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Adsorption of bacterial lipopolysaccharides on carbonized rice husks obtained in the batch experiments

The scope of this study is the measurement of endotoxin adsorption rate for carbonized rice husk. It showed good adsorption properties for LPS. During the batch experiments, several techniques were used and optimized for improving the material's adsorption behavior. Also, with the results obtained it was possible to differentiate the materials according to their adsorption capacity and kinetic characteristics.
Keywords: lipopolysaccharide, carbonized rice husk, adsorption, surface modification.

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Адсорбция бактериального липополисахарида на поверхности карбонизованной рисовой шелухи на основе результатов серийных экспериментов

Результатом данной работы явилось изучение селективной сорбции липополисахарида (ЛПС) токсического шока наноструктурированными карбонизованными материалами на основе карбонизованной рисовой шелухи. С помощью серийных экспериментов были использованы различные подходы для оптимизации адсорбции ЛПС. Полученные в ходе исследовании результаты позволяют использовать перспективный карбонизованный материал для селективной сорбции ЛПС из различных растворов.
Ключевые слова: липополисахарид, карбонизованная рисовая шелуха, адсорбция, модификация поверхности.

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Сериялы тәжірибелер арқылы карбонизделген күріш қауызы бетіне бактериалды липополисахариті адсорбциялау

Жұмыста токсикалық шок липополисахаридін (ЛПС) наноқұрылымды карбониздеген материал – карбонизделген күріш қауызына таңдамалы сорбциялау нәтижелері сөз етіледі. Сериялық тәжірибелер арқылы ЛПС адсорбциясын оптимизациялау үшін әртүрлі тәсілдер қолданылды. Зерттеу жұмысының нәтижелері перспективт карбонизделген материалды әртүрлі сұйықтықтардан ЛПС молекуласын таңдамалы сорбциялауға болатындығын көрсетті.
Түйін сөздер: липополисахарид, карбонизделген күріш қауызы, адсорбция, беткейді модификациялау.

Bacterial lipopolysaccharides (LPS) are the major outer surface membrane components present in almost all Gram-negative bacteria and act as extremely strong stimulators of innate or natural immunity in diverse eukaryotic species ranging from insects to humans. These include affecting structure and function of organs and cells, changing metabolic functions, raising body temperature, triggering the coagulation cascade, modifying hemodynamics and causing septic shock. Because of this toxicity, the removal of even minute amounts is essential for safe parenteral administration of drugs and also for septic shock patients' care. The absence of a general method for endotoxin removal from liquid interfaces urgently requires finding new methods and materials to overcome this gap. Nanostructured carbonized plant parts is a promising material that showed good adsorption properties due to its vast pore network and high surface area [1, 2].

Endotoxins and septic shock. Gram-negative sepsis is perhaps the most important infectious disease problem in hospitals today. Despite recent advances in our understanding of the pathophysiological mechanisms of sepsis and improved antimicrobial therapy, the mortality rate from gram-negative sepsis remains frustratingly high, particularly after the onset of shock. At the pathophysiological level, the development of gram-negative sepsis involves a complicated series of effects based on the composition of the bacterial cell wall. The structure of the LPS molecule is shown schematically in Figure1. Chemically, endotoxins are *lipopolysaccharides* (frequent synonyms of *endotoxin* are *LPS* and *pyrogen*) that consist of three biologically, chemically, genetically and serologically different parts. These are a non-polar lipid component, called lipid A, the so-called core oligosaccharide and a heteropolysaccharide representing the surface antigen (O-antigen).

The physiological effects of endotoxin *in vivo* and the biochemical mechanisms underlying these effects have been extensively investigated. The administration of small doses of endotoxin to animals affects their hemodynamics, body temperature, blood clotting, cellular and humoral immunities, and other important physiologic parameters; large doses are lethal. In most species, the injection of LPS is associated with a rapid onset of fever, hypotension, and neutropenia. Humans are very sensitive to the pyrogenic activity of LPS and demonstrate fever at a small fraction of the LPS dosage required to cause the febrile response in rabbits [3, 4].

