A Review on Preparation and Characterization of Activated Bio-Char

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Authors’ contributions

This work was carried out in collaboration among all authors. Author DA studied analyzed and wrote the first draft of the manuscript. Authors RNP and RKS managed the analyses of the study, helps in literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Activated charcoal (AC) is a carbonaceous material with high surface area due to high degree of micro-porosity which makes it a very versatile adsorbent for utilization in industrial, medicinal, environmental and other fields. Ligno-cellulosic biomass (eg. pine needles, paddy stubbles, other waste agricultural residue) can be a prominent raw material for activated bio-char due to its abundance, slow decomposition (which lead to fire/ air pollution). The main factors which affect the AC characteristics (surface area, micropore volume, mesopore volume) are biomass properties, impregnation ratio, impregnation time, activation temperature and activation time. The specific surface area, micropore, mesopore decreases after optimum value with continually increase in impregnation ratio and impregnation time. The activation temperature and impregnation ratio also affect the AC yield significantly. Sorption capacity get affected by adsorbent doses, contact time, agitation speed, adsorption temperature due to availability of active binding sites, adsorptive forces/ bonds.

Keywords: Activated charcoal; micropore; mesopore; impregnation; sorption capacity.

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1. INTRODUCTION

Activated bio-char is a carbonaceous material with higher surface area due to high degree of micro-porosity. 1 g of activated carbon has surface area of about 1000 m² which make it a very versatile adsorbent in industrial, medicinal, environmental and other fields. Charcoal is the ancient form of man-made carbon was used in metal manufacturing process since 8000 BC [1]. At about 460 B.C. Phoenicians applied charcoal filters for purification of drinking water and during 460-370 B.C. Other applications of activated carbon during that time were for fuel, wood preservation, and treating ulcers, intestinal disorder, chlorosis etc. The thermochemical approach for both carbonization and activation was adopted during early stage of production [1,2,3]. It was also stated that in 1914, the production of chemically activated carbon with ZnCl2 was introduced at industrial scale. In the era of 1st world war, granular activated carbon (GAC) was found effective in adsorption of poisonous gases. In 1st World War, the use of poisonous gases paved the way for the development and large-scale production of granular activated carbon (GAC) which was seen to be effective in the adsorption of such gases. Then, a variety of raw materials and production techniques came into use in the manufacture of activated carbon. The commercial activated carbon fall into three categories: powdery activated carbon (PAC, 1-150 μm), granular activated carbon (GAC, 0.5- 4 mm), and extruded activated carbon (EAC, 0.8-4 mm). The particle size is the key difference between PAC and GAC. The smaller size of PAC provides rapid diffusion and also affects the adsorption kinetics of species. In accordance with this, activated carbon with comparatively smaller particle size require lesser adsorption time. However, small particle size results in clogging, high pressure drop so they are not preferred in water/wastewater treatment systems. The application of PAC includes consecutive dosing of AC into stream (gas/liquid) and then proper separation (settling or filtration) to obtain treated stream. Advantages of using PAC are lesser storage cost, lesser dosing costs, and flexibility in adjustment of dose quantity. The only limitation is PAC regeneration since it requires extraction of fine particles from treated stream [2].

The anthropogenic carbon dioxide, methane, ozone and chlorofluorocarbon contribute largely to the GHG emissions/ global warming. Among them, CO2 contribute significantly and is accountable for 75% of the total greenhouse gases [4]. It has been reported that fossil fuel fired power plants individually contribute 86% of total anthropogenic gas emissions and the rest is cumulative impact of vehicular sector, chemical processes, deforestation [5]. The continuously increasing pollution is a major concern since CO2 concentration is expected to rise 550 ppm by 2035 which will lead to average 2°C temperature rise, extinction of 15-40% species [6]. The use of activated carbon as CO2 adsorbent is comparatively advantageous due to low cost, wide availability of raw material, high thermal and chemical stability, ease of production process, easy regeneration process, and hydrophobic nature.

2. UTILIZATION OF BIOMASS THROUGH THERMOCHEMICAL CONVERSION

Bio-char is a popular adsorbent for air and water purification. Employing agricultural/forest residue as raw material for manufacturing activated carbon avail economically, preserves environment and waste/residue management. So, it can be seen as a prominent, abundantly available raw material in industries [7].

