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Source Water Purification of Bamboo Activated Carbon Prepared from Bamboo Charcoal by Using the Multi-layer Filtration Method

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Bamboo charcoal (BC) was used as a precursor to prepare bamboo activated carbon (BAC) by the method of steam activation with at 700, 800 and 900°C of activation temperatures and 60, 90 and 150 min of activation duration, respectively. Using the standing, filtration and lab-designed multi-layer filtration methods, the feasibility of source water purification was investigated herein. The yield of BAC was 58.02 to 90.59%. The iodine value (362–891 mg/g) of BAC was higher than that (277 mg/g) of BC. After treatment by the standing and the filtration methods, except for turbidity and total bacterial count, the other tested items could reach drinking water quality standards. The lab-designed multi-layer filtration method designated with filter paper, BC, BAC and filter paper as the 1st, 2nd, 3rd and 4th filter-layer individually located in the glass funnel with 5 ± 2 and 10 ± 2 mL/min of flow rate to purify the source water. The turbidity, total hardness, nitrite nitrogen, coliform, and total bacterial count of the treated water resulted in 1.30 NTU, 89.02 mg/L, 0.05 mg/L, 0.00 CFU/mL and 26.00 CFU/mL, and reached drinking water quality standards in Taiwan. In the Ames tests, the residual bacteria rate of the cytotoxicity for source water and the treated water was higher than 80% of the control, indicating a lack of cytotoxicity. The mutagenicity of both was not over spontaneous revertants of the control group by more than two times, signifying no mutagenicity. The BAC offers the potential development of water quality purification, and with the multi-layer filtration method has the consulted value of purification from source water.

Key words: Ames Test, Bamboo Activated Carbon (BAC), Multi-Layer Filtration Method, Source Water, Steam Activation

INTRODUCTION

According to the annual statistics of the Forestry Bureau reports (2015) from 1998 to 2013, the average percent of various bamboos in Taiwan's total bamboo forest area accounted for about 6%. The average number of various bamboos harvested amounted to about 1.24 billion pieces. Bamboo products occupy an important relation to the practicality of life, and have significant economic value in Taiwan. However, the 921 earthquake in 1999 seriously impacted the local traditional bamboo industries. For the recovery of the bamboo industries and to achieve Taiwan bamboo resource utilization, since 2002, the Forestry Bureau has scheduled the project "Promotion and Transformation of Bamboo Industries" to develop new types of bamboo products, especial for bamboo charcoal.

Bamboo charcoal (BC) has a particular pore structure, surface functional group, chemical stability, mechanical strength, acid resistance, alkali resistance and heat resistance, and can provide strong adsorption characteristics; as a result, it has been used extensively in water purification (Lin *et al.*, 2003). In general, activated carbon (AC) is a good adsorbent for gaseous and liquid adsorption and is widely applied in the purification, de-

colorization, and removal of toxic substances, as well as the treatment of waste water (Manocha, 2003; Yorgun *et al.*, 2009; Sun and Jiang, 2010). Hence, the BC, after being refined by activation, becomes bamboo activated carbon (BAC) that still retains the charcoal's characteristics, and its absorption capacity is higher than that of BC (Weng, 2010).

Previous work (Lin *et al.*, 2014) has established some referable results for the adsorbability of BC and BAC. The iodine value (886–1068 mg/g) of BAC, refined from Moso BC, is higher than that (141–623 mg/g) of Moso BC. The true density (2.11 g/cm^3) and BET surface area ($791.22 \text{ m}^2/\text{g}$) of BAC are obviously higher than those (1.68 g/cm^3 and $138.70 \text{ m}^2/\text{g}$) of BC. BAC belongs to the micro/mesopore multi-structure because the average pore diameter is 2.22 nm. Lee *et al.* (2014) and Weng (2010) reported that the BET specific surface area of BAC, prepared by using the method of physical activation, can reach commercial AC, normally ranging from 500 to $1500 \text{ m}^2/\text{g}$ (Huang, 2002). According to the Brunauer–Deming–Deming–Teller (BDDT) classification, the nitrogen adsorption-desorption isotherms of the BAC are classified as type IV with the hysteresis loop, presenting the possibility of adsorption-desorption porosity; in addition, BAC can be used as functional water purifying material (Liu, 1998; Lin *et al.*, 2014; 2015).

