A METHOD TO REGENERATE EXHAUSTED CARBON

Field of the Invention

The present invention relates to a method to regenerate exhausted carbon. More particularly, the present invention relates to a method to obtain a biological activated carbon from exhausted carbon.

Background of the Invention

Odorous compounds such as ammonia (NH_3) and hydrogen sulfide (H_2S) emitted from Wastewater Treatment Plants (WWTPs) are widely treated using an integrated system of chemical scrubbers followed by activated carbon (AC) tank. However, the chemical scrubbing is generally expensive in terms of the recurrent use of chemicals; it is also environmentally unfavourable in producing secondary pollutants. Meanwhile, the used carbons require either costly regeneration or treatment before disposal as hazardous wastes (1).

Biofiltration, as a relatively new and exciting technology for controlling odours, has attracted an increasing popularity due to its low operation cost and nature of treating odour in an ecological and environmental-friendly way. Development of novel packing media is one of the key issues in biofiltration process to overcome the inefficiency relative to the natural media used, such as the need for an adequate residence time, limited lifetime, and subject to bed compaction. Recently, the use of AC as packing material for odour biofiltration has been explored and proven to be greatly effective with such advantages as: shortening residence time and thus reducing the bioreactor size; higher removal efficiency due to combining adsorption and biodegradation; acting as a buffer for fluctuating loadings; good resistance to crushing; and much extended lifetime (2-4).

However, improvements and/or alternative methods are needed to shorten the start-up period and cut down the investment cost of using a large amount of carbons (5), as a packing material in odour biofiltration.

Summary of the Invention

In accordance with a first aspect of the invention, there is provided a method of obtaining a microbial culture for regenerating exhausted carbon, the method comprising:

a) obtaining a seed sludge from a wastewater reclamation environment;

b) culturing the seed sludge under conditions suitable for enrichment of autotrophic sulfide-oxidizing bacteria; and

c) assessing the culture for ability to regenerate exhausted carbon.

In a related aspect, the method of obtaining a microbial culture for regenerating exhausted carbon wherein the exhausted carbon is H_2S-exhausted carbon.

The bacterial number in culture is obtained by standard method of plate counting, in the unit of CFU (sulphide-oxidizing bacteria) /ml culture. As latter the pre-deposited sulfur in exhausted carbons served as the sole energy source, the better growth of bacteria indicated the larger release of sulfur from exhausted carbons, i.e., a better regeneration.

Advantageously, the ability of the culture to regenerate exhausted carbon is to check for (1) increased content of the bacterial oxidation end product (i.e. sulphate) in the liquid phase and (2) the remaining sulfur content on the carbon surface.

In this invention, suitable conditions for the enrichment of autotrophic sulfide-oxidizing bacteria reply on the bacterial seeds and nutrient medium, which includes sulphur compounds as an energy source, e.g. thiosulfate (TS) medium, as described in the examples. The seed sludge is typically incubated for between 15 and 25 days, such as about 20 days, to allow for the growth of the autotrophic sulfide-oxidizing bacteria. Typically, the culture is subdivided into fresh medium every 4-6 days, as described in the examples.

The ability of the enriched culture to regenerate exhausted carbon can be assessed by incubating the culture with exhausted carbon, together with a mineral salt medium suitable for the growth of sulphur chemolithoautotrophs (e.g. as described in the examples) for a defined period, such as from about 15 to 30 days, at from 25°C to 30°C; measuring the combustible sulphur content of the exhausted carbon after the incubation

period, e.g. with a thermo-analytical analyzer (see the examples); and comparing with the combustible sulphur content of the exhausted carbon prior to incubation with the culture. A culture is typically considered to be suitable for regenerating exhausted carbon if it can produce at least a 40% reduction in combustible sulphur content of a chemically impregnated exhausted carbon having an initial combustible sulphur content of at least 5 wt%, over an incubation period of 20 days at 25°C (10g of exhausted carbon added to 200 ml of medium with 2 $x10^7$ CFU of bacteria).

By "enrichment", it means any process that uses selective culture media and incubation conditions to select specific microorganisms from the natural environment. The enriched mixed microbial culture of the present invention is obtained from such a process.

By "autotrophic sulfide-oxidizing bacteria", it is meant any bacteria which can oxidize the reduced sulfur compounds such as H_2S, S_o, $S_2O_3^{2-}$, and assimilate carbon from carbon dioxide without supplying organic carbon source.

Advantageously, the microbial culture is obtained from natural sources that are economical and easy to cultivate.

In accordance with a second aspect of the invention, there is provided a microbial culture for regenerating exhausted carbon, obtained by the method of the first aspect of the invention.

In a related aspect, the microbial culture for regenerating exhausted carbon wherein the exhausted carbon is H_2S-exhausted carbon.

In accordance with a third aspect of the invention, there is provided a method for regenerating exhausted carbon, the method comprising incubating the exhausted carbon with a microbial culture including autotrophic sulfide-oxidising bacteria to form a regenerated carbon comprising autotrophic sulfide-oxidising bacteria immobilised on the carbon.

In a related aspect, the method for regenerating exhausted carbon wherein the exhausted carbon is H_2S-exhausted carbon.

Preferably, the autotrophic sulfide-oxidizing bacteria are present in, or derived from activated sewage sludge.

Preferably, the sulphur compounds in the exhausted carbon are the sole energy source for the autotrophic sulfide-oxidizing bacteria. Advantageously, the pre-deposited or pre-adsorbed sulphur compounds, including all kinds of reduced inorganic sulphur and organic sulphur in the exhausted carbon serve as an energy source for microbial growth. The initial attachment of the microorganisms on the carbon surface may also serve to enhance the release of pre-adsorbed sulphur from the carbon.

