarbons in the liquefaction products may be generated through different pathways, which are dehydration of alcohols, feedstock hydrocarbons breaking down, decarboxylation of fatty acids, and recombination of the resultant radical fragment [20]. The alcohols present from both bio-oil may be derived from the reduction of the acids [20].

**Table 3** GCMS result for bio-oil produced from Azolla and duckweed

NAME	RETENTION TIME	AREA	
		AZOLLA	DUCKWEED
Succinic anhydride	2.52	52703	N/A
Trimethylene oxide	2.53	N/A	28056
2-Propanamine	2.55	N/A	35101
Formic acid, ethenyl ester	2.60	11202	N/A
Acetic acid, 1-methylethyl ester	2.81	68461	51846
Butane, 2-chloro-2-methyl-	2.85	44342	37917
2-Propanone, 1-chloro-	3.06	10267	6581
Methanamine, N-methoxy-N-nitroso-	3.23	N/A	1320
Serine	3.24	N/A	1320
Propane, 1,2-dichloro-	3.27	N/A	1009
2-Butanone, 3-chloro-	3.48	6135	5459
Acetic acid, [(aminocarbonyl)amino]oxo-	3.62	N/A	1075
2-Propanone, 1,1-dichloro-	3.68	22639	20486
Propane, 1,1'-sulfonylbis-	3.88	77117	N/A
2-Propanone, 1,1-dichloro-	3.99	5363	4913
Trimethylene oxide	4.13	1518	N/A
Propanoic acid, 2,2-dimethyl-	4.45	3232	N/A
Propanoic acid, 2-hydroxy-2-methyl-, methyl e	4.50	301	315
Hydrazinecarboxylic acid, 1,1-dimethylethyl e	4.55	N/A	464
2-Hexanol	4.67	92701	76433
Butane, 2,3-dichloro-2-methyl-	4.86	16699	15467
Succinic anhydride	4.90	1771	N/A
Heptane, 1-chloro-	5.17	N/A	2619
Hexane, 1-chloro-	5.18	2662	N/A
d-Alanine	5.22	N/A	1218
3-Nitropropanoic acid	5.28	1199	N/A
2-Propanone, 1,3-dichloro-	5.54	1953	2137
2-Propenoic acid, ethenyl ester	5.70	4847	N/A
Pentane, 3-bromo-	5.91	1050	N/A
3-Hexanol, 2-methyl-	5.69	1301	N/A
4-Heptanol	5.69	N/A	1791
Diisopropyl sulfide	5.85	N/A	676
Methyl 3-hydroxytetradecanoate	5.86	843	N/A
Cyclopentane, 1,2-dichloro-, trans-	5.89	N/A	4819
1-Butene, 2-chloro-3-methyl-	5.90	4439	N/A
Ethane, 1,1,2,2-tetrachloro-	5.98	2269	1824