A previous study (Lin *et al.*, 2015) reported that the AC with multiple micro/mesopores can be used as functional water purifying material. As AC has a developed pore structure, it can adsorb the trace amounts of organic contaminants in water (Kihn *et al.*, 2000; Andersson *et*

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al., 2001), and significantly reduce the chemical oxygen demand and total organic carbon in water (Kunio *et al.*, 2001; Seyed *et al.*, 2004; Omri *et al.*, 2013). It is also effective on the turbidity and chromaticity of the physical standards for drinking water (Anu *et al.*, 2006). In addition, the AC has an inhibitory effect on total bacterial count and coliform in water (Ogawa *et al.*, 2011), and conforms to the biological criteria of water standards of the Environmental Protection Administration of Taiwan (EPA). In Taiwan, source water includes groundwater, reservoirs and rivers (Shiu, 2000), and all water sources must conform to the Water Quality Standard for Source Water (EPA, 1997) before they can be used as a water source of tap water supplied as industrial water for the public (EPA, 2013c).

On the other hand, the massive amounts of chemical substances, environmental accumulation, and chemical reactions pollute the environment and pose hazards to human health. Mutagenicity is a change in the hereditary property in the reproduction process of deoxyribonucleic acid storing gene information in biological stomatocytes caused by toxic chemical substances. If the source water is severely polluted, there may be mutagenicity, increasing the probability of cell mutation, and if the chlorine dosage is too high in the source water, there may be high carcinogenic risk. The gene mutation caused by chemical substances can be tested by the Ames test (Ames *et al.*, 1975; Maron and Maron, 1983). For this study, BAC prepared from BC via steam activation was employed to purify source water by using the standing, filtration and lab-designed multi-layer filtration methods; the source water and water treated by the prepared BAC were examined to evaluate the preliminary safety of cytotoxicity and mutagenicity of the Ames test, in order to hopefully obtain clean water from source water in remote mountain areas or/and post-disaster.

MATERIALS AND METHODS

Specimen and its basic properties

Bamboo charcoal

The bamboo charcoal (BC) was Moso bamboo (*Phyllostachys heterocycla* Milf), manufactured in earth kilns at a temperature of 700–800°C (Hung, 2004; Lin and Hwang, 2006) as the precursor; it was ground and sieved to the size of 10–40 mesh, and then dried in a vacuum oven overnight at 105°C.

Source of water specimen

The source water as the blank group was obtained from Chiayi Lantan Lake, a reservoir in Southern Taiwan, on September 21, 2016 with about 30°C temperature and 78% of relative humidity.

Preparation of bamboo charcoal carbon

Two methods are commonly used for preparing activated carbon: physical and chemical activation. Considering the residue of chemical activating agent, the prepared bamboo activated carbon (BAC) for the study used the physical activation method. The precursor, 30 g of oven dried BC, was activated in a closed container of

super-high temperature vacuum carbonization activation equipment (Chi-How Heating Co., Ltd.). Nitrogen (N₂ gas) was added to make the container oxygen free. The heating rate was set at 10°C/min to carbonization temperature in the range of 700, 800 and 900°C. The steam activation was carried out at 700, 800, and 900°C with the activation duration for 60, 90 and 150 min, respectively. The flow rate of steam was maintained at 200 mL/min. The BACs were then cooled by N₂ gas to a normal temperature and taken out. The aforesaid preparation conditions referred to earlier studies (Chang *et al.*, 1998; Juang *et al.*, 2000; Tseng *et al.*, 2007; Lee *et al.*, 2014; Lin *et al.*, 2014 and 2015). The equation for the BAC yield (*Y*) is $Y (\%) = (\text{bone dry weight of BAC} / \text{absolute dry weight of BC}) \times 100$.

Characterization of BC and BAC

pH value

The pH value of BC and BAC was determined according to CNS 697 (1965). BC and BAC weighing 10 mg were separately placed on a flask equipped with a boiler-reflux condenser, and 30 mL of distilled water was added to the flask and boiled for 5 min. The supernatant liquid was determined using a pH meter (Cyber Scan pH 510) after cooling the mixture to room temperature.

Iodine value

Iodine value was determined according to JIS K 1474 (1991). BC and BAC were crushed separately and run through a sieve to obtain particles with a size between 40 and 60 mesh, and the equation for determining the iodine adsorption value was $I = (10 - K \times f) \times 12.69 \times 5/M$; (*I* iodine adsorption value (mg/g); *K* volume of sodium thiosulfate solution used for titration (mL); *f* ratio of 0.1 N sodium thiosulfate solution to 0.1 N iodine solution; *M* absolute dry weight of BC or BAC).