Preferably, the exhausted carbon is pre-treated, prior to incubation with the microbial culture, with a solution of a strong base which reacts with sulphur compounds present on the exhausted carbon to release water-soluble reduced sulphur compounds from the exhausted carbon. More preferably, the strong base is a soluble hydroxide, such as sodium hydroxide. Advantageously, the sodium hydroxide pre-treatment releases or desorbs the sulphur compounds, or part of the sulphur compounds, that are deposited in the exhausted carbon. The release of the sulphur compounds provide an energy source for bacterial growth and, hence, biofilm growth. The release of the sulphur compounds also allows for the regeneration of active space for further adsorption (e.g. bio-regeneration). More preferably, the exhausted carbon is agitated in 1 N sodium hydroxide for at least about 24 hours. The pH of the solution is then adjusted to neutral to supply a proper environment for the growth of the sulfide-oxidizing bacteria.

Preferably, the microbial culture forms a layer of sulfide-oxidising biofilm on the carbon.

By "biofilm", it is meant to refer to any complex aggregation of microorganisms marked by the excretion of a protective and adhesive matrix.

Preferably, the exhausted carbon is a chemically-impregnated carbon, such as Addsorb VA3, a coal-based activated carbon (Jacobi Carbons). The exhausted carbon is typically obtained from wastewater treatment plants or natural gas purification plants (where the exhausted carbon may also include organosulfur compounds).

Preferably, the incubation period of the exhausted carbon with the microbial culture is about 25 to 30°C for about 15 to 30 days in sterilized mineral salt medium suitable for the growth of sulphur chemolithoautotrophs (e.g. as described in the examples). With a longer incubation time it was observed the number of microorganisms on the surface of the carbon did not increase much. Supplying enough oxygen and ensuring a good mixing, e.g. by shaking, is necessary during the incubation period.

In a related aspect, the present invention provides the use of a culture comprising autotrophic sulphide-oxidising bacteria to regenerate exhausted carbon.

In accordance with a fourth aspect of the invention, there is provided a biological activated carbon (BAC) regenerated from exhausted carbon obtained or obtainable in accordance with the third aspect of the invention.

In a related aspect, the biological activated carbon (BAC) is regenerated from H_2S-exhausted carbon obtained or obtainable in accordance with the third aspect of the invention.

Preferably, the carbon comprises immobilised autotrophic sulfide-oxidising bacteria.

Preferably, the carbon is chemically-impregnated carbon, such as Addsorb VA3, a coal-based activated carbon (Jacobi Carbons).

In a related aspect there is provided a biological activated carbon (BAC) regenerated from H_2S-exhausted carbon, which carbon comprises immobilised autotrophic sulfide-oxidising bacteria.

In one embodiment, the biological activated carbon comprises at least 10^7 CFU sulphide-oxidising bacteria per g (dry weight) of carbon, such as at least 10^8 CFU.

In accordance with a fifth aspect of the invention, there is provided the use of a BAC according to the fourth aspect of the invention for the biofiltration of odorous gases or the removal of H_2S and/or other organosulfur compounds present from natural gas.

By "exhausted carbon", it is meant to any activated carbon that has been at least partially exhausted with H_2S and/or organosulphur compounds).

Advantageously, the present invention allows wasted or exhausted carbon to be re-used in wastewater treatment plants (i.e. bio-regeneration). It prolongs the carbon service life, reduces the start-up time of biofilters and producing a much cheaper packing material. . The further release of the sulphur compounds by sulfide-oxidizing bacteria from exhausted carbon also allows for the regeneration of active space for further adsorption (i.e., bio-regeneration).

The present invention may also allow for the simultaneous bio-regeneration and subsequent use of the regenerated biological active carbon which may then be used as packing material for the biofiltration of off gas.

Brief Description of the Figures

In order that the present invention may be fully understood and readily put into practical effect, there shall now be described by way of non-limitative examples only preferred embodiments of the present invention, the description being with reference to the accompanying illustrative figures.

In the figures:

Figure 1 is a graph showing sulfate concentration and pH profiles during biofilm development;

Figure 2 is a graph showing the combustible sulphur content of AP and VA samples;

Figure 3 is a scanning electron microscope (SEM) photograph showing the biofilm on BVA3 surface developed for 15 days;

Figure 4 is a graph showing sulfate concentration profiles during biofilm development with pre-treatment;

Figure 5 is a graph showing the combustible sulphur content for VA samples with pre-treatment;

Figure 6 is a scanning electron microscope (SEM) photograph showing the biofilm on BVA3 surface developed for 8 days with pre-treatment;

Figure 7 is a graph showing DTG curves for bottom VA samples without and with pre-treatment; and

Figure 8 is a graph showing micropore volume and area for VA samples without and with pre-treatment.

Example

The following examples are presented to further illustrate and explain the present invention and should not be taken as limiting in any regard.

All references herein mentioned are hereby incorporated by reference.

