1-Supplementary (S₁)

1. Biological Oxygen Demand (BOD)

Test Procedure

The BOD test takes 5 days to complete and is performed using a dissolved oxygen test kit. The BOD level is determined by comparing the DO level of a water sample taken immediately with the DO level of a water sample that has been incubated in a dark location for 5 days. The difference between the two DO levels represents the amount of oxygen required for the decomposition of any organic material in the sample and is a good approximation of the BOD level.

1-Take 200ml of water sample and 70ml of aerated water in BOD bottle. (The sample needed to be diluted with aerated water to give the amount of oxygen needed for decomposition of organic materials in the water by microorganisms).
2-Take 270 ml of aerated water in BOD bottle as blank sample (two blank bottles).
3-Record the DO level (ppm) immediately using the dissolved oxygen prob.
4-Place the water sample and the blank samples in an incubator in complete darkness at 20°C for 5 days.
5- After 5 days, take another dissolved oxygen reading (ppm) using the dissolved oxygen prob.
6- BOD value is calculated by the following equation:

\[
\text{BOD} = \frac{\text{Difference in DO} - \text{Error} \times 270}{\text{volume of sample}}
\]

- Difference in DO: is the difference in the DO value between before and after incubation.
- Error: is the difference in the DO value between the two blank samples.
- 270: is the total volume of water in the BOD bottle.

2. Chemical Oxygen Demand (COD) mg/l

Closed Reflux, Colorimetric Method

COD test is based on the principal that most organic compounds are oxidized to CO₂ and H₂O by storing oxidizing agents under acid conditions. COD was measured using closed flux methods. COD concentrations were determined colorimetrically. A total of 2.5 ml
samples were added to COD reaction vials, containing 3.5 ml of silver sulphate/sulphuric acid and 1.5 ml digestion solution and digested at 150°C for 2 hours. A blank sample containing distilled water was also prepared. After digestion, vials were allowed to cool, inverted twice and allowed to stand before measuring absorbance at 600 nm. The spectrophotometer was zeroed using the blank vial.

1 Apparatus
   Digestion vessels 15 x 180 mm with screw caps.
   Oven to operate at 150 ± 2°C.
   Spectrophotometer for use at 600 nm

2 Reagents
   a) Digestion solution: Add to about 500 mL H₂O 10.216 g K₂Cr₂O₇, previously dried at 103°C for 2 hours, 167 mL conc. H₂SO₄ and 33.3 g HgSO₄. Dissolve (this will take many hours, mixing during this time is not required, however mix after dissolving), cool to room temperature and dilute to 1000 mL.
   b) H₂SO₄/Ag₂SO₄: Add 10 g Ag₂SO₄ to 1 L conc. H₂SO₄, let stand overnight to dissolve. Mix carefully after dissolving.
   c) Stock KHP: 850 mg potassium hydrogen phthalate, dried at 120°C for 24 hours, is dissolved in 1000 mL H₂O.

   The COD of this stock solution is 1000 mg O₂/L.

3 Preparation of standards
   Prepare standard solutions containing a known COD concentration by diluting a known volume, A, of the stock KHP to 100 mL.

<table>
<thead>
<tr>
<th>mL A/100 mL</th>
<th>COD mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>30</td>
<td>300</td>
</tr>
<tr>
<td>40</td>
<td>400</td>
</tr>
<tr>
<td>50</td>
<td>500</td>
</tr>
<tr>
<td>60</td>
<td>600</td>
</tr>
<tr>
<td>70</td>
<td>700</td>
</tr>
<tr>
<td>80</td>
<td>800</td>
</tr>
<tr>
<td>90</td>
<td>900</td>
</tr>
</tbody>
</table>

4 Procedure
Wash the digestion tubes and caps with 4 M H2SO4 before first use to prevent contamination.

1. Transfer 2.5 mL of standard or sample to the digestion tube and add 1.5 mL digestion solution
2. Carefully run 3.5 mL H2SO4/Ag2SO4 down inside of tube so an acid layer is formed under the sample-digestion solution layer. Tightly cap tube and swirl several times to mix completely, do not invert the tubes!
3. Place the tubes in a preheated oven of 150°C during 2 hours
4. Allow them to cool, mix the content and let particles settle
5. The next day: Transfer the content gently and without mixing to a 1 cm cell and measure the absorbance at 600 nm against water
6. Plot the absorbance against the known COD in order to get a calibration line.
7. Read absorbance of samples and compare to calibration line, determine the mathematical equation of this line.