True density

The BC and BAC particle size was 40 to 60 mesh, and dried in an oven at 105°C for 24 h. By using the Ultracycrometer 1000 (Quantachrome Instrument Co. Ltd.) in the Divisions of Forest Utilization in the Taiwan Forestry Research Institute, the experimental details (Hwang *et al.*, 2013) of the density were investigated. Three replicas of each BC and BAC were performed.

Characterization measurements

The pore structure characteristics of BAC were measured by nitrogen adsorption-desorption isotherms at 77 K using a Micromeritics ASAP 2020 at a relative pressure (*P/P₀*) ranging from 10⁻² to 1. The BET specific surface area (*S_{BET}*) was determined using the standard BET equation applied to a relative pressure range from 0.06 to 0.2. A t-plot and Barret-Joyner-Halenda (BJH) (Barrett *et al.*, 1951) analysis software were used to calculate the external surface area (*S_{ex}*), micropore volume (*V_{mi}*) and pore size distribution. The total pore volume (*V_{tot}*) was estimated as the liquid volume of nitrogen at a high relative pressure of 0.98, and the average pore diameter (*D*) was calculated using the equation $(4V_{\text{tot}} / S_{\text{BET}}) \times 10^3$ (Hu and Srinivasan, 1999).

Water purification methods

The standing, filtration and lab-designed multi-layer filtration methods were employed to purify the source water. The purification methods are described as follows;

Standing method: the BC and BAC and the source water were mixed by a weight ratio of 1: 10 (wt%), and kept still for 30, 60, and 120 min, respectively (Zhou and Lu, 2010).

Filtration method: the BC and BAC were placed in a glass funnel, the source water flowed from the top to the bottom under gravity, and were controlled by valve; the flow velocity was 10 ± 2 mL/min and 5 ± 2 mL/min (Ogawa *et al.*, 2011), and the weight ratio of BC and BAC to source water was 1: 10 (wt%).

Multi-layer filtration method: this method, designed in the Lab of Environment Functional materials, National Chiayi University in Taiwan, was designated with filter paper, BC, BAC and filter paper as the 1st, 2nd, 3rd and 4th filter-layer individually located in the glass funnel with 5 ± 2 and 10 ± 2 mL/min of flow rate to purify the source water. The weight ratio of BC and BAC to source water as the 2nd and 3rd filter-layer was still 1:10 (wt%).

Water tests

Three types of water tests for testing source water and the treated water were included, physical, chemical and biological items. In addition, the surface tension and contact angle of the treated water were investigated.

Physical item

The turbidity was tested by the water turbidity detection method – turbidimetric method, as published by EPA (NIEA W219.52C, 2005).

The total hardness was tested by the inwater total hardness detection method – EDTA (Ethylenediaminetetraacetic acid) titration, as published by EPA (NIEA W208.51A, 2006). The equation was total hardness (represented by CaCO_3 , mg/L) = $A \times B \times 1000 / V$, where A is the titration volume of EDTA for source water and the treated water titration minus titration volume of Blank; V is source water or the treated water volume (mL); B is the milligrams of CaCO_3 equivalent to 1 mL EDTA volumetric solution.

The surface tension (dyne/cm) of source water and the treated water was tested by Surface Tensiometer (CBVP-A3, Face Co). The experimental procedures refer to (Luiz Fernando and Roger, 2005).

The measured contact angle ($1/2 \theta$) of source water and the treated water was measured by the sessile drop method with a contact angle goniometer (Contact-angle meter CA-D, Face Co). The contact angle (θ) is double the measured contact angles between the water and the surface of the China fir (*Cunninghamia lanceolata*) specimen with the top of polyurethane (PU) sand sealer.

Chemical item

The pH value was measured by the inwater hydrogen ion concentration measuring method – electrode method, as published by EPA (NIEA W424.52A, 2008).

The nitrite nitrogen was tested by the inwater nitrite nitrogen detection method – spectrophotometry, as published by EPA (NIEA W418.52C, 2013b). The equation

is nitrite nitrogen concentration (mg/L) = $A \times 50 / V$, where A is the concentration value (mg/L) measured by calibration curve; V is source water or the water specimens volume (mL). The sodium nitrite standard solution 0.1 $\mu\text{g}/\text{mL}$ was used to discuss the changes in the content adsorbed by BC and BAC, respectively.

Biological item

The coliform was tested by the commercial quick test method; namely, Chromocult coliform was used to quickly test the aerobic or facultative anaerobic, gram-negative, and asporulate coliform bacteria. The equation was coliform count (CFU/100mL) = [(red colony + violet colony) \times 100] / source water or the treated water volume \times extension rate.