Materials and methods

Enrichment of autotrophic sulfide-oxidizing microbial culture

The activated sludge, collected from a wastewater reclamation plant in Singapore, was used for enrichment of autotrophic sulfide-oxidizing microbial culture. The seed sludge was enriched in ~ 20-days to allow the growth of sulphur chemolithoautotrophs using thiosulfate (TS) medium. The TS medium was consisted of 10.0 g/L $Na_2S_2O_3 \cdot 5H_2O$ as energy source, and a buffered mineral salt solution (pH = 7.0 ± 0.2) in the following

composition (g/L): KH_2PO_4, 3.0; K_2HPO_4, 3.0; NH_4Cl, 0.4; $MgCl_2 \cdot 6H_2O$, 0.46; and 10 ml of trace element solution in 1 liter of medium. The composition of trace element solution (g/L) was: Na_2-EDTA, 10.0; $FeCl_2 \cdot 4H_2O$, 3.58; $CuCl_2 \cdot 2H_2O$, 0.137; $ZnSO_4 \cdot 7H_2O$, 11.0; $MnCl_2 \cdot 4H_2O$, 2.5; $CoCl_2 \cdot 7H_2O$, 0.59; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.5; and $CaCl_2 \cdot 2H_2O$, 10.0. The acclimation of activated sludge was performed in the TS medium with pH decreasing to 2 in about 4-6 days to complete one generation. 1/10 of the mixture was then transferred into fresh TS medium for a new cycle. After 3 to 4 transfers, the culture was harvested by centrifugation (3000 rpm for 15 min) in the phase of stable growth, and was washed in sterile mineral media three times before further use.

The standard plate count method (Black 2005) was used for counting viable sulfide-oxidizing cells in the liquid. The pH of liquid was measured using a Horiba F-53 pH meter, and the concentration of sulfate (the final oxidized product) was determined by turbidimetric method in accordance with the standard methods (6).

Preparation of H_2S-exhausted carbons

Two types of AC, both pellet-shaped in 4 mm of diameter, were selected. AP460 (designated as "AP") was obtained from Calgon Carbon Corporation, which is a type of non-impregnated carbon. AddSorb VA3 (designated as "VA") from Jacobi Group, is chemically impregnated carbon, and is currently the most widely used type of AC for H_2S removal.

The H_2S-exhausted carbons were obtained from the carbon breakthrough capacity tests following the standard adsorption method (ASTM-D6646-03). Details can be found in reference (7). Glass tubes (4.8 cm diameter; 22.9 cm packing height) used for the tests were packed with each type of AC and operated at room temperature. The 1% (v/v) H_2S together with moist air was diverted into the bottom of the carbon bed at 5.2 L/min. The H_2S concentration was monitored at the outlet of the bed with a Gas Detection Tube (Draeger Safety Asia, Singapore) until a breakthrough of H_2S at 50 ppm concentration was obtained. Double confirmation of H_2S concentration in gas was done using Jerome 631-XE H_2S Analyzer (Arizona Instrument, USA). The exhausted carbons were collected from top, middle, and bottom of the bed, and designated with additional letter "E", and termed as "1, 2, 3" for the top, middle and bottom carbons, respectively. For example,

AP-E1 represents the exhausted carbon from the top of bed packed with AP carbon. A complete analysis of the exhausted carbons from different locations of the bed packed with different carbons was conducted, in terms of surface pH, sulphur content, structural properties. The methods used for these analyses are iterated in the subsequent texts.

Biofilm development on exhausted carbon

Biofilm development on exhausted carbons was conducted in batch experiments. 10 g of exhausted carbons were added into 500 ml flasks containing 200 ml of sterilized mineral salt medium. One ml of enriched microbial culture containing 1.9 $\times 10^7$ CFU/ml of autotrophic sulfide-oxidizing bacteria in the microbial culture was inoculated into the flasks. Control experiments were also conducted under the same experimental conditions but without adding microbial inoculums. Both experimental and control flasks were covered with cotton stoppers and incubated on a rotary shaker at 150 rpm at room temperature. During the whole stage, no external sulphur source was added into the flasks except for the sulphur previously deposited on the exhausted carbons.

Bacterial count for autotrophic sulfide-oxidizing bacteria on carbon surface was determined using TS medium agar, which was prepared using autoclave-sterilized mineral salt agar (mineral salt medium with 15% Bacto agar) and filter-sterilized TS solution. The bacterial cells were de-attached from carbon and suspended in solution following the method as described by Chung et al., (4). Triplicate plates were prepared to get the average count.

Carbon identification

A thorough analysis of carbons (fresh, exhausted and biocarbons) was conducted using various approaches and methods described below. Three analyses for each sample were carried out for repeatability and precision of the measurement.

For measurement of surface pH and sulfate content of carbons, 1 g of samples was soaked in 50 ml of ultra pure water and swirled in an autoshaker for 24 h to reach

equilibrium. The resulted solution was passed through a 0.2 μm membrane filter to remove particles. The pH and the sulfate concentration of the filtered solution were then determined by the methods above mentioned.

The Micrometrics BET Analyzer model ASAP 2010 was used to measure the porosity of all carbon samples. For the morphological observations of carbon surface and biofilm development, the carbon pellets were fixed, dried and viewed with a scanning electron microscope (SEM) (Stereoscan 420, Leica, Cambridge Instruments) as described previously (3). To identify the deposited sulphur species on carbons, thermal analysis was conducted using a thermal gravimetric analyzer (TGA) (Netzsch STA 409). The combustible sulphur content of carbons was determined using a thermo-analytical analyzer (PE2400 series II CHNS/O analyzer, PerkinElmer Instruments).

Pretreatment of exhausted carbon

Without any pretreatment, exhausted carbons from both AP and VA were used for biofilm development in the first instance. After that, chemical pretreatment was performed for the VA exhausted carbon only, to enhance the desorption of sulphur compounds from exhausted carbons into liquid phase and ensure an efficient biofilm development. The chemical pretreatment involved adding 50 ml of 1 N NaOH solution into 10 g of exhausted carbon in a 500 ml flask, shaking 24 hours for a complete reaction. And then, pH was adjusted back to neutral and the solution containing sulphur-bearing compounds is ready for biofilm development.