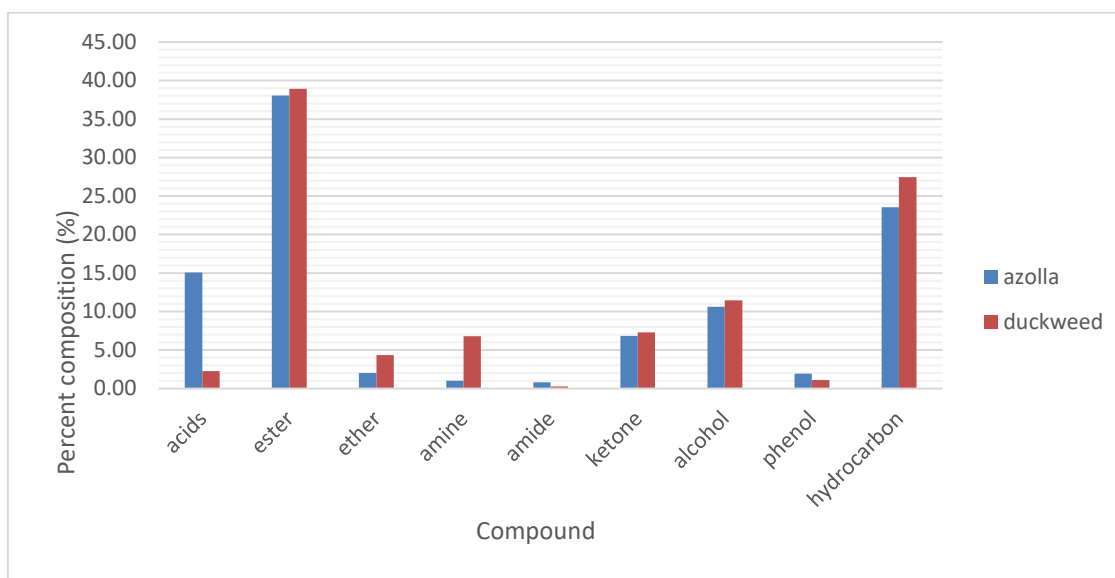
1,3,7-Octatriene	6.11	1564	1857
Propanoic acid, 2,3-dichloro-, methyl ester	6.16	N/A	963
Carbamic acid, diethyl-, methyl ester	6.23	N/A	2122
Isopropylsulfonyl chloride	6.27	6258	5090
1-Propene, 1,2,3-trichloro-, (Z)-	6.31	5437	4770
Propane, 1,2-dichloro-2-methyl-	6.47	9606	11812
Chlorodimethylethylsilane	6.66	7876	7186
1-Octanamine, N-methyl-	6.70	N/A	1801
Pentanal, 2,3-dimethyl-	6.77	1064	N/A
2-Nonanone	6.78	N/A	922
Octane, 2-bromo-	6.85	N/A	5467
Bis(2-chloroethyl) ether	6.86	6267	4265
Diethyl Phthalate	6.89	2534	N/A
Benzenepropanenitrile, .beta.-hydroxy-	7.04	4977	4265
Thiourea	7.07	N/A	2089
1-Propanethiol	7.08	2309	N/A
2,2-Bis(chloromethyl)-1-propanol	7.20	4819	4503
Propane, 1,3-dichloro-	7.27	7881	7857
Propane, 1,3-dichloro-	7.34	6539	6768
Butane, 1-chloro-3-methyl-	7.52	1923	N/A
2,2-Bis(chloromethyl)-1-propanol	7.53	N/A	1737
Benzene, 1-chloro-2-propyl-	7.56	N/A	1502
1,3-Dichloro-2-butene	7.57	1476	N/A
Oxirane, 2,3-dimethyl-	7.95	N/A	725
Succinic anhydride	8.07	3703	N/A
N-Acetylmethionine	8.25	963	N/A
Propane, 1,3-dichloro-	8.46	N/A	910
n-Heptadecylbenzene	8.58	2135	2105
2-(N-Morpholino)ethanesulfonic acid	8.59	N/A	494
Cyclopropanecarboxylic acid, 3-(2,2-dichloro-	8.65	N/A	1043
Nithiamide	8.71	584	N/A
Propane, 2-bromo-1-chloro-	8.76	2312	2492
Carbonic dihydrazide	8.79	N/A	705
1,4-Dioxane	8.88	N/A	1793
Benzaldehyde, 2,5-dimethyl-	8.93	5819	N/A
1,3-Bis-(methylthio)-2-methoxypropane	8.99	493	N/A
2-Butanone, 3-chloro-	9.10	6089	4859
Butane, 1-chloro-3,3-dimethyl-	9.33	N/A	989
Diethyl Phthalate	9.50	72181	N/A

Diethyl Phthalate	9.58	31075	N/A
Diethyl Phthalate	9.62	27260	N/A
Diallylethylamine	9.66	N/A	2881
Diethyl Phthalate	9.58	31075	N/A
Phthalic acid, monoethyl ester	9.71	3253	N/A
1,4-Dioxane, 2,3-dichloro-	9.77	N/A	1747
Diethyl Phthalate	9.54	16047	N/A
Decane, 1-chloro-	10.21	N/A	586
Benzonate	10.22	1353	N/A
Propane, 1,2-dichloro-2-methyl-	10.41	6647	5450
Diethyl Phthalate	10.50	4503	N/A
Propane, 2-bromo-1-chloro-	10.56	N/A	3006
1-Dodecanol	10.80	N/A	2239
Cyclooctasiloxane, hexadecamethyl-	10.92	3174	N/A
Dodecanoic acid, methyl ester	11.13	N/A	18720
Hexadecane	11.22	N/A	716
Hexatriacontane	11.29	N/A	892
Dodecanoic acid	11.34	N/A	1744
Aziridine, 2-methyl-	11.37	2986	N/A
2-Acetylbenzoic acid	11.56	5830	N/A
1,4-Dioxane	11.73	5445	N/A
n-Pentadecanol	12.16	N/A	609
Decane, 1-iodo-	12.32	N/A	808
Phenol, 4-(aminomethyl)-2-methoxy-	12.39	2140	N/A
Methyl tetradecanoate	12.43	N/A	11182
1,6-Hexanediamine, N,N'-dimethyl-	12.44	N/A	1493
Heptadecane, 3-methyl-	12.58	N/A	1203
Heptasiloxane, hexadecamethyl-	12.69	26554	N/A
Hexadecane, 1-iodo-	12.70	N/A	791
n-Heptadecanol-1	12.86	1057	N/A
Octadecanoic acid, 2-oxo-, methyl ester	12.90	N/A	632
Formic acid, ethenyl ester	13.20	3113	N/A
1,4-Dioxane	13.18	2096	N/A
d-Alanine	13.19	1074	N/A
Formic acid, ethenyl ester	13.23	1074	N/A
Heptasiloxane, hexadecamethyl-	13.61	40663	N/A
Hexadecanoic acid, methyl ester	13.68	31986	N/A
Hexadecanoic acid, methyl ester	13.75	N/A	101155
Pentadecanoic acid	13.89	N/A	4018
Azeleonnitrile	14.10	435	N/A