The total bacterial count was tested by the inwater total bacterial count detection method – mixed dilution method, as published by EPA (NIEA E204.55B, 2013a). The equation is total bacterial count (CFU/mL) = total colony counts in the selected culture dish/sum of actual volumes of source water or the treated water in the selected culture dish.

Ames tests

The Ames test is done as proposed by Ames *et al.* (1975) and Waleh *et al.* (1982). The *Salmonella typhimurium* (*S. typhimurium*) TA98 and TA100 were used as test strains (Mortelmans and Zeiger, 2000). The phosphoric acid buffer solution was used as the control (Control), and source water before and after purification by the multi-layer filtration method was repeated to calculate the colony counts. After cytotoxicity testing, the residual bacterial count (Survival, %) has to be higher than 80% of the residual bacterial count of the Control, meaning that the specimen has no toxic response to cells (Waleh *et al.*, 1982). Survival (%) = [(the bacterial count of the tested water / the bacterial count of the Control)] \times 100. In the mutagenicity test, if the specimen induced by spontaneous revertants is two times higher than the Control's, i.e. mutagenicity ratio (MR) above 2.0, it means the specimen has mutagenicity (Ames *et al.*, 1975). MR = induced revertants per plate/spontaneous revertants per plate (Control).

Statistical analysis

The results are represented by mean (standard deviation). The statistical analysis was conducted using SPSS 12 (Statistical Product and Service Solutions) and Duncan's multiple range test ($p < 0.05$). Different letters represent significant difference, while the same letter (same subset) represents no significant difference.

RESULTS AND DISCUSSION

Characterization of BC and BAC

Yield

The characterizations of AC are dependent on the activation temperature and duration (Chang *et al.*, 1998 and 2003; Zhang *et al.* 2004; Tseng *et al.*, 2007; Huang *et al.*, 2010; Wu *et al.*, 2010; Peng *et al.*, 2010). The activation temperature influences the precursor by means of

Table 1. Yield, pH value and iodine value of bamboo charcoal (BC) and BAC

	Specimen	Yield (%)	pH value	Iodine value (mg/g)
BC	—	—	7.87 (0.10) ^a	277.55 (10.10) ^a
	T700–60 ¹⁾	79.34 (3.92) ^{d2)}	9.87 (0.28) ^c	362.17 (20.75) ^b
	T700–90	73.37 (1.40) ^{cd}	10.25 (0.17) ^c	825.70 (16.92) ^d
	T700–150	58.02 (7.93) ^a	10.21 (0.22) ^c	891.17 (43.45) ^{de}
	T800–60	90.59 (0.96) ^e	10.12 (0.01) ^c	613.52 (11.72) ^c
BAC	T800–90	74.18 (0.90) ^{cd}	8.92 (0.74) ^b	933.45 (31.54) ^d
	T800–150	66.39 (0.94) ^c	10.00 (0.27) ^c	886.00 (9.44) ^e
	T900–60	88.47 (3.71) ^e	9.90 (0.09) ^c	584.50 (17.58) ^c
	T900–90	62.75 (1.08) ^b	11.10 (0.04) ^d	870.43 (12.98) ^{de}
	T900–150	66.39 (2.39) ^{ab}	10.50 (0.81) ^{cd}	886.00 (10.41) ^{de}

¹⁾ Bamboo activated carbon (BAC; activation temperature–activation duration)

²⁾ Mean (standard deviation) with the different superscripts are significantly different ($\rho < 0.05$) by Duncan's multiple range test

the pyrolytic decomposition decreasing the volatile compounds and tar, as well as increasing carbonaceous gasification (Teng and Hus, 1999). The yield of BAC was from 58.02 to 90.59%, using steam activation at activation temperatures of 700, 800 and 900°C and activation duration of 60, 90 and 150 min, respectively (Table 1). Zhou *et al.* (2003) report that the steam activation method produces oxygen that results in much more carbonaceous surface oxidization. The difference in the yield of BAC was significantly (5%) in accordance with Duncan's multiple range test.

pH value

As shown in the Table 1, the pH value of BC was 7.87. When the activation temperature and duration increased, the BAC became alkaline, 9.87–11.10%. This is because the AC prepared from precursor contains abundant natural mineral substances of Ca, Mg, Fe, K, Mn, and P metal ions, and the different compounds are generated and discharged. Also, a higher temperature is required for the reaction to form oxides, which may be because its pH is alkaline (Gundale and Deluca, 2006; Neary *et al.*, 1999). The results revealed that the pH value of BAC was significantly (5%) different in accordance with Duncan's multiple range test, except for the BAC prepared at the activation temperature of 700°C with different activation duration.