Results and discussion

Characteristics of H_2S-exhausted carbons

In carbon breakthrough tests, variable total capacities of H_2S adsorption, 3.3 and 8.3% H_2S/AC, were found for AP and VA carbon, respectively. The generated H_2S-exhausted carbons were characterized for their surface properties, such as sulphur content, pH,

and structural parameters. The results are listed in Table 1, and compared with that of fresh carbons.

Table 1 Sulfur content, surface pH and structural parameters of carbon samples

Sample	Sulfur content (w/w % S/AC)		pH	Structural parameters			
	S-combustible sulphur	S-sulfate		V_{mic} (cm^3/g)	S_{BET} (m^2/g)	S_{mic} (m^2/g)	S_{ext} (m^2/g)
AP	0.25	---	7.6	0.26	972	563	409
AP-E1	0.75	0.12	7.1	0.246	920	529	391
AP-E2	1.74	0.3	6.3	0.221	871	482	389
AP-E3	3.79	0.59	4.7	0.151	642	355	288
VA	0.26	---	9.9	0.18	910	400	510
VA-E1	2.85	0.18	8.9	0.167	845	378	466
VA-E2	5.73	0.56	5.3	0.142	776	322	454
VA-E3	10.19	1.27	4.2	0.075	641	178	463

---: non-detectable

V_{mic}: Micropore volume; S_{BET}: BET surface area; S_{mic}: Micropore area; S_{ext}: External surface area

The combustible sulphur and sulfate contents in exhausted carbons increased largely from top (outlet, labeled "1") to bottom (inlet, labeled "3") of the H_2S adsorption bed for both carbons. It indicated the full usage of carbon at the bottom, but the top carbon still remained similar as fresh carbon with only a few sulphur detected, due to little H_2S left in the gas stream at outlet (7). The content of combustible sulphur was obviously higher than that of the corresponding sulfate in exhausted carbons. The combustible sulphur could be any sulphur-bearing species (but excluding S (VI), such as sulfate) formed through either physical or chemical adsorption. As sulfate (VI), the fully oxidized sulphur species, cannot be used as food for sulfide-oxidizing bacteria, only the combustible sulphur content in exhausted carbons can indicate the amount of sulphur compounds that can be potentially used by bacteria.

The pH values of exhausted carbon decreased from the top to the bottom of adsorption bed (Table 1). The change of pH accounted for the dissolution of H_2S in the water film

and the formation of sulphuric acids in the carbon. Fresh VA carbon had an alkaline surface pH while that of AP was close to neutral.

BET surface area (S_{BET}) reduced significantly from top to bottom exhausted carbons, due to the much larger deposition of sulphur products in carbon pores at inlet than at outlet of the bed (8). The external surface area (S_{ext}) can be used to indicate the available surface for bacteria growth whilst nearly all of the internal surface areas are inaccessible, due to the large size of microorganisms (~ 1 μm) (9). Meanwhile, micropore volume (V_{mic}) and area (S_{mic}) are believed to correlate to the adsorption capacity of AC as generally micropores serve as active sites for adsorption. In view of re-using the exhausted carbons to develop biofilm, it can be seen that these exhausted carbons all had decreased micropore volume and area (i.e., H_2S adsorption capacity) from top to bottom, which were lower than that of the corresponding fresh carbons. It indicated that the biocarbon developed from exhausted carbons might perform differently in physical/chemical adsorption with that from fresh carbons. In addition, there was not much change in external surface area for VA exhausted carbons in comparison to its fresh carbon whilst an evident reduction for AP exhausted carbons was observed, implying a larger space available for bacterial attachment in VA exhausted carbons. This can probably be linked to the presence of salts as a result of neutralization reaction in the case of caustic carbon (VA) (10).

Biofilm development on exhausted carbons

The exhausted carbons demonstrated different properties (Table 1), which could result in different biofilm developments. It is worthy to investigate the suitability of exhausted carbons for developing biocarbon.

The increase of sulfate concentrations and drop of pH were employed as two indicators to monitor the sulphur oxidation during biofilm development on exhausted carbons. The effectiveness of microbial sulphur-oxidation was confirmed through the comparison of batch incubation of H_2S-exhausted carbons with and without bacterial cultures. The results from VA carbons are shown in Figure 1, as a representative. Three experiments

(with bacteria) were referred to BVA1, 2 and 3 while correspondingly their controls (without bacteria) were VA1, 2 and 3 involving exhausted carbons from the top, middle and bottom of bed, respectively.

Figure 1 shows abrupt changes in sulfate concentrations and pH in the solution after one-day incubation for all experiments and controls. These sudden changes were attributed to their different sulfate contents and surface pH (Table 1). The sulfate contents of controls (VA1, 2, and 3) appeared relatively constant with time progressing after the 1st day up to the 28th day. There was no significant difference in sulfate contents and pH between control and experiment solution with the top carbon (VA1 and BVA1), most likely attributed to the very low sulphur content here, resulting in little release of sulphur into liquid phase thus negligible use by bacteria. Nevertheless, for BVA3 and BVA2 in comparison to their counterparts (VA3 and VA2), sulfate contents increased obviously with time, accompanied by a gradual pH drop. The widening deviation of both sulfate content and pH value with time between the experiment and control trials gave a clear indication to the occurrence of microbial sulphur oxidation.

On the 14th day (Figure 1), the pH in liquid with bottom carbons was adjusted back to neutral, to verify the bacterial function. Results showed that more sulfate was produced in the experiment over control flasks right after the changes made, confirming that the bacteria indeed played an important role in sulphur release from the VA exhausted carbons.