The pine needles biomass is the result of regular leaf shredding (from middle of March to July) in continuously growing pine forest [8,9]. These chir pine (Pinus roxburghii) forests are abundantly available in north western Himalayan range with 1.09 million km² cover across Nepal, Bhutan, China, Pakistan, and India. India has shown pine needle productivity of 6.3 t/ha per year [8]. Shredded pine needles make thick layer of foliage on the forest bed, which have comparatively poor biodegradability due to its high lignin content and lead to hindrance in natural growth of soil flora. In addition to that, the shredded biomass is also a major cause of summer forest fire and results in destroying soil fauna and affects wildlife, cattle [10]. Utilization of pine needle biomass for production of activated bio-char benefits the environment by conserving soil flora, fauna by reducing the chances of forest fire. The average fire frequency per fire season (mid-February to mid-June) across forest divisions in Uttarakhand was 30.5 ± 7.2 which also leads to significant GHG emissions [8]. In addition, the activated bio-char also serves environment by getting implemented as adsorbent for soil, wastewater remediation and engine emission control etc. Similarly, coconut shell biomass has minimal economic value and their slow degradation, costly disposal method...
cause environmental pollution. It can be considered as a suitable precursor for activation due to its lower ash content and excellent natural structure [11,12,13].

India generates more than 600 MT of biomass waste from different crops and produces 140 MT of rice straw alone annually [14]. The paddy straw/ stubbles burning in combine harvested paddy field is commonly practiced among farmers and contribute significantly to the GHG emissions, smog in Delhi/ NCR region, neighboring states of Punjab and Haryana. Burning of rice straw in the field produces ash, along with occasional presence of some heavy metals which have a negative effect on soil and therefore, reduce crop productivity. So, the pine needle and rice straw can be considered as major waste biomass in north Indian region which have slow biodegradation and lead to fire hazards. A tonne of rice straw emits 3.7 kg particulate matter, 1.0 kg volatile organic compounds, 3.1 kg nitrogen oxides, 0.7 kg sulphur oxides and 34.7 kg of carbon monoxide upon burning. These gaseous emissions are responsible for various health risks like asthma, bronchitis, decreased lung function and also increase fog incidences even in distant cities [15]. So, these ligno-cellulosic biomass can be suggested as prominent precursor for production of bio-char and activated bio-char.

Biomass is the fourth largest source of energy in the world, supplying about 14% of the primary energy [16]. The waste biomass can be effectively utilized by converting it to bio-oil and bio-char through pyrolysis (Fig. 1) as suggested by the kinetic studies of ligno-cellulosic biomass. Bio-oil can be defined as mixture of complex organic compounds which can be converted to fuel/ energy, phenols, ketones, aldehydes, and alcohols. Bio-char is another product obtained from thermal decomposition through pyrolysis process in absence or in presence of limited amount of oxygen at a temperature range of 400-600°C and atmospheric pressure as described in Fig. 1. Another by-product of pyrolysis process is producer gas which is a mixture of H₂, CO, CH₄, and CO₂. The quick condensation of volatiles results in bio-oil formation and the remaining uncondensed volatiles can be collected as producer gas/ pyrolysis gas, which can further be utilized as fuel for direct combustion, gas engines/gas turbines, and to generate electricity.

3. PROCESS ANALYSIS OF BIO-CHAR FORMATION

The degradation of biomass is process of decomposition of hemicellulose, cellulose and lignin. Researchers found that DTG peaks at around 290°C and 327°C is due to the thermal degradation of hemicellulose and cellulose, respectively due to the release of volatile matter from rice straw. In the third stage, a peak between 440°C and 480°C was formed due to decomposition of lignin. Increase in fixed carbon was observed (31% to 65%) with increasing torrefaction temperature is due to the depletion of volatile matter and complete decomposition of hemicellulose and cellulose (from 300°C and 350°C). The energy yield, mass yield decreases with an increase in torrefaction temperature due to moisture loss and thermal decomposition of lignocellulose which forms volatile gaseous products [14]. The hemicellulose pyrolysis is completely under 350°C, cellulose between 250-500°C, and lignin pyrolysis spread even over 500°C. The pyrolysis of cellulose and hemicellulose produces more gaseous products than that of lignin at the same temperature [16]. Similarly, experiments on wood result in decomposition of hemicellulose at 200–325°C, cellulose at 240-375°C, lignin at 280 to 500°C [17,18].