Iodine value

The iodine value of BAC was 362.17–933.45 mg/g (Table 1), which was higher than the value of BC, 277.55 mg/g. The iodine value of BAC prepared with duration at 90 min at 800°C and 900°C for over 90 min was the highest range, 870.43–933.45 mg/g. According to Duncan's analysis, there was obvious difference in the iodine value of the BAC due to the increase in activation temperature or duration. This indicates that a higher temperature and duration results in expansion on micropores or a burn-off on the carbonaceous surface because of uneven activation; that is, the micropores' neighbor is ruined and its pore scale is expanded (Zhou *et al.*, 2003; Aworn *et al.*, 2008).

In order to evaluate the absorption and porosity of BAC in the following experiments, including the true density, nitrogen adsorption–desorption isotherms and pore size distribution, the selected BAC was prepared by using steam-activation with a flow rate from 200 mL/min at activation temperatures of 700, 800 and 900°C and the activation duration of 90 min.

True density

The true density of BAC was from 1.90 to 2.02 (g/cm³). Salvador and Jiménez (1999) report that true density is the density of the solid material, excluding the volume of any open and closed pores. A larger true density generally is needed if AC is to be considered as a material of liquid absorption, such as water purification. The results are between 1.68–1.82 g/cm³ (Lin *et al.*, 2010) and 2.11–2.17 g/cm³ (Lin *et al.*, 2014).

Characterization measurements

The SBET, S_{ext} (specific surface area, external surface; from t-plot and BJH analysis) and V_{tot} (total pore volume) of the selected BAC increased as the activation temperature increased (results are not shown in the Table). Commercial AC exhibits SBET values ranging from 500 to 2000 m²/g (Rodríguez-Reinoso and Molina-Sabio, 1992). The results obtained demonstrated that the SBET of BAC was in the range of commercial AC, except for BAC at 700°C. The percentage of V_{mf}/V_{tot} (micropore volume/ V_{tot}) showed that BAC has some micropores over 86%, but physical activation has pore-drilling and expansion effects at 800°C, producing multiple micropores (Lua and Guo, 2000; Yun *et al.*, 2001). The average pore diameters of BAC were from 1.94 to 2.18 nm. When the activation temperature increases, as the expansion effect is greater than the pore-drilling effect, multiple mesopores are produced (Walker and Almagro, 1995). According to the Bruauer, Deming, Deming and Teller (BDDT) classification, the selected BAC was Type IV, indicating the micro-mesopore content of the adsorbents, and was H4 type hysteresis loops with most of the microporous structures in accordance with the International Union of Pure and Applied

Chemistry (IUPAC). The range of the mesopores drastically increased from 2 to 4 nm, indicating the creation of micropores to produce mesopores. The formation of mesopores is known to be enhanced by an increase in the activation temperature or the flow rate of steam (Zhou, 2003; Aworn *et al.*, 2008).

Water purification

Physical item

The turbidity and total hardness of the source water from Chiayi Lantan Lake was 2.39 NTU and 243.62 mg/L, respectively (Table 2). The source water purified separately by prepared BC and BAC with the standing and filtration methods for the turbidity and total hardness were 16.29 to 40.20 NTU and 9.37 to 224.99 mg/L, respectively (results were not shown in Table). The results of total hardness meet the quality standard for drinking water in Taiwan at 300 mg/L (calculated by CaCO_3). The basic functional groups of carbon surface help to reduce the total hardness of water; moreover, the inorganic substance is likely to exchange ions with the carbon surface functional groups, and form the complex absorption (McCafferty *et al.*, 2000). However, the results of turbidity were higher than the standard at 2 NTU. This study used the multi-layer filtration method to retry the

source water purification. As shown in Table 2, the turbidity was 1.30 to 1.86 NTU with the flow velocity of 10 mL/min, meeting the quality standard for drinking water, with about 22.18 to 45.16 percent removal (PR), but with the 5 mL/min it was 1.85 to 2.18 NTU after filtration. According to the results of Duncan's multiple range test, the multi-layer filtration method treatment results of different filtration flow velocities had significant difference, indicating that the decrease of turbidity is influenced by the multi-layer filtration method at a flow velocity of 10 mL/min.

