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Oxidation of activated carbon fibre and its adsorption of amylase

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Activated carbon fibre was oxidized by combustion of nitrocellulose and oxidation with nitric acid to introduce the nitrogen and oxygen groups on its surface. The pore structure and chemical groups were investigated by nitrogen adsorption and FTIR. The result showed that the pore structure and surface morphology were not much affected but amide and hydroxyl groups could be introduced after oxidation. The adsorption capacity of amylase was markedly increased, and the hydrolysis activity of starch was maintained after 3 runs.

Key words: *activated carbon fibre; surface modification; adsorption; amylase; hydrolysis*

1. Introduction

Porous carbons have been widely used as adsorbents, catalyst/catalyst supports, electronic materials and energy storage materials due to their high surface area and large pore volume [1]. The advantages of activated carbon fibre are the smaller fibre diameter which minimizes diffusion limitations and allows rapid adsorption /desorption, more concentrated pore size distribution. The novel porous carbon has been widely used in separation, purification and catalytic processes.

Activated carbon fibres display a better adsorption capacity for small molecules and a higher storage capacity/volume for hydrogen. They have also been used as electrode materials in high performance electric double-layer capacitors [4]. Not only the pore structure but also the surface chemical composition of the activated carbon fibre significantly influence its adsorption capacity, catalytic activity or its catalyst support performance. Heteroatoms on the surface of the activated carbon fibre determine its surface chemical properties and play a significant role in device applications. The two most important heteroatoms are oxygen and nitrogen which affect the surface acidic, basic and hydrophilic properties of activated carbon fibres [5–7].

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Chemical oxidation of activated carbon fibre is a frequently used method to introduce heteroatoms onto its surface. Various reagents have been used as oxidizers: concentrated nitric or sulfuric acid, sodium hypochlorite, permanganate, bichromate, hydrogen peroxide, transition metals, ozone-based gas mixtures, etc. Among various oxidation treatments, oxidation of nitric acid is the most widely used method for increasing the total acidity that is produced from wet oxidation treatment [8]. Recently, it was reported that the oxygen- and nitrogen-containing groups on activated carbon fibre surface could be introduced by nitrocellulose combustion oxidation at 300 °C [9], the modified activated carbon fibre showed higher adsorption capacities for ammonia and carbon disulfide.

It is well known that carbon possesses good biocompatibility [10]. Enzymes as biocatalysts with high specificity, high catalytic efficiency and bio-degradability, are widely applied in industrial process and medical sciences, because they can increase the rate of chemical reaction by lowering the activation energy. Enzyme immobilization on a support may improve its thermal behaviour, it may be repeatedly and continuously used and simplify the separation of enzymes from reaction media, thus, the recovery of enzyme and purification of the final products become more reliable, simple and efficient [11].

α-Amylases belong to an enzyme group widely used in food, paper, textile, distillery, fermentation and brewing industries. The hydrolysis of starch to products with low molecular weight, catalyzed by α-amylase (1,4- α-d glucan glucanohydrolase) is one of the most important commercial enzymatic processes [12]. The immobilization of α-amylase was investigated by many researchers. For example, α-amylase was immobilized on zirconia or alumina via adsorption and exhibited higher activity [13, 14], it was covalently immobilized on phthaloyl chloride-containing amino group functionalized glass beads forming amide bonds between amino groups on protein and acid chloride groups on the glass surface [15]. α-amylase was also immobilized in modified ordered mesoporous silicas through the reaction of free –CHO (following alkylamine and glutaraldehyde procedures) with $–NH_2$ of enzyme for hydrolysis of starch, thermal and pH stability of the immobilized enzyme was improved [16]. Although activated carbon fibre displayed developed pore structure, it has not been widely used as an enzyme support because its surface chemical property was generally hydrophobic, its oxygen and nitrogen content was very small, and it could not interact strongly with enzymes. Thus it is necessary to investigate the properties of modified activated carbon fibre as an enzyme support material. Activated carbon fibre was treated by nitrocellulose combustion and nitric acid oxidation to modify its surface chemical composition, and the adsorption of amylase and hydrolysis for starch were investigated and discussed.