Remarks:
1. Use an adjustable Eppendorf pipette
2. Run the standard and samples in duplicate
3. For each set of samples also analyses simultaneously one or two standards with a COD value comparable to the samples.

References
Supplementary (S_2)

1. Characterization of the dried biomass

The following characteristic properties of the dried were studied

1.1 Proximate Analysis-

ASTM defines proximate analysis as the determination by prescribed methods of moisture, volatile matter, ash & fixed carbon. The proximate analysis of the given activated carbon sample will be followed by the procedure given below.

a) Moisture Content-

A small amount of the sample of the dried biomass was put in a petri-dish or crucible, covered with a lid & weighed using a weighing balance. The crucible was placed in the hot air oven at 105°C with its lid removed & dried for 1.30 hrs. The crucible was taken out, immediately covered with the lid, cooled in a desiccator & weighed.

\[
M = 100 \frac{(B-F)}{(B-G)} \quad \text{Eq.} \quad 1-S_2
\]

Where:

Wt of empty petri-dish = G

Wt of empty petri-dish + Activated carbon sample (before heating) = B

Wt of empty petri-dish + moisture free sample (after heating) = F

b) Ash content

The crucible was ignited in the muffle furnace at 750+ 25°C for 1.5 hours. The crucible was placed in the desiccator, cooled to room temperature & weighed. A known amount of the sample of the dried biomass was dried in the hot air oven at 150°C for 3 hours was put in the crucible & the crucible was placed back in the muffle furnace at 750+25°C for 1.5 hours. The crucible was taken out of the furnace, placed in the desiccator, cooled to room temperature & weighed.
A=100(F-G)/(B-G)  

Where:

Wt of empty silica crucible = G
Wt of empty crucible + activated carbon sample (before heating) = B
Wt of empty crucible + ash (after heating) = F =

c) Volatile matter content

A known amount of sample of the dried biomass was put in the crucible. The crucible was placed in an muffle furnace at 920±10°C, covered with lid, & placed for exactly 7 minutes. The crucible was taken out, allowed to cool & weighed.

\[ VM=100\left(100(B-F)-M(B-G)\right)/\left((B-G) (100-M)\right) \]  

Where:

Wt of empty crucible with lid = G
Wt of empty crucible + lid + sample (before heating) = B
Wt of empty crucible + lid + sample (after heating) = F
M=Moisture content in %

d) Fixed carbon

\[ \%\text{ of fixed carbon}=100-(M+A+V M) \]  

2. Compositional analysis of the raw lingo-cellulosic materials: the biomass materials were subjected to compositional analysis using the gravimetric method. Materials used in this study were all unscreened.
a) Extractives: 2.5 g of dried raw biomass was loaded into the cellulose thimble. With the Soxhlet extractor set up, 150 mL of acetone was used as solvent for extraction. Residence times for the boiling and rising stages was carefully adjusted to 70°C and 25 min respectively on the heating mantle for a 4 h run period. After extraction, the sample was air dried at room temperature for few minutes. Constant weight of the extracted material achieved in a convection oven at 105°C. The %/(w/w) of the extractives content was evaluated as the difference was in weight between the raw extractive-laden biomass and extractive-free biomass.1-3

b) Hemicellulose: 1 g of extracted dried biomass was transferred into a 250 mL Erlenmeyer flask. 150 mL of 500 mol/m³ NaOH was added. The mixture was boiled for 3.5 h with distilled water. It was filtered after cooling through vacuum filtration and washed until neutral pH. The residue was dried to a constant weight at 105°C in a convection oven. The difference between the sample weight before and after this treatment is the hemicellulose content (%w/w) of dry biomass.1-4