![Fig. 1. Schematic of pyrolysis process](image-url)
A study on date pits shows decrement in yield with temperature increment attributed to decomposition of cellulose (long polymer of glucose without branches) and hemicellulose (constituted of various branched saccharides). The final product yield become constant after 700°C due to complete decomposition of cellulose, hemicellulose. The decomposition of lignin occurs gradually till 900°C [19].

The heating/ burning properties of raw material used for bio-char production analyzed with proximate and ultimate analysis and few are presented in Table 1. Pine needles has caloric value of 4690 k cal/kg, pH 6.1 and volatile content 72.4%, hemicellulose 11.4%, cellulose 18.2%, lignin 9.1%, organic carbon 34%, TKN 0.73%, C/N ratio 46.6%, and ash content 2.5% [20]. The higher volatile matter in pine needles (around 78%) makes it suitable for the pyrolysis process, more reactive and easily devolatilized and also produces less char [21]. Lower ash content of pine needle (3.38%) is beneficial since, high ash content restrict the burning rate and energy conversion by fouling, slag formation at elevated temperatures. The wheat straw (4%), barley straw (6%) and switchgrass (4.5%) also possess lesser ash content as coal (8%) Fixed carbon content was calculated as 13.77% [22]. The composition of bamboo charcoal was carbon 44.40%, hydrogen 5.17%, oxygen 43.00% and ash 7.43%, pH 7.85 [7]. The proximate analyses of palm shell assert the carbon as 18.7%, moisture 7.96%, ash 1.1%, volatile matter 72.47%, C 50.01%, H 6.9%, N 1.9%, S 0, O 41%, cellulose 29%, hemicellulose 47.7% and lignin 53.4% [23]. The fixed carbon content increases (13.43% to 49.8%) and volatile matter decreases (79.09% to 42.5%) upon activation of N-biochar [24].

Various studies reveal that several independent operating parameters have significant effect on the yield of bio-char. The yield of the solid product (30.7 to 3.2 wt.%) and liquid product (48.3 to 17.8 wt.%) decreased and gaseous product increased (16.3 to 71.3 wt.%) with decrease in precursor particle size. Similarly, as temperature increases (500 to 700°C), the yield of gaseous product increases and both liquid and solid decrease [16].

### 3.1 Process Parameters in Bio-Char Activation

The bio-char is generally less preferred fuel source as compared to bio-oil due to handling, incompatibility with engines, storage, and secondary pollutants issues (higher ash content). In spite of its incompatibility as bio-fuel in automobile sector, several other merits make it economically feasible byproduct of pyrolysis process. Recently several research studies emphasized on the application of bio-char in agricultural sector, water purification, air purification etc. Several researchers explored the potential application of biomass char in soil, composting, activated carbon, water/air remediation.

The industrial production of powdery activated carbon initialized in the beginning of 20th century and patents were obtained for physical (thermal) and chemical activation of carbon [1]. The standard procedure for preparation of activated carbon is illustrated in Fig. 2. Various researchers implemented the physical and chemical activation processes individually or sequentially with different biomass, activation time, impregnation ratio, impregnation time, and activation agent as depicted in Table 2. It was evident from a study that activation agents decompose and form inorganic components. These inorganic compounds react with carbon and accelerate the gasification process of the impregnated biomass [29]. For activation, rice husk was calcined under inert atmosphere of nitrogen, at temperature 773 K (rate 1.4 K/min) for 2 h using a retort-like stainless steel reactor. The sample was heated from room temperature to 1123 K (heating rate 5 K/min) in nitrogen gas flowing at 300 mL/min. Then, nitrogen was substituted for steam as activating agent (flowing rate 1.7 g/gram of carbonized matter/ hour) for 105 min to obtain activated bio-char [30]. Another researcher solidified the mixture of pecan shell and phosphoric acid solution at 170°C in muffle furnace for 1 h before activation. Then, activation of biomass was performed at different temperature levels under air flow of 0.6 L/min [31].