2. Experimental

Oxidation of activated carbon fibre. Commercially viscose-based activated carbon fibre (designated as ACF-0), activated at 850 °C with steam, was supplied by Zichuan Carbon Fiber Limited Company, Qinhuangdao (P.R. China). Firstly, the ACF-0 was

dried at 120 °C for 2 h to remove the adsorbed steam and organic molecules, then, it was impregnated with nitrocellulose acetone solution and the loading amount was fixed at 1 wt. % of nitrocellulose at room temperature for 4 h. Finally, the impregnated activated carbon fibre was heated to 300 °C for 30 min in air and labelled as ACF-NC. ACF-0 was impregnated in 5 M nitric acid for 12 h, then, it was leached with distilled water until pH of approximately 7 has been attained. Finally, it was dried at 80 °C and designated as ACF-HNO_3.

Characterization of activated carbon fibres. Nitrogen adsorption isotherms were performed at 77.4 K with a Micromeritics ASAP 2010 analyzer. The surface area was calculated using the Brunauer–Emmett–Teller (BET) model. The pore volume, pore size distribution and the average pore diameter were determined by the density function theory. Before nitrogen adsorption measurements, the activated carbon fibres were outgassed at 300 °C for 15 h. Infrared spectra were recorded on a Nicolet Impact 380 FT-IR spectrometer using KBr pellets. Scanning electron micrographs were obtained on a JEM-3010 using a copper grid type sample holder. Before observation, the samples were sputtered with gold for 2 min in order to avoid charging.

Adsorption of amylase and hydrolysis of starch. In the adsorption experiments, 1 g of activated carbon fibre was mixed with equal volumes of 0.1 M phosphate buffer and α-amylase solution. It was shaken at room temperature for 1 h and then filtered. The adsorbed amount of α-amylase was obtained as the difference between the hydrolysis capacity of the original enzyme solution with that of the filtrate.

The hydrolysis activities of free and the immobilized enzymes were examined in a batch reactor. 1 wt. %, 2 wt. % and 5 wt. % starch solution was prepared by dissolving soluble starch in distilled water and the pH was adjusted to 4.7 by 0.1 M HCl. Then 1 g of activated carbon fibre containing α-amylase was placed in a test vial. Subsequently, 100 cm^3 of starch solution was added and the system was incubated in a water bath with constant shaking at 40 °C. 1 cm^3 of solution was drawn each 10 min to determine the amount of hydrolyzed maltose. The reaction was stopped by adding 1 cm^3 of 3,5-dinitrosalicylic acid reagent. Incubation was also performed in a boiling water bath for 5 min. The amount of reducing sugar (maltose) was determined spectrophotometrically at 540 nm.

3. Results and discussion

3.1. Pore structures of activated carbon fibres

The specific surface areas and pore volumes of the ACF-0, ACF-NC and ACF-HNO_3 are 651 m^2/g, 696 m^2/g and 682 m^2/g, 0.35 cm^3/g, 0.38cm^3/g and 0.36 m^3/g, respectively, indicating the ACF-NC and ACF-HNO_3 have a more developed pore structure.

Figure 1 shows the nitrogen adsorption isotherms and pore size distributions of ACF-0, ACF-NC and ACF-HNO_3. The nitrogen isotherms were planar at the relative pressure higher than 0.1 which suggested that the activated carbon fibres mainly had micropores. The ACF-NC has a wider pore size distribution, especially in the 0.7–0.8 nm range than that of ACF-0. It implies that not only the carbon of external surface but the micropore surface are oxidized during the combustion of nitrocellulose. The pore size distribution of ACF-HNO_3 was slightly wider than that of ACF-0.

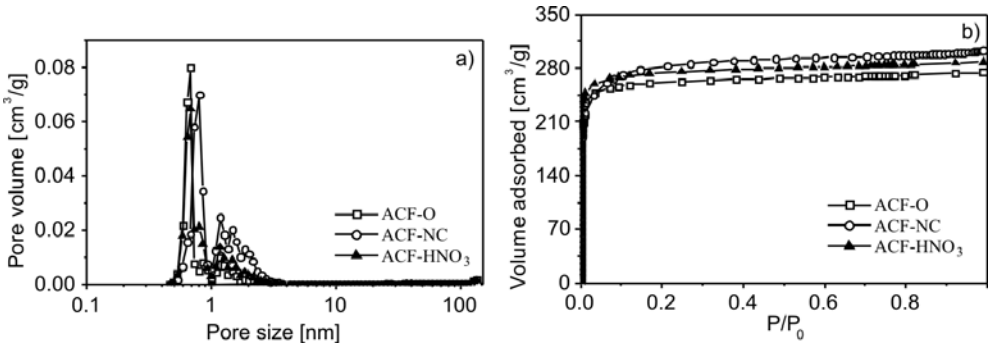

Fig. 1. The nitrogen adsorption isotherms (a)
and pore size distribution (b) of ACF-0, ACF-NC and ACF-HNO_3

Thus specific surface area, micropore volume and pore diameter of activated carbon fibre all increase slightly after combustion of nitrocellulose or oxidation of nitric acid.