Combustible sulphur analysis of the control and experimental carbons after 28 days of biofilm development was conducted, and the results are shown in Figure 2, in comparison with that of exhausted carbons. The difference of sulphur contents between the exhausted and control was attributed to the dissolution of sulphur compound into aquatic solution and that between the control and experiment accounted for bacterial oxidation. It was found that for VA carbons a clear decrease of combustible sulphur contents occurring in experiment from that in control, indicating that bacteria could play an important role in releasing combustible sulphur from VA exhausted carbons, followed by oxidation to sulfate in the liquid phase. The decrease of combustible sulphur content

in experiment of VA carbon (Figure 2) was supposed to correlated to the increase of the sulfate content in liquid (Figure 1). A thorough study of sulphur balance and distribution in carbon and solution is thus worthy, and it will be carried out in a near future.

The biofilm development on VA exhausted carbons was observed through SEM (Figure 3) and sulfide-oxidizing bacteria count. Too much biomass could block the carbon external pores that make the adsorption capacity loss. Alternatively, a weak biofilm is not efficiently enough in removing contaminants. Hence, the overall consideration of the biofilm development is to optimize the combination of the two effects (adsorption and biodegradation). Counting bacteria on carbon surface can quantify the density of the biomass, as described below.

Morphological observation, carried out after 15 days of biofilm development, showed that rod-shaped bacteria (~1 μm in length) distributed partially on carbon surface. Bacterial counts from the 5th to 28th day of biofilm development revealed that there are sulfide-oxidizing bacteria approximately 1.0 to 5.8×10^5 CFU/g dry AC present on three BVA carbons. BVA1 with low sulphur content presented relatively less bacteria attachment, while for BVA3 with higher sulphur content, higher bacteria numbers could be observed.

Similar trials were conducted with AP exhausted carbons, but there was almost no difference found between control and experiments, in terms of sulfate content and pH in liquid (data not shown). This indicated that in AP's case the function of bacteria to oxidize the deposited sulphur was limited. The possible reasons are: (1) the smaller external surface areas of AP, compared to VA exhausted carbons (Table 1), which was not beneficial to the biofilm development on AP under limited energy source; (2) the less desorption of pre-adsorbed sulphur from AP carbons compared to VA, due to different adsorption mechanisms associated. For impregnated carbon (VA), sulphur deposits were concentrated on the carbon surface, which could be easier released than the case of non-impregnated carbon (AP) where the sulphur compounds were mostly deposited in micropore (10,11). Figure 2 also revealed that almost similar levels of combustible sulphur were presented in the exhausted, control and experimental samples of AP. It confirmed that the oxidizable sulphur compounds previously deposited on AP could not

be effectively released into liquid phase, thus inaccessible to bacteria. Previous studies (12,13) also reported that contaminants in carbon would first desorb from the solid phase, then followed by biodegradation in liquid phase.

Overall, it was evidenced that the exhausted VA carbons could provide sulphur compounds for microbial oxidation thus to develop biofilm using pre-deposited sulphur as the sole energy source. However, AP was not recommended for this purpose. This was in accordance with their different extents in release of combustible (oxidizable) sulphur compounds from exhausted carbon (Figure 2). Other researches (14,15) also observed that carbon type is one of a key issues for desorption of other types of adsorbed chemicals, in view of bio-regeneration. In the following section, enhanced biofilm development on VA exhausted carbons was further investigated.

Enhanced biofilm development on exhausted carbons by adding NaOH

Although a green light of developing biofilm on VA exhausted carbon was seen based on pre-deposited sulphur as the sole energy source, the development process was slow and the amounts of bacteria attached on the surface of exhausted carbon were significantly lower than those of other studies where additional energy source was provided continuously (4). Combustible sulphur analysis revealed that a large amount of oxidizable sulphur was still remained in the VA biocarbons (Figure 2). Therefore, it is critical to significantly enhance the desorption of these oxidizable sulphurs so as to ensure a good biofilm development.

NaOH solution was added in VA exhausted carbons as a pre-treatment step to enhance the desorption of sulphur compounds into aquatics phase, followed by the normal biofilm development procedure. Figure 4 shows the sulfate concentration profiles in liquid phase for control and experimental conditions. It can be seen that the sulfate contents in three BVA flasks increased largely with time at the first 8 days while those of controls remained constant. In addition, compared to the sulfate concentration in liquid of BVA without adding NaOH (Figure 1), pre-treatment indeed increased greatly the quantity of

sulphur bio-oxidization. In a quantitative comparison, sulfate generation in liquid on the 24th day was found at 8.56% S-sulfate/AC for BVA3 with adding NaOH, while it was only 1.85% S-sulfate/AC without pretreatment on the 28th day. The pH (data not shown here) in experimental flasks decreased and was adjusted to 7 when it dropped to below 3 while in the controls, the pH remained always constant.

The results of analyzing combustible sulphur content (Figure 5) in VA carbons with pretreatment showed that a large amount of combustible sulphurs in exhausted carbons were removed by NaOH adding. Further big release of sulphur was achieved by bacterial function, resulting in much less sulphur remained in experiments than those in controls. Compared to the case without pretreatment (Figure 2), the remaining combustible sulphurs of BVA were much fewer for the case with pretreatment.

After the pretreatment followed by biofilm development run for 8 days, the VA carbon pellet was collected for SEM observation. The results (Figure 6) showed that biofilm was well developed on bottom exhausted carbons (BVA3), which was also supported by the plate count of bacteria attached on the biocarbon: 2.6×10^8 CFU/g dry carbon. Amounts of bacteria attached were much higher than those of BVA without pretreatment. The reason associated is that, due to NaOH adding, much more oxidizable sulphur released, which benefited to the initial formation of a biofilm on carbon surface. It could then be much easier for the immobilized bacteria than suspended ones to access the pre-deposited sulphur on exhausted carbon, evidenced by much higher combustible sulphur released due to bacterial contribution (Figure 5). Bacterial cell attachment to a sorbent was previously shown to facilitate the degradation of other pre-adsorbed chemicals (16,17). It is possible that the attached bacteria may be exposed to a higher chemical concentration (i.e., sulphur compounds in this study) within the inner boundary diffusion film compared to that of bulk solution, resulting in enhanced biodegradation.