Another chemical activation by phosphoric acid and potassium hydroxide was performed with different impregnation ratios (20, 40, 60 and 80%, w/w %) and then heating in a charcoal kiln [7]. A research conclude that the activation agent act as acid catalyst to accelerate bond cleavage reaction and development of crosslinks during heating and condensation process. H₃PO₄ activation includes reaction of acid with biomass, formation of phosphate and polyphosphate bridges that connect and crosslink polymer fragments. These phosphate ions remain on
Table 1. Proximate and ultimate analysis of rice husk, rice straw, corncob and pine needles [25,26,27,28]

| Contents                     | Rice husk | Rice straw | Corncob | Pine needles |
|------------------------------|-----------|------------|---------|--------------|
| Volatile matter (%)          | 72.8      | 74.7       | 78.7    | 72.4         |
| Fixed carbon (%)             | 9.3       | 15.2       | 16.2    | 26.1         |
| Ash (%)                      | 17.9      | 10.1       | 0.9     | 1.5          |
| C (%)                        | 48.9      | 45.2       | 45.5    | 48.21        |
| H (%)                        | 6.2       | 6.5        | 6.2     | 6.57         |
| N (%)                        | 0.8       | 0.8        | 1.3     | -            |
| O (%)                        | 44.1      | 47.5       | 47.0    | -            |
| Chemical components          |           |            |         |              |
| Hemicellulose (%)            | 28.6      | 35.7       | 31.0    | 11.4         |
| Cellulose (%)                | 28.6      | 32.0       | 50.5    | 18.2         |
| Lignin (%)                   | 24.4      | 22.3       | 15.0    | 9.1          |

Fig. 2. Process flow chart of physical and chemical activation of biomass

activated bio-char surface even after washing [32]. A study revealed the production of activated bio-char from phosphoric acid impregnated coffee ground, pomelo skin and its implementation as adsorbent for ethylene and n-butane. Impregnated coffee grounds were ultrasonically treated (1 h), oven dried at 373 K (12 h), washed and calcined in muffle furnace at 723 K (1 h) for activation. Pomelo skin biomass was activated by impregnation, hydrothermal treatment at 373 K (12 h), drying, washing and calcined at 723 K (1 h) [33]. Activated carbon from dried pineapple waste biomass (leaves, stem, crown) was prepared by ZnCl₂ impregnation for 24 h, dried at 110°C for 24 h, pyrolysed (600°C, 1 h), washed and dried at 100°C for 24 h [34]. Adsorption quality of activated carbon of rice husk char (flash pyrolysed) can be enhanced by recovering amorphous silica of char with Na₂CO₃. Further, the resultant carbonaceous material has been subjected to physical activation (800°C) using steam and carbon dioxide as activating agents [35].
**Table 2. Effect of activation process and time on physico-chemical properties**

| Activation method                                                                 | Activation time (min) | $S_{\text{BET}}$ (m$^2$/g) | $V_{\text{tot}}$ (cm$^3$/g) | Yield (%) | References |
|----------------------------------------------------------------------------------|-----------------------|-----------------------------|------------------------------|-----------|------------|
| Physical activation (CO$_2$ + steam), Microwave heating                         | 75                    | 2195                        | 1.293                        | 39.2      | [36]       |
| Physical activation (steam), Conventional heating                                | 120                   | 1926                        | 1.26                         | 39.1      | [37]       |
| Physical (inter atmosphere), Conventional heating                                | 60                    | 378                         | 0.26                         | -         | [38]       |
| Physical (without activation agent), Conventional heating                        |                       | 663                         | 0.23                         | 23.2      | [39]       |
| Physical activation (CO$_2$), Conventional heating                               | 120                   | 1964                        | 0.98                         | -         | [40]       |
| Chemical (KOH), conventional heating                                             | 120                   | 2451                        | 1.21                         | 23.6      | [41]       |
| Combination of physical and chemical activation (ZnCl$_2$ & CO$_2$)             | 60                    | 1837                        | 1.27                         | 30        | [31]       |
| Combination of physical and chemical activation (KOH & CO$_2$)                  | 60                    | 1408                        | 0.77                         | 25        | [31]       |