The surface tension of the liquid is used for evaluating wetting on the solid surface. The contact angle is defined as the angle between the solid surface and a tangent, drawn on the drop-surface, passing through the triple-point atmosphere-liquid-solid (Luiz Fernando and Roger, 2005). Both are generally considered in investigating the coatings or adhesion operation on the wood surface, but for this study, they are considered the source water, after filtration with different BAC, perhaps water molecules become meticulous (smaller) and conducive to the absorption of the human body after drinking. The surface tension and contact angle (θ) of source water are 73.60 dyne/cm and 115° , respectively (Table 3). The surface tension and the contact angle of the water speci-

Table 2. Turbidity and total hardness ¹⁾ and its percent removal of water specimens after treating by the multi-layer filtration method

Purification method, water specimen and flow velocity		Turbidity (NTU)	PR (%) ²⁾	Total hardness (mg/L)	PR (%)	
Source water		2.39 (0.73) ^{b3)}	–	243.62 (13.25) ^{d)}	–	
Multi-layer filtration method	T700 ⁴⁾	5 mL/min	2.18 (0.16) ^{b)}	193.98 (2.99) ^{c)}	20.38	
		10 mL/min	1.86 (0.07) ^{ab)}	200.08 (9.13) ^{c)}	17.87	
	T800	5 mL/min	2.03 (0.12) ^{b)}	15.06	89.02 (6.63) ^{a)}	63.46
		10 mL/min	1.30 (0.17) ^{a)}	45.61	121.81 (0.00) ^{a)}	50.00
	T900	5 mL/min	1.85 (0.08) ^{ab)}	22.59	121.81 (13.25) ^{a)}	50.00
		10 mL/min	1.81 (0.12) ^{ab)}	24.27	117.13 (6.63) ^{a)}	51.92

¹⁾ Turbidity and total hardness of water quality standard for drinking water source is 2 NTU and 300 mg/L (Environmental Protection Administration, 2013c)

²⁾ Percent removal (PR, %) = [(turbidity (or total hardness) of blank – turbidity (or total hardness) of water specimen after treating with BAC) / turbidity of blank] × 100

³⁾ See the Table 1 ²⁾

⁴⁾ Bamboo activated carbon (activation temperature)

Table 3. Surface tension ¹⁾ and contact angle of water specimens after treating by the filtration method

Water specimen–flow velocity	Surface tension (dyne/cm)	Contact angle θ (°)
Source water	73.60 (0.39) ^{b2)}	115 (2.84) ^{a)}
T700–10mL/min ³⁾	72.30 (0.51) ^{a)}	119 (3.85) ^{a)}
T800–10mL/min	72.30 (0.33) ^{a)}	116 (5.99) ^{a)}
T900–10mL/min	72.20 (0.33) ^{a)}	117 (4.15) ^{a)}

¹⁾ The tests was examined on the surface of China fir specimen with the top of polyurethane (PU) sand sealer

²⁾ See the Table 1 ²⁾

³⁾ Water specimen (treated with BAC at activation temperature–flow velocity)

men after treating by the filtration method with 10 mL/min of flow velocity with BAC preparing by 700, 800 and 900°C of activation temperatures were 72.20–72.30 dyne/cm and 116–119°, respectively. As expected, the surface tension decreased and the contact angle increased, meaning the water molecules and liquid surface tension of source water after treatment became smaller, even though they were insignificantly different from the source water in accordance with Duncan's multiple range test. The same results are shown in Table 2; the total hardness decreased after filtration. In other words, the hard water or moderately hard water would become soft water, with Ca²⁺, Mg²⁺, Sr²⁺ and Fe²⁺ removed by treatment with AC (Lin *et al.*, 2015).

Chemical items

The pH value and the nitrite nitrogen concentration of source water were 7.26 and 0.08 mg/L, respectively. After the use of the BC and BAC standing and filtration methods, the pH value increased to 8.10–10.14 and the nitrite nitrogen decreased to 0.02–0.08 mg/L (results not

shown in the Table). The pH value and the nitrite nitrogen concentration of the treated water, purified by the multi-layer filtration method, were 8.84–9.80 and 0.05–0.08 mg/L, respectively, with different flow velocity (Table 4). The results of the nitrite nitrogen meet the quality standard for drinking water in Taiwan at 0.1 mg/L, even for source water. The pH value increases because AC becomes alkaline by means of the Lewis Bases-like effect (Zhu *et al.*, 2012), and AC surface produces more basic functional groups with a higher activation temperature (over 800°C) (Park and Kim, 2001; Zhu *et al.*, 2012). For the decrease of the nitrite nitrogen concentration, the basic alkaline functional group of AC can adsorb nitrite nitrogen (Cabal *et al.*, 2009; Nunell *et al.*, 2012).