Sulfur reactions associated in developing biofilm with pre-treatment

Sulfur deposits in exhausted carbons were previously formed in the H_2S adsorption process. The transformation of these sulphur species is the basis for understanding the biofilm development mechanism. The possible sulphur-involving reactions in the course of biofilm development were proposed based on the experimental observations.

(1) Identification of sulphur species on carbon

In order to identify the deposited sulphur species on the VA carbons, thermal analysis was conducted. The results for the bottom portion of VA are presented in Figure 7, as a representative. In the case of the fresh carbon, its DTG curve in the temperature range of 200 to 500□ was almost featureless. An increase in weight loss at the temperature range was proportional to an increase in the content of sulphur deposits. From previous work, it was learnt that the peak centered at about 240-300□ was assigned to the presence of bonded SO_2, and the peak centered at about 380-400□ represented elemental sulphur (8,18). In this example, for exhausted samples (VA-E3), control (VA3) and experimental (BVA3) samples without pretreatment, only one peak was observed at about 400□, suggesting elemental sulphur probably dominated in these VA carbons.

Moreover, the DTG peak of carbon from control was smaller than that of the exhausted one, showing the effects of nutrient water and shaking which was able to remove water soluble sulphur species (8,19). Furthermore, an even smaller DTG peak was found for experimental carbon than that of control, suggesting some sulphur compounds removed from the carbon through bacterial degradation.

Curves VA3-Na and BVA3-Na represented the control and experimental samples with pretreatment. Three well-defined peaks were found around 280, 410 and 680□ in the curve of VA3-Na control. It showed that NaOH adding may remove a large quantity of elemental sulphur from exhausted carbon, and generate some products including possibly sulphur oxide (at peak 280□) and new sulphur species (at peak 680□). For BVA3-Na after bacterial oxidation, only a small peak around 300□ remained, which

might be the remaining bonded SO_2 that cannot be used by bacteria or those sulfates adsorbed in biofilm.

(2) Identification of sulphur species in liquid

After adding NaOH solution into carbons and shaking for 24 hours, the solution became yellow color only in the flasks containing middle and bottom carbons with higher sulphur contents, but for fresh carbon without sulphur and top carbon with little sulphur, no change in solution color was observed. After that, HCl solution was added to adjust the pH back to neutral when white precipitates were produced in the flasks containing middle and bottom carbons. And again, no evident change was found for solutions with fresh and top carbons. Preliminary sulphur analysis using standard methods (6) found that the yellow solution contained mainly $S_2O_3^{2-}$, S^{2-} and a little of elemental sulphur (data not shown), but after the addition of HCl solution, the main sulphur species in solution was elemental sulphur.

Therefore, the possible sulphur reactions associated in developing biofilm with pre-treatment were proposed as the following (20,21):

(1) After adding NaOH solution into carbons:

SO_2 (ads) (on carbon) + 2NaOH (aq) $\rightarrow$ Na_2SO_3 (aq) + H_2O

S (ads) (on carbon) $\leftrightarrow$ S (aq)

3 S (aq) + 6NaOH (aq) $\rightarrow$ 2Na_2S (aq) (colorless) + Na_2SO_3 (aq) + 3H_2O

S (aq) + SO_3^{2-} (aq) $\rightarrow$ $S_2O_3^{2-}$ (aq)

Then, Na_2S reacted with elemental sulphur to yield polysulfide compounds:

Na_2S (aq) + S (aq) $\rightarrow$ Na_2S_2 (aq) (yellow color)

Na_2S_2 (aq) + xS (aq) $\rightarrow$ Na_2S_{x+2} (aq) (x=0-4) (yellow color)

(2) After adding HCl to neutralize the solution:

S_x^{2-} (aq) + $2H^+ \rightarrow H_2S$ + (x-1) S (white precipitates)

$S_2O_3^{2-}$ (aq) + $2H^+ \rightarrow SO_2 + H_2O$ + S (white precipitates)

(3) Formation of biofilm initially on the carbon surface by utilizing oxidizable sulphur in liquid as food for bacteria.

$H_2S + 2O_2 \rightarrow 2H^+ + SO_4^{2-}$ (with sulfide-oxidizing bacteria)

$S + 3/2O_2 + H_2O \rightarrow 2H^+ + SO_4^{2-}$ (with sulfide-oxidizing bacteria)

$SO_3^{2-} + 1/2\ O_2 \rightarrow SO_4^{2-}$ (with sulfide-oxidizing bacteria)

Suspended bacteria + desorbed oxidizable sulphur → a thin layer of biofilm on carbon

4) Further enhanced sulphur compounds release from carbon due to the presence of initial biofilm on carbon, resulting in more bacteria growth on carbon surface:

The thin layer of biofilm + sulphur deposits on carbon → well developed biofilm

Based on the above results, the biofilm development on exhausted carbons could be considered as a combination of the following aspects: 1) Part of the sulphur compounds could desorb from the exhausted carbon into liquid phase by water washing or pretreatment; 2) The desorbed oxidizable sulphur compounds can be biodegraded by suspending bacteria in liquid phase, thus allowing sulphur-oxidizing bacteria to grow in the solution; 3) Some active bacteria might attach on the carbon surface to form a biofilm initially at external surface of carbon, and then they were exposed to a steeper concentration gradient of sulphur compounds shortening the diffusion distance between bacteria and substrate; 4) Following that, further desorbed sulphur compounds passing through the biofilm would undergo biodegradation, resulting in more bacteria growth on carbon surface.