3.2. Surface functional groups of activated carbon fibres

The elemental compositions of activated carbon fibre before and after oxidation are listed in Table 1. The carbon content markedly decreased and the oxygen content significantly increased after oxidation, the hydrogen and nitrogen contents also slightly increased.

Table 1. Elemental compositions [wt. %] of samples

Sample	C	H	N	O
ACF-0	83.63	2.87	2.01	11.48
ACF-HNO_3	79.44	3.01	2.12	15.43
ACF-NC	69.43	3.27	2.34	24.95

In order to investigate the change of surface acidicity and basicity, the Boehm method was used [17]. The differences of the surface functionalities on basic ACFs and acidically treated ACFs determined by the Boehm titration are listed in Table 2.

Oxidation with HNO_3 introduces a large number of carboxylic groups; nitrocellulose oxidation can form phenolic and basic groups.

Table 2. Surface functionalities of ACFs by the Boehm titration [molecule/nm^2]

Sample	Phenol	Lactone	Carboxyl	Acidic groups	Basic groups
ACF	0.58	0.28	0.02	0.88	0.12
ACF-HNO_3	0.31	0.64	0.44	1.39	0.06
ACF-NC	0.83	0.07	0.00	0.90	0.87

The FT-IR spectra of ACF samples are shown in Fig. 2. For the ACF-NC, the asymmetry of the peak ranging from 3000 cm^{-1} to 3400 cm^{-1} indicates strong hydroxyl interaction and the presence of overlapping N–H bands. The peak at 1700 cm^{-1} may be attributed to overlapping of N–H in-plane bending and the conjugated –C=O modes [18]. The two correlative peaks at 1456 cm^{-1} and 1395 cm^{-1} indicate the -C=O belonged to the carbonate groups [18]. Moreover, there are a number of overlapping bands appearing between 1400 cm^{-1} and 1000 cm^{-1}, which indicate the presence of C-O-C, C-O-N or >C-N groups. A wider and stronger peak at 3460 cm^{-1} corresponds to the hydroxyl for ACF-HNO_3.

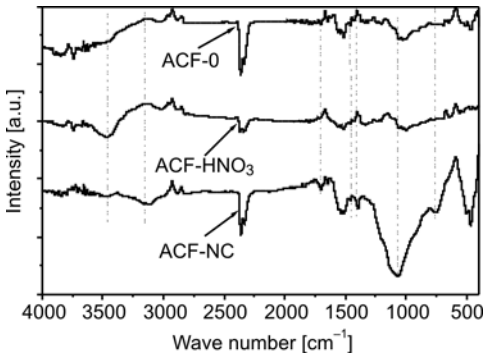

Fig. 2. The FT-IR spectra of: ACF-0, ACF-NC and ACF-HNO_3

FT-IR spectra show that ACF-NC, unlike ACF-0, contains functional groups primarily consisting of phenolic hydroxyls, imide and ethers. The spectra also show that ACF-HNO_3 has more surface hydroxyl groups that does ACF-0.

3.3. The SEM morgraphology of samples

The SEM images of activated carbon fibres are shown in figure 3. The surface of ACF-0 looks smooth, the spots on the surface are the adsorbed dirt. The least dirt was found on the surface of ACF-HNO_3; most of the dirt was leached during nitric acid impregnation. This also shows that the nitric acid oxidation does not damage the fibre surface.