Tridecanoic acid	13.91	22710	N/A
Ethanol, 2-bromo-	14.09	1526	N/A
Octadecanal	14.37	15889	N/A
Dehydroabietylamine	14.23	N/A	1444
Heptadecanoic acid, methyl ester	14.36	N/A	1358
Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-, tri	14.61	35226	N/A
Phenol, 4-(aminomethyl)-2-methoxy-	14.70	5497	N/A
n-Nonadecanol-1	14.85	3275	N/A
1,2-Ethanediamine, N-(2-aminoethyl)-	14.96	N/A	1122
4-Decenoic acid, methyl ester, Z-	14.98	N/A	389
Octadecanoic acid, methyl ester	15.16	N/A	67744
Cyclohexanone, 4-(methylthio)-	15.23	N/A	1047
Formic acid, ethenyl ester	15.38	2158	N/A
Semioxamazide	15.50	N/A	2563
1(2H)-Naphthalenone, octahydro-4-hydroxy-,	15.58	N/A	2106
1-Heptadecanol, acetate	15.91	N/A	1440
3,4-Methylenedioxy-.beta.-nitrostyrene	16.10	1661	N/A
Hexasiloxane, tetradecamethyl-	15.80	33979	N/A
Trimethylene oxide	16.10	1661	N/A
Formic acid, ethenyl ester	16.30	N/A	929
4-Penten-1-ol, 3-methyl-	16.69	N/A	1056
Trimethylene oxide	17.05	N/A	3084
Succinic anhydride	17.06	2999	N/A
1,2-Oxathiolane, 2,2-dioxide	17.08	3706	N/A
Hexasiloxane, tetradecamethyl-	17.36	22083	N/A
Formic acid, ethenyl ester	17.48	4555	N/A
2(R),3(S)-1,2,3,4-Butanetetrol	17.85	N/A	1595
Trimethylene oxide	17.92	4086	N/A
Benzene, (azidomethyl)-	18.42	5887	N/A
Acetic acid, hydrazide	18.46	N/A	938
1,3,6-Trioxocane	18.53	5073	N/A
2,5-Pyrrolidinedione, 1-methyl-	18.56	N/A	3168
Semioxamazide	18.56	N/A	2132
2-Octanamine	19.32	979	N/A
Stearic acid hydrazide	19.40	N/A	414
1,2-Ethanediamine, N-(2-aminoethyl)-	19.45	N/A	2412
1,2-Oxathiolane, 2,2-dioxide	19.62	N/A	1661

Heptasiloxane, hexadecamethyl-	19.70	13825	N/A
Methionine	19.89	2205	N/A
d-Alanine	20.19	1620	N/A
Formic acid, ethenyl ester	20.20	3794	N/A
9-Octadecenoic acid, 12-(acetyloxy)-, methyl e	20.31	N/A	9759
Hydrazinecarboxylic acid, ethyl ester	20.42	N/A	1371
Succinic anhydride	20.67	N/A	2815
Formic acid, ethenyl ester	20.72	3918	1334
3,6-Dimethylpiperazine-2,5-dione	20.72	3918	N/A
Propanamide	20.77	787	N/A
Formic acid, ethenyl ester	21.88	5555	N/A



**Figure 1.** Chemical composition (%) for bio-oils produced from duckweed and azolla

#### 4. Conclusion

A thorough comparison of the oil yield and chemical compositions from methanolysis of duckweed and azolla had been carried out. A higher oil yield was produced from the liquefaction of azolla than that of duckweed. Azolla produced 35% oil yield which is 3% higher than the duckweed's. However, the hydrocarbon, alcohol, ester amine, ketone and ether compounds of duckweed were higher than the compounds in the bio-oil produced from azolla. The duckweed is a potential feedstock for bio-oil with a reduced content of acids. Both aquatic biomasses showed high ester content with comparable compounds of ketones, phenol and hydrocarbon. The obtained results showed that duckweed and azolla are the potential feedstocks from aquatic biomass to be further investigated for bio-oil use.

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