Too much biomass attachment may block the carbon external pores and make the total adsorption capacity loss, resulting in limited buffer capacity of biocarbon during transient loading (22). Therefore, the free biofilm coverage on the carbons with exposure areas can benefit to keep adsorption capacity in odour biofiltration process. Alternatively, a

weak biofilm is not efficiently enough in removing contaminates (23,24). Hence, the overall consideration of the biofilm development is to optimize the combination of the two effects, biodegradation and adsorption. Further work should be conducted to investigate the odour removal performance of these developed biocarbons. It is estimated that the start up time of using exhausted carbon in biofiltration will be shorten compared to fresh carbon, as energy source (the sulphur deposits) already existed in exhausted carbon surface.

This example investigates the combined functions of biofilm development on exhausted carbon and bioregeneration of exhausted carbon. The simultaneous bioregeneration of H_2S exhausted carbon in the course of biofilm development was assessed based on analyzing sulphur compounds and BET porosity of the developed BVAs.

It is evident that a portion of sulphur compounds was released from exhausted carbons via biodegradation, in the course of biofilm development. There was less combustible sulphur found in experimental samples than those in controls (Figures 2 and 5), corresponding to higher sulfate contents detected from experimental flasks than those from control ones (Figures 1 and 4). Regeneration efficiencies of combustible sulphur in exhausted carbon are shown in Table 2.

Table 2 Regeneration efficiency of combustible sulphur in VA exhausted carbon (%)

Sample	Without pretreatment			With pretreatment		
	Control	Experiment	Net contribution of bacteria	Control	Experiment	Net contribution of bacteria
Top	13.7	33.0	19.3	65.6	93.0	27.4
Middle	14.5	47.3	32.8	54.5	93.4	38.9
Bottom	7.8	18.0	10.2	65.8	92.3	26.5

Around 18-47% of overall regeneration efficiencies were achieved for exhausted VA carbons without any pretreatment, among which ~10-33% of sulphur removal was attributed to biodegradation. Pretreatment of adding NaOH enhanced the overall regeneration efficiency of combustible sulphur in exhausted carbon to 92-93%, with the

net contribution of bioregeneration increased to ~ 26-39%. It confirmed the effect of bacterial occurrence in enhancing the release of oxidizable sulphur. A decrease in intensities of the sulphur peak in DTG curves (Figure 7) also showed directly the effect of biological regeneration of VA exhausted carbon.

Removal of pre-deposited sulphur compounds on exhausted carbon can re-open adsorption sites or free carbon pores, which can be re-used for further adsorption of targeted contaminants. Micropore volume (V_{mic}) and micropore area (S_{mic}) of different VA samples, including original carbon, exhausted carbons from top, middle and bottom of bed, and biocarbons developed from exhausted VA without and with pretreatment, are shown in Figure 8. It clearly indicated the decrease of V_{mic} and S_{mic} of exhausted VA carbons from top to bottom, resulted from the increasing occupy of sulphur compounds in micropores (8). In developing biofilm without pretreatment, the V_{mic} and S_{mic} of BVA increased largely from those of exhausted carbons, indicating the regeneration effects. Furthermore, with pretreatment the generated biocarbons (BVA-Na) had further enlarged V_{mic} and S_{mic} which reached almost the same level of that of original carbon. In more details, the bottom exhausted VA carbon remained only about 40% of the initial V_{mic} and S_{mic} in original carbons, which were renewed in biocarbons generated without pretreatment at respectively 67% and 74% of the initial V_{mic} and S_{mic}. With pretreatment, the V_{mic} and S_{mic} of the developed biocarbon were further increased to 80% and 95% of initial ones, showing a significant recovery of carbon adsorption capacity. This resulted from the removal of sulphur that had earlier deposited within the micropores of the carbon (25).

This example shows that, without supplying any external energy source, the sulfide-oxidizing bacteria could access partially the pre-adsorbed sulphur in VA exhausted carbon for bacterial growth and then form a biofilm on carbon surface. Therefore, it is highly feasible to develop biocarbon from exhausted carbons, benefiting to sustainable bio-regeneration and reuse of these carbons in odour biofiltration. The guideline for developing biocarbon from exhausted carbon includes carbon type, utilizable sulphur (energy for bacterial growth), carbon external surface area (space for bacterial attachment), and micropore volume and area (active site for physical/chemical adsorption). Pretreatment trials by adding NaOH proved to be effective to enhance the

development of biofilm on exhausted carbons. The sulphur-associated reaction occurring during developing biofilm on exhausted carbon was proposed. Additionally, the function of bioregeneration in the course of biofilm development on exhausted carbon was also preliminarily assessed, which supported the idea of simultaneous bioregeneration of exhausted carbon for a sustainable biofiltration system.

Whilst there has been described in the foregoing description preferred embodiments of the present invention, it will be understood by those skilled in the technology concerned that many variations or modifications in details of design or construction may be made without departing from the present invention.

References

(1) Devinny, J. S.; A, D. M.; S, W. T. *Biofiltration for air pollution control*; Lewis Publishers: Boca Rotan, FL, 1999.

(2) Yan, R.; Ng, Y. L.; Chen, X. G.; Geng, A. L.; Gould, W. D.; Duan, H. Q.; Liang, D. T.; Koe, L. C. C. *Water Science and Technology* 2004, *50*, 299-308.

(3) Duan, H. Q.; Koe, L. C. C.; Yan, R. *Applied Microbiology and Biotechnology* 2005, *67*, 143-149.