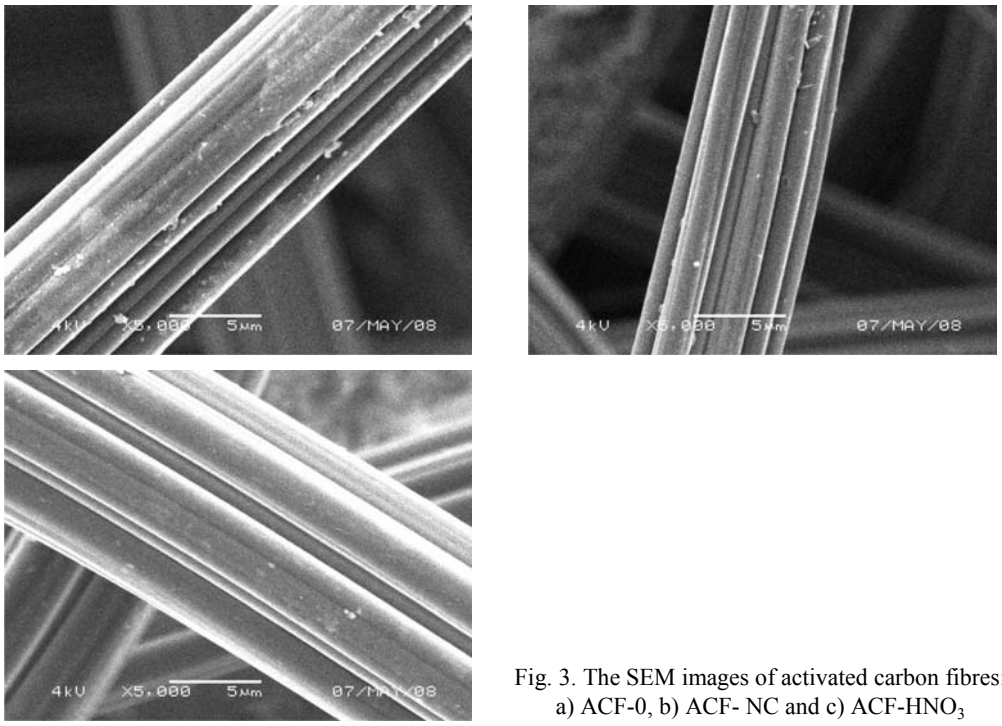

Fig. 3. The SEM images of activated carbon fibres: a) ACF-0, b) ACF- NC and c) ACF-HNO_3

The surface of ACF-NC was less contaminated than that of ACF-0, some impurities were washed during impregnation of nitrocellulose; this suggests that all nitrocellulose had been completely combusted. By comparing the SEM images, the activated carbon fibre surface was not destroyed after oxidation with nitric acid and combustion of nitrocellulose. This proves that it is a simple method to introduce nitrogen/oxygen atoms on the surface of activated carbon fibre, and one in which no damage occurs to the surface.

3.4. Adsorption of amylase and hydrolysis of starch

The adsorption capacities of α-amylase on ACF-0, ACF-NC ACF-HNO_3 are 2.0 mg/g, 9.9 mg/g and 7.9 mg/g, respectively. Because the pore structures of ACFs were similar, the surface oxygen- and nitrogen-containing groups are the only markedly affected factor characterizing the α-amylase adsorption, especially the phenolic hydroxyls, which could form a hydrogen-bond with the $–NH_2$ of an α-amylase molecule.

Compared with free α-amylase, the hydrolysis activity of α-amylase immobilized on ACF-0, ACF-NC and ACF-HNO_3 was decreased to 20%, 48% and 34%, respectively. This suggests that active sites of α-amylase were affected by the activated carbon fibre pore structure, which could change the interaction between the α-amylase and starch molecule. In addition, the immobilized α-amylase molecule was fixed on

the pore channel and the mass transfer was also restricted, so the apparent hydrolysis activity was decreased.