**Table 3. Effect of activation process, precursor on properties of activated bio-char**

| Sr. no. | Precursor           | Activation process          | $S_{\text{BET}}$ (m$^2$/g) | $V_{\text{micro}}$ (cm$^3$/g) | References |
|---------|---------------------|----------------------------|-----------------------------|-------------------------------|------------|
| 1.      | Apricot stones      | Steam activation           | 1092                        | 0.37                          | [42]       |
| 2.      | Almond tree pruning | Physical (CO$_2$) activation | 840                         | 0.39                          | [43]       |
| 3.      | Coffee residue      | Physical (steam) activation | 469                         | 0.17                          | [44]       |
| 4.      | Palm shell          | Physical (CO$_2$) activation | 519                         | 0.22                          | [45]       |
| 5.      | Rice bran           | Physical (CO$_2$) activation | 652                         | 0.06                          | [46]       |
| 6.      | Coconut shell       | Physical (steam) activation | 663                         | 0.23                          | [13]       |
| 7.      | Coconut shell       | Chemical activation        | 1266                        | 0.68                          | [48]       |
| 8.      | Coconut shell       | Chemical activation followed by physical activation | 2114 | 1.42 | [48] |

**3.2 Characterization of Activated Bio-Char**

Bio-char as well as original feedstock both characterized for ash content and volatile content following ASTM E1755-01 and ASTM E 872 methods. Fixed carbon determined by difference from the ash and volatile content. Higher heating value (HHV) determined using an oxygen bomb calorimeter. Physical structure characterization will be performed on Micromeritics ASAP Surface Analyzer through nitrogen adsorption at 77K and morphological characterization using a Scanning Electron Microscope (SEM). Samples should be pre-treated by degassing at 200 °C to 3 μm Hg. Surface area can be calculated using BET equation, total pore volume from the amount of nitrogen adsorbed at a relative pressure around 0.95 and micropore volume obtained with t-plots method. Boehm titration is one of the most widely used methods to quantify acidic groups with different strengths on activated carbons.

The effect of raw biomass used as well as the activation parameters eg. activation temperature, activation agent, impregnation ratio and impregnation time on characteristics of activated biochar was studied by several researchers (Table 2). The activation chemicals impregnated in carbonized samples induce formation of microporosity and distribution of chemicals in precursor before carbonization governs the pore size distribution of activated carbon. Mesopores are developed due to reaction between biomass and activation agent at temperature range 300-350°C [31]. Activated carbon's surface has distinct pores and become rough, irregular shaped since it loses the fibrous characteristics at 500°C during pyrolysis [24].

Physical activation (steam/ CO$_2$) of apricot stones, almond tree pruning, coffee residue, palm shell, rice bran, coconut shell provide activated bio-char with micropore volume of 0.37, 0.39, 0.17, 0.22, 0.06, 0.23 cm$^3$/g, respectively.
Highly microporous activated bio-char was obtained with sequential chemical, physical activation of coconut shell which is followed by chemically activated coconut shell as indicated in Table 3. It implies that higher value of specific surface area can be attained with chemical activation process as compared to physical activation [42-48]. The values of $S_{\text{BET}}$ with chemical activation of coconut shell can reach 1266 m$^2$/g. The sequential activation by chemical and physical methods improve the available adsorptive surface area value to 2114 m$^2$/g [49]. The strong bases, NaOH and KOH activated bio-char acquire BET surface area higher than 3000 m$^2$/g.

As seen from Fig. 3, with increase in impregnation ratio, the development of micropore, mesopore volume and surface area initially increase and then decrease after attaining the peak as depicted in figure [31]. The mesoporous surface area continually increase with increase in impregnation ratio but the specific surface area of activated carbon increases till 1:1 impregnation ratio. The micropore surface areas were obtained by subtracting mesopore surface area from the corresponding BET surface area. The maximum surface area was recorded for 800-2:1:1 (carbonization temperature in °C– activation time, h–impregnation ratio). It was concluded that the mesopore volume and mesopore surface area increases significantly with increase in impregnation ratio and explained as collapsing, merging of micropores into larger mesopores with increment impregnation ratio [47,50]. The optimum impregnation time recorded as 10 min (for maximum surface area, mesopores) and 20 min (for maximum micropores) [23].

The activation time significantly affect the porous structure of activated carbon. It provides complete moisture removal and partial volatile evolution which lead to formation of basic structure of activated carbon. Continual increment after optimum activation time, the specific surface area decreases due to enlargement of pores [52,53]. Activation process involves removing tar substances from biomass pore space and leads to creation of microporosity in carbon structure [54]. The development patterns indicate that widening of micropores is not the primary origin of mesopores [31]. The surface area ($S_{\text{BET}}$), and the micropore volume ($V_{\text{DR}}$) of date stone activated carbon, increases with increase in activation time at 700°C temperature. At 700°C, recycling of carbon atom into glycoside rings which leads to formation of grapheme structure through partial graphitization and longer residence time results in more pores and higher surface area [36,55].