Biological items

The AC with the strongly alkaline solution condition can cause bacterial death and/or the bacteria to be adsorbed (Uraki *et al.*, 2008). As shown in Table 5, the coliform of the source water was 158.50 CFU/mL, and the treated water after purifying with the multi-layer fil-

Table 4. pH value and nitrite nitrogen ¹⁾ of water specimens after treating by the multi-layer filtration method

Purification method, water specimen and flow velocity		pH value	Nitrite nitrogen (mg/L)
Source water		7.26 (0.04) ^{1b)}	0.08 (0.00) ^d
T700 ³⁾	5 mL/min	9.37 (0.06) ^{ef}	0.08 (0.00) ^d
	10 mL/min	9.21 (0.10) ^e	0.07 (0.00) ^c
Multi-layer filtration method	T800	5 mL/min	9.43 (0.01) ^f
		10 mL/min	8.84 (0.03) ^d
	T900	5 mL/min	9.80 (0.07) ^g
		10 mL/min	9.21 (0.05) ^e

¹⁾ pH value and nitrite nitrogen of water quality standard for drinking water source is 6.0–8.5 and 0.1 mg/L (Environmental Protection Administration, 2013c)

²⁾ See the Table 1 ²⁾

³⁾ See the Table 2 ⁴⁾

Table 5. Coliform and total bacterial count ¹⁾ and its percent removal of water specimens after treating by the multi-layer filtration method

Purification method, water specimen and flow velocity		Coliform (CFU/mL)	PR (%) ²⁾	Total bacterial count (CFU/mL)	PR (%)
Source water		158.50 (61.5) ^{3b)}	–	1688.18 (9.09) ^f	–
T700 ⁴⁾	5 mL/min	7.00 (0.00) ^a	95.58	144.00 (16.00) ^{cd}	91.47
	10 mL/min	4.00 (1.00) ^a	97.48	161.00 (9.00) ^d	90.46
Multi-layer filtration method	T800	5 mL/min	10.50 (2.50) ^a	124.52 (9.98) ^c	92.54
		10 mL/min	5.00 (4.00) ^a	96.85	145.00 (3.00) ^{cd}
T900	5 mL/min	1.50 (0.50) ^a	99.05	26.00 (1.00) ^a	98.44
		0.00 (0.00) ^a	100.00	73.25 (1.25) ^b	95.63

¹⁾ Coliform and total bacterial count of water quality standard for drinking water source is 0.06 CFU/mL and 100 CFU/mL (Environmental Protection Administration, 2013c)

^{2), 4)} See the Table 2 ^{2), 4)}

³⁾ See the Table 1 ²⁾

tration method was 1.50–10.50 CFU/mL with the flow velocity of 5 mL/min, not meeting the quality standard for drinking water (0.06 CFU/mL); even the PR was about 95.58 to 99.05%. With BAC prepared in 90°C of activation temperature at the multi-layer filtration with 10 mL/min of flow velocity, it met the quality standard for drinking water, and the PR was 100% (0.00 CFU/mL). For the total bacterial count, the source water was 1688.18 CFU/mL. The PR (total bacterial count) for water specimens reached 90.46–92.54% (161.00–124.52 CFU/mL), but it was higher than the quality standard for drinking water, 100 CFU/mL (Table 5). Meanwhile, the source water after treatment by the multi-layer filtration method with the BAC prepared from BC at 90°C of activation temperature and 90 min of activation duration with 5 and 10 mL/min of flow rate was able to reach drinking water quality standards, 100 CFU/mL. The total bacterial count of the treated water was 26–73 CFU/mL because the total bacterial count in water effectively decreases as the AC characterizations increase (Ogawa *et al.*, 2011).

Ames test

The results of the cytotoxicity of the source water and the treated water after the multi-layer filtration method at the flow velocity of 5 and 10 mL/min for *S. typhimurium* TA98 and TA100 strains are shown in Table 6. The bacterial count of the Control without S9 mixture was 2673 in TA98, and 1290 in TA100; the TA98

with S9 mixture was 1790, and TA100 was 2530. The bacterial count of source water and water specimens without the S9 mixture was 2140–3527 in TA98 and 1437–1717 in TA100, and Survival was 80–133%. The TA98 and TA100 with the S9 mixture were 2437–3040 and 2237–2763, respectively, and Survival was 88–169%. The source and the treated waters had no cytotoxicity. It is said that that if the Survival of the source and the treated waters in TA98 and TA100 strain tests is higher than 80% of the Control; the mutagenicity can be carried out (Waleh *et al.*, 1982). The results of mutagenicity are shown in Table 7. The revertants of *S. typhimurium* TA98 and TA100 induced by source water, purifying with the multi-layer filtration method at the flow velocity of 5 and 10 mL/min with and without the S9 mixture, had not exceeded double the spontaneous revertants of the Control, meaning they had no mutagenicity (Ames *et al.*, 1975). It indicated that that the source water before and after purification had no mutagenicity toward *S. typhimurium* TA98 and TA100.