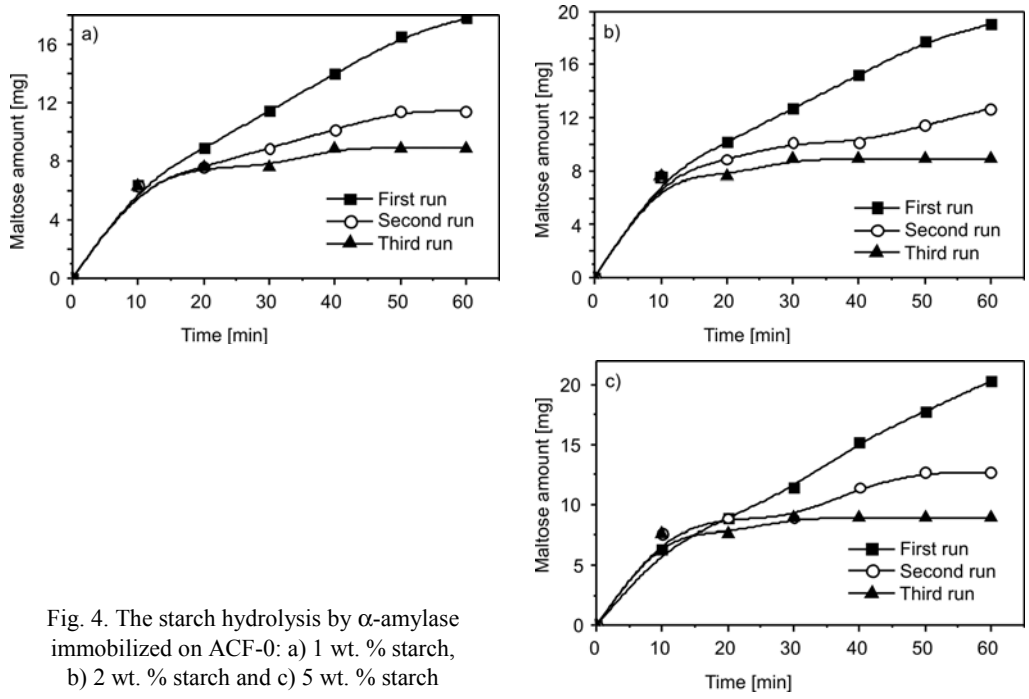

Fig. 4. The starch hydrolysis by α-amylase immobilized on ACF-0: a) 1 wt. % starch, b) 2 wt. % starch and c) 5 wt. % starch

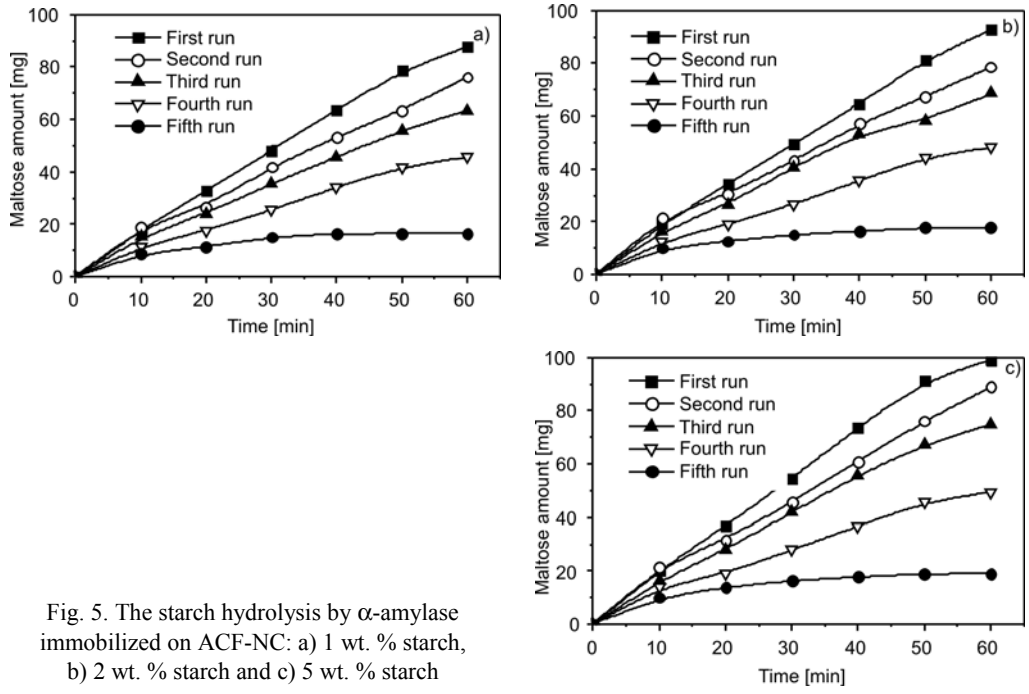

Fig. 5. The starch hydrolysis by α-amylase immobilized on ACF-NC: a) 1 wt. % starch, b) 2 wt. % starch and c) 5 wt. % starch