![Fig. 3. Effect of impregnation ratio on BET surface area [31,32,34,50,51]](image-url)
The activated carbon is been widely used for the removal of organic matter, dyes, heavy metals, pollutant gases and water contamination due to higher specific surface area available for adsorption [56-59]. The ibuprofen removal percentage increases with increase in adsorbent doses and an optimum adsorption was obtained for 2 g of adsorbent. Despite this, the sorption capacity decreases with increase in adsorbent dose due to aggregation which consequently leads to reduction in surface area. [24]. For a constant contact time duration (240 min), highest amount of methylene blue adsorption (376 mg/g) obtained by using 0.5 g biochar-due to availability of more active binding sites on the surface of PLAC-B [34]. Similar trends were also observed by several researchers by varying the adsorbent doses and keeping pH, agitation speed and contact time constant [60]. The optimum ibuprofen adsorption capacity was recorded at 120 min contact time. At this point, equilibrium attained after all the vacant sites occupied by IBP molecule and further increase in contact time cannot contribute much in adsorption due to availability of lesser vacant sites. Although, increasing agitation speed (upto 160 rpm) affects adsorption capacity significantly (95.86%) [24].

Activated bio-char of pineapple waste biomass (ZnCl₂ impregnated, 1:1) shows rapid adsorption of methylene blue during initial stage (120 min) of contact time but fail to approach equilibrium [34]. Methylene blue removal from aqueous solution using silk cotton, coconut tree saw dust, maize cob, and banana pith activated carbon was recorded maximum 48% (in 24 h), 93.4% (in 24 h), 100% (in 1 h), and 93.35% (in 24 h), respectively [61]. The longer residence time of char at elevated temperatures results in higher micropore volume [55]. Also, an increase in temperature during adsorption results in a reduced sorption potential due to breakdown of adsorptive forces, weak interaction forces (hydrogen bonds and Van der Waals forces) [24,62].

The yield of activated carbon decreases with increase in activation temperature from 500-650°C (at constant activation time) and with increase in activation time from 0.5-6 h (at constant temperature) [55]. Similarly, progressive reduction in activated bio-char yield with increase in activation time was also reported for coconut shell activation [36]. The higher reaction rate between char and steam (as compared to CO₂) leads to lower activated bio-char yield [36,63,64]. K₂CO₃ impregnated activated carbon experienced intensified dehydration, elimination reaction and release of volatile matter which leads to reduction in yield of final product [23]. With increase in impregnation ratio, the yield decreases and burn-off increases of palm shell [65]. These finding indicate that the yield of activated bio-char decreases with increase in activation temperature, activation time and impregnation ratio of activation agent.

Several studies found that activated bio-char sorption technologies are highly efficient and economical as compared to conventional methods for contaminant removal or wastewater treatment [66-69]. Other water treatment technologies have several limitations as compared to activated bio-char. For example, membrane filtration (such as ultrafiltration and reverse osmosis) have high cost of filter media/ clogging/ dye layer build-up problem, chemical techniques (chlorination, ozonation, and wet air oxidation) include toxic by-products [70], titanium oxides or ferrous ions as catalysts have ferrous sludge, and electrochemical destruction involves high cost [66]. These shortcomings of other methods paved the way for activated bio-char as a prominent adsorbent for contaminants.

4. CONCLUSIONS

Degradation of cellulose, hemicellulose and lignin occur at temperature range of 300-350, 250-500 and 280-500°C, respectively. Chemical activation of biomass is generally done by impregnation (H₃PO₄, ZnCl₂, KOH), oven drying at 100±10°C, calcined in muffle furnace (400-600°C), washing and drying. Mesopores are developed due to reaction between biomass and activation agent at temperature range 300-350°C. With increase in IR, the development of micropore, mesopore volume and mesopore surface area initially increase and then decrease after attaining the peak. Agitation speed, adsorbent dose, and temperature affect significantly the sorption capacity of activated bio-char.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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