(4) Chung, Y. C.; Lin, Y. Y.; Tseng, C. P. *Journal of Chemical Technology and Biotechnology* 2004, *79*, 570-577.

(5) Webster, T. S.; Devinny, J. S.; Torres, E. M.; Basrai, S. S. *Biotechnology and Bioengineering* 1997, *53*, 296-303.

(6) APHA *Standard methods for the examination of water and wastewater*; 20th ed.; American Public Health Association: Washington DC, USA, 1999.

(7) Yan, R.; Liang, D. T.; Tsen, L.; Tay, J. H. *Environmental Science and Technology* 2002, *36*, 4460-4466.

(8) Yan, R.; Chin, T.; Ng, Y. L.; Duan, H. Q.; Liang, D. T.; Tay, J. H. *Environmental Science and Technology* 2004, *38*, 316-323.

(9) Black, J. G. *Microbiology: principles and explorations*; 6th ed.; Chichester Wiley: New York, 2005.

(10) Bandosz, T. J.; Bagreev, A.; Adib, F.; Turk, A. *Environmental Science and Technology* 2000, *34*, 1069-1074.

(11) Bandosz, T. J. *Carbon* 1999, *37*, 483-491.

(12) Silva, M.; Fernandes, A.; Mendes, A.; Manaia, C. M.; Nunes, O. C. *Water Research* 2004, *38*, 2677-2684.

(13) Klimenko, N.; Smolin, S.; Grechanyk, S.; Kofanov, V.; Nevynna, L.; Samoylenko, L. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 2004, *230*, 141-158.

(14) Aktas, O.; Cecen, F. *Journal of Chemical Technology and Biotechnology* 2006, *81*, 1081-1092.

(15) De Jonge, R. J.; Breure, A. M.; van Andel, J. G. *Water Research* 1996, *30*, 875-882.

(16) Park, J. H.; Feng, Y.; Ji, P.; Voice, T. C.; Boyd, S. A. *Applied and Environmental Microbiology* 2003, *69*, 3288-3298.

(17) Harms, H.; Zehnder, A. *Applied and Environmental Microbiology* 1995, *61*, 27-33.

(18) Bandosz, T. J. *Journal of Colloid and Interface Science* 2002, *246*, 1-20.

(19) Bagreev, A.; Rahman, H.; Bandosz, T. J. *Environmental Science and Technology* 2000, *34*, 4587-4592.

(20) Patnaik, P. *Handbook of inorganic chemicals* McGraw-Hill: New York, 2003.

(21) Brock, T. D.; Madigan, M. T. *Biology of microorganisms*; 6th ed.; Prentice Hall: Imprint Englewood Cliffs, N.J., 1991.

(22) Madjid, M. T.; University of Toronto 1998.

(23) Scholz, M.; Martin, R. J. *Water Research* 1997, *31*, 2959-2968.

(24) Duan, H.; Koe, L. C. C.; Yan, R.; Chen, X. *Water Research* 2006, *40*, 2629-2636.

(25) Bagreev, A.; Rahman, H.; Bandosz, T. J. *Advances in Environmental Research* 2002, *6*, 303-311.

CLAIMS

1. A method of obtaining a microbial culture for regenerating exhausted carbon, the method comprising:

a) obtaining a seed sludge from a wastewater reclamation environment;

b) culturing the seed sludge under conditions suitable for enrichment of autotrophic sulfide-oxidizing bacteria; and

c) assessing the culture for ability to regenerate exhausted carbon.

2. A microbial culture for regenerating exhausted carbon, obtained by the method of claim 1.

3. A method for regenerating exhausted carbon, the method comprising:
incubating the exhausted carbon with a microbial culture including autotropic sulfide-oxidising bacteria to form a regenerated carbon comprising autotrophic sulfide-oxidising bacteria immobilised on the carbon.

4. A method according to claim 3, wherein the autotrophic sulfide-oxidizing bacteria are present in, or derived from activated sewage sludge.

5. A method according to any one of claims 3 or 4, wherein sulphur compounds in the exhausted carbon are the sole energy source for the autotrophic sulfide-oxidizing bacteria.

6. A method according to any one of claims 3 to 5, wherein the exhausted carbon is pre-treated, prior to incubation with the microbial culture, with a solution of a strong base which reacts with sulphur compounds present on the exhausted carbon to release water-soluble reduced sulphur compounds from the exhausted carbon.

7. A method according to claim 6, wherein the strong base is sodium hydroxide.

8. A method according to any one of claims 6 or 7, wherein the carbon is agitated in the solution of strong base prior to incubation with the microbial culture.

9. A method according to any one of claims 3 to 8, wherein the microbial culture forms a layer of sulfide-oxidising biofilm on the carbon.

10. A method according to any one of claims 3 to 9, wherein the exhausted carbon is a chemically-impregnated carbon.

11. A method according to any one of claims 3 to 10, wherein the incubation period is about 25-30°C for about 15 – 30 days.

12. A method according to any one of claims 3 to 11, wherein the incubation medium is sterilised mineral salt.

13. A method according to any one of claims 1, 3 to 12, wherein the exhausted carbon is H_2S-exhausted.

14. A microbial culture according to claim 2, wherein the exhausted carbon is H_2S-exhausted.

15. A biological activated carbon (BAC) regenerated from exhausted carbon obtained by the method of any one of claims 3 to 13.

16. A BAC according to claim 15, wherein the carbon comprises immobilised autotrophic sulphide-oxidising bacteria.

17. A BAC according to claim 15 or 16, wherein the carbon is chemically-impregnated carbon.

18. A BAC according to any one of claims 15 to 17, wherein the exhausted carbon is H_2S-exhausted.

19. Use of a BAC according to any one of claims 15 to 18 in biofiltration of odorous gases.