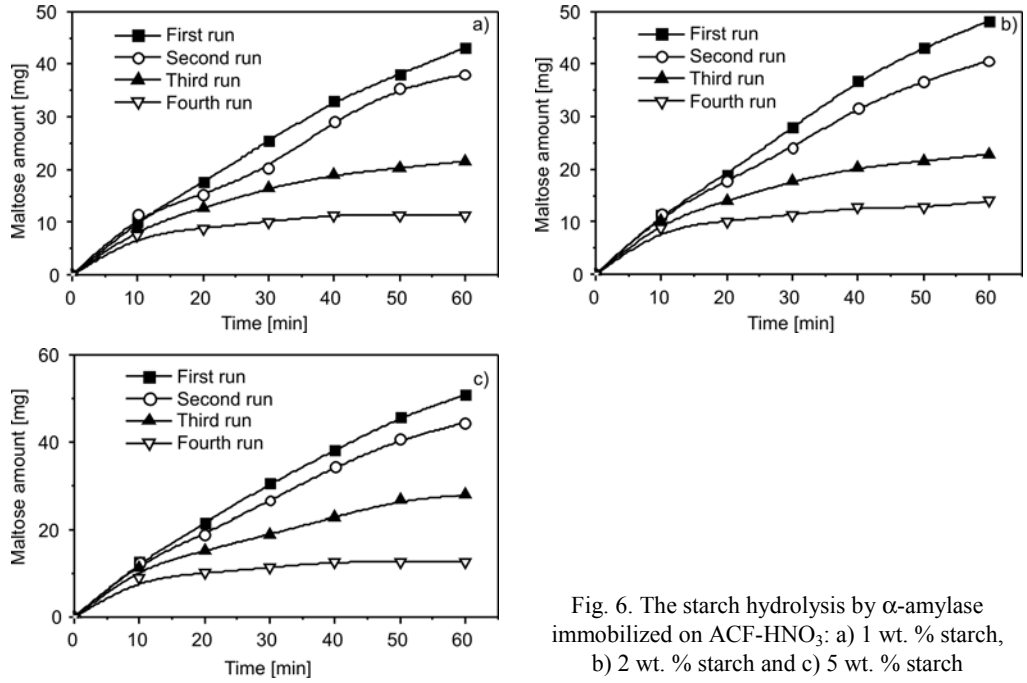

Fig. 6. The starch hydrolysis by α-amylase immobilized on ACF-HNO_3: a) 1 wt. % starch, b) 2 wt. % starch and c) 5 wt. % starch

The results of starch hydrolysis by immobilized α-amylase are shown in Figs. 4–6. The amount of maltose hydrolyzed by α-amylase immobilized on same ACF was independent of the starch concentration. Maltose was also hydrolyzed by α-amylase: the results show that if immobilization occurs on the same ACF, the quantities of hydrolyzed maltose and hydrolyzed starch remain the same. This suggested that the starch concentration was sufficient. The greater the quantity of amylase immobilized on ACF, the higher was the amount of hydrolyzed maltose. As for the ACF-0, the quantity of hydrolyzed maltose was 20 mg after 60 min for the first run; it decreased to 12 mg in the second run and to 8 mg in the third run. As for the ACF-NC, the amount of hydrolyzed maltose was 100 mg after 60 min for the first run; the adsorbed amylase maintained higher hydrolysis activity after three runs. Its activity decreased after a fourth run, and the maltose amount was then 50 mg. As for the ACF-NC, the amount of hydrolyzed maltose was 50 mg after 60 min for the first run; the adsorbed amylase maintained higher hydrolysis activity after two runs. Its activity decreased after 3 runs, and the maltose amount was then 20 mg.

The most important advantage of the immobilized enzyme was its reusability. The surface groups of activated carbon fibre also affected the desorption behaviour of α-amylase. Most of the α-amylase immobilized on ACF-0 would be desorbed during the hydrolysis, and the retained activity of α-amylase immobilized on ACF-0 was 10% after 2 runs. However, α-amylase immobilized on ACF-NC and ACF-HNO_3 exhibited more than 24% and 14% activity (relative to free α-amylase) after 4 runs

and 3 runs, respectively. This indicated that there existed a strong interaction between α-amylase and the surface groups of ACF-NC and ACF-HNO_3.

4. Conclusion

Activated carbon fibre could be oxidized by nitric acid and nitrocellulose combustion to modify its surface chemical property. The carbon content of activated carbon fibre markedly decreased and the oxygen content significantly increased after oxidation. The hydrogen and nitrogen content also increased to a certain degree. The Boehm titration and FTIR results indicated that the oxidation with HNO_3 introduces a large number of carboxylic groups; while the nitrocellulose oxidation could form phenolic and basic groups (amide). The adsorption was increased 5 times and 3.5 times, respectively. Immobilized amylase showed higher hydrolysis activity for starch after 3 runs.

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