c) Lignin: 0.3 g of dried extracted raw biomass was weighed in glass test tubes and 3 mL of 72% H₂SO₄ was added. The sample was kept at room temperature for 2 h with carefully shaking at 30 min intervals to allow for complete hydrolysis. After the initial hydrolysis, 84 mL of distilled water was added. The second step of hydrolysis was made to occur in an autoclave for 1 h at 121°C. The slurry was then cooled at room temperature. Hydrolyzates were filtered through vacuum using a filtering crucible. The acid insoluble lignin was determined by drying the residues at 105°C and accounting for ash by incinerating the hydrolyzed samples at 575°C in a muffle furnace. The acid soluble lignin fraction was determined by measuring the absorbance of the acid hydrolyzed samples at 320 nm. The lignin content was calculated as the summation of acid insoluble lignin and acid soluble lignin.5

d) Cellulose: The cellulose content (%w/w) was calculated by difference, assuming that extractives, hemicellulose, lignin, ash, and cellulose are the only components of the entire biomass.1-3

References


2- Supplementary (S₃)

Biosorption kinetics

The pseudo-first-order kinetic model

Pseudo-first-order model can be expressed by Eq. (1-S₃). The values of log(qₑ – qₜ) were calculated from the kinetic data and plotted against time t. A linear fit to the experimental data gives a straight line with a slope $k_1/2.303$ and an intercept log(qₑ).¹

\[
\log (q_e - q_t) = \log(q_e) - \frac{K_1}{2.303} t
\]

Where, $q_t$ is the amount of COD, BOD and Oil& Grease adsorbed at time t (mg g⁻¹); $k_1$ is the rate constant of pseudo-first-order in mg g⁻¹ min⁻¹.

The pseudo-second-order model

Pseudo-second-order model can be expressed by Eq. (2-S₃). The values of $t/q_t$ were calculated from the kinetic data and plotted against time t. A linear fit to the experimental data should give a straight line with a slope $1/q_e$ and an intercept $1/(k_2.q_e^2)$.²

\[
\frac{t}{q_t} = \frac{1}{K_2.q_e^2} + \frac{t}{q_e}
\]

Where, $k_2$ is the rate constant of pseudo-second-order in g mg⁻¹ min⁻¹.

Isotherm studies

Langmuir isotherm

As formulated in Eq. 4-S₂, the linear plot of $C_e/q_e$ versus $C_e$ gives a slope $1/q_m$ and an intercept $1/(q_m.K_L)$.³

\[
\frac{C_e}{q_e} = \frac{C_e}{K_L} + \frac{1}{q_m}
\]

Equation 3-S₃
Where, $q_m$ is the maximum sorbate uptake under the given condition, also known as the monolayer biosorption capacity (mg g$^{-1}$); $K_L$ is the Langmuir biosorption coefficient related with the free energy of sorption (L mg$^{-1}$).

In addition, the dimensionless separation factor constant ($R_L$), can be estimated from the Langmuir isotherm by Eq. (4-S$_3$).

$$R_L = \frac{1}{1 + K_L C_0}$$  

4-S$_3$

Where $C_0$ is the initial concentration of BOD, COD and oil & grease. Values of $R_L > 1$ and $0 < R_L < 1$ show unfavorable and favorable adsorptions, respectively, while values of $R_L = 1$ and $R_L = 0$ show a linear adsorption and irreversible adsorption, respectively.

**Freundlich isotherm**

Freundlich model can be applied to multilayer adsorption, taking place on the heterogeneous adsorbent surface with different adsorption sites.$^4$ As formulated in Eq. (6-S$_2$), a linear plot of $\ln (q_e)$ versus $\ln (C_e)$ gives a slope $1/n$ and an intercept $\ln(K_F)$.$^5$

$$\ln (q_e) = \ln (k_f) + \frac{1}{n} \ln (C_e)$$  

5-S$_3$

Where,

$K_F$ is a constant relating the biosorption capacity (the ability of biosorbent to adsorb); $1/n$ is the heterogeneity factor of the adsorbent, relating the biosorption intensity: the closer $1/n$ values to zero, the more heterogeneous the adsorption surface.

**References**

1 S. Lagergren, Handlingar, 1898, 24 (4), 139.