CONCLUSIONS

This study evaluated BC and the BAC with the standing, filtration, multi-layer filtration methods for purifying source water, and performed the Ames test to ensure the preliminary safety of water quality before and after purification. After treatment by the standing and filtration methods, the turbidity, total hardness, nitrite nitro-

Table 6. Cytotoxicity of source water and water specimens after treating with bamboo activated carbon by multi-layer filtration method toward *Salmonella typhimurium* TA98, TA100 without (–S9) or with S9 (+S9) mixture

Water specimen	TA98				TA100			
	–S9	Survival ¹⁾ (%)	+S9	Survival (%)	–S9	Survival ¹⁾ (%)	+S9	Survival (%)
Control ²⁾	2673 (127) ³⁾	100.0	1790 (164)	100.0	1290 (298)	100.0	2530 (333)	100.0
Source water	2140 (408)	80.0	2437 (318)	136.1	1547 (463)	119.9	2730 (64)	107.9
5 mL/min ⁴⁾	2547 (290)	95.3	2567 (172)	143.4	1437 (312)	111.4	2237 (365)	88.4
10 mL/min	3527 (573)	131.9	3040 (428)	169.8	1717 (74)	133.1	2763 (158)	109.2

¹⁾ Survival (%) = [(the bacterial count of test group / the bacterial count of control group)] × 100

²⁾ Control: only with 0.1 mL phosphate buffer saline

³⁾ Mean (standard deviation)

⁴⁾ Flow velocity

Table 7. Mutagenicity of source water and water specimens after treating with bamboo activated carbon by multi-layer filtration method toward *Salmonella typhimurium* TA98, TA100 without (–S9) or with S9 (+S9) mixture

Water specimen	TA98				TA100			
	–S9	MR ¹⁾	+S9	MR	–S9	MR ¹⁾	+S9	MR
Control ²⁾	290 (111) ³⁾	1.0	174 (15)	1.0	176 (6)	1.0	208 (12)	1.0
Source water	154 (35)	0.5	271 (6)	1.6	154 (5)	0.9	274 (18)	1.3
5 mL/min ⁴⁾	46 (5)	0.2	199 (21)	1.1	40 (4)	0.2	64 (15)	0.3
10 mL/min	184 (60)	0.6	243 (87)	1.4	57 (1)	0.3	69 (3)	0.3

¹⁾ MR (Mutagenicity ratio) = induced revertants per plate/spontaneous revertants per plate (Control)

^{2), 3) and 4)} Control: see Table 6 ^{2), 3) and 4)}

gen, colifandm group and total bacterial count of purified source water were 16.29 NTU, 28.11 mg/L, 0.02 mg/L, 0.00 CFU/mL and 151.00 CFU/mL, respectively. The drinking water quality standards of Taiwan are 2 NTU, 300 mg/L, 0.1 mg/L, 0.06 CFU/mL and 100 CFU/mL. Conclusively, after treating by multi-layer filtration method with the BAC prepared from BC at 900°C and 90 min of activated duration with 10 mL/min of flow rate, the treated water turbidity was 1.81 NTU; the pH value in water was increased in solution; The total hardness was 117 mg/L; The nitrite nitrogen concentration could be reduced; the percent removal of coliform was 100%, and the total bacterial count in water was removed by 95.63%. The water tests conform to the water quality standard for drinking water in Taiwan. The bacterial survival rate of cytotoxicity testing for source water before and after purification was higher than 80% of the Control, meaning there was no cytotoxicity. The mutagenicity results showed that the spontaneous revertants of the source and the treated water were not exceeded by two times, indicating no mutagenicity. Therefore, BC can be used to prepare BAC that is more functional to purify water, especially by using the method of multi-layer filtration, in order to offer clean water from source water in remote mountain areas and/or post-disaster.

AUTHOR CONTRIBUTION

Han Chien LIN designed this paper, performed the experiments, and wrote the paper. Ling-Tseng LIU analyzed the data and the statistical analysis. Noboru FUJIMOTO participated in the design of the study, supervised the work and provided facilities and resources. All authors assisted in editing of the manuscript and approved the final version.

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