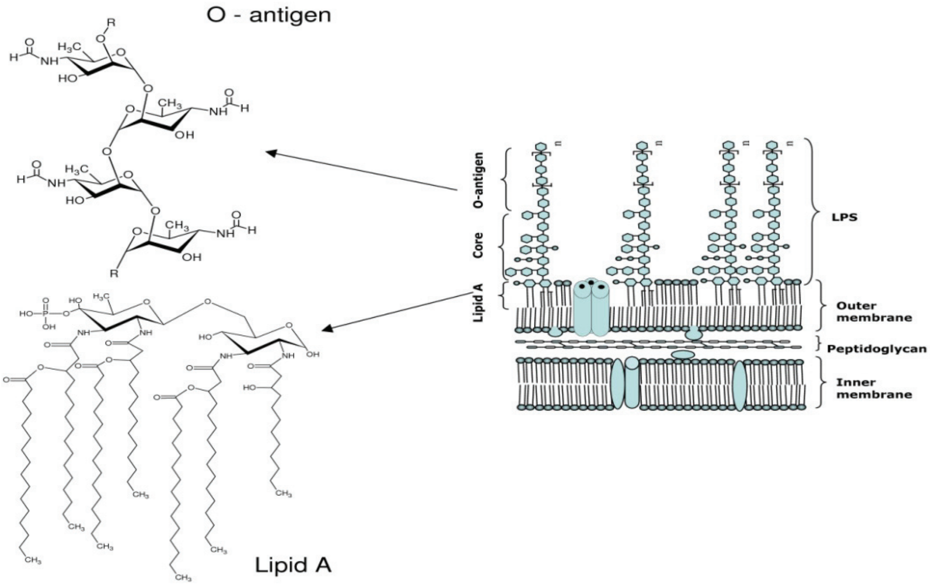

Figure 1 – Structure of lipopolysaccharide

Specific elimination (absorption) of LPS from blood plasma represents one of the most promising methods for sepsis management. The resin-based adsorbents used for this purpose so far, still have many drawbacks, such as high costs, sophisticated manufacturing and low adsorption capacity. In this respect, activated charcoal and its derivatives possess many advantageous characteristics.

Nanostructured carbonized materials and their adsorption characteristics. Activated carbons are known as excellent adsorbents. Their important applications are the adsorptive removal of color, odor, and taste, and other undesirable organic and inorganic pollutants from drinking water, in the treatment of industrial waste water; air purification in inhabited spaces, such as in restaurants, food processing, and chemical industries; for the purification of many chemical, food, and pharmaceutical products; in respirators for work under hostile environments; and in a variety of gas-phase applications. Their use in medicine and health applications to combat certain types of bacterial ailments and for the adsorptive removal of certain toxins and poisons, and for the purifications of blood, is being fast developed.

Carboneous adsorbents obtained from plant raw material are very versatile because of its extremely high surface area and micropore volume. The samples were been used had been carbonized according to the procedure developed at the

Laboratory of Hybrid Technologies in the Institute of Combustion Problems, Almaty, Kazakhstan. A flow set-up was used with following parameters: temperature range 250-800°C in argon flow (50-90 cm^3/min). Different temperatures would create a different pore system and therefore it would change the properties of the activated carbon.

Carbon surface has a unique character. It has a porous structure which determines its adsorption capacity, it has a chemical structure which influences its interaction with both polar and nonpolar molecules. Besides, it has active sites in the form of edges, dislocations and discontinuities which determine its chemical reactions with other atoms. Thus, the adsorption behavior of an activated carbon can't be interpreted on the basis of surface area and pore size distribution alone. Previous studies have proved that activated carbons having equal surface but different activation treatments show markedly different adsorption properties /5/.

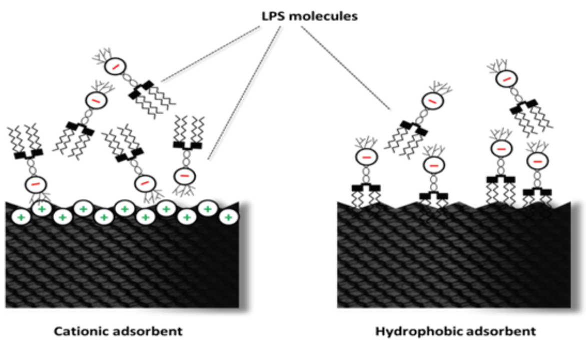

Figure 2 – Two approaches to promote selective LPS adsorption

Due to dualistic chemical nature of endotoxins, two approaches can be used to promote selective LPS adsorption. The first is based on administration of positively charged groups into the adsorbent's surface, which increases interaction with phosphate groups of LPS molecule. The other enhances the role of hydrophobic forces between the non-polar groups. Surface modification by polyethyleneimine (PEI) is one of the most efficient methods of negative charge creation on a surface.

Materials and Methods

Carbonized Materials and their modification. Rice husks (RH), activated at different temperatures served as main raw material for the adsorbent's preparation. The materials used in this study differ in surface properties because of the initial raw material used, because of temperature regime during their manufacturing. The materials used are listed in the Table 1.

Due to the high porosity and paramagnetic properties of the activated carbon, several steps are necessary before the carbonized materials can be used. First of all, the small-size fraction (dust) must be removed from the activated carbon sample as the measurement techniques used use light absorption, dust could interfere with the results.

Beside of dust, air-liquid interface during the experiment must be avoided as air can occupy the sites of adsorption and therefore it must be eliminated so there won't be competition for the adsorption sites between air bubbles and the adsorbent under study. One way to do this is by autoclaving the material. The autoclaving process was done at 1 bar, 121°C for 1 hour.

Table 1 – Characteristics of the materials used

Material used	Carbonization temperature, °C	Modification agent and its concentration, (M)	Porosity, cm^3/g	Specific area, m^2/g	Hemoglobin adsorption rate, mg/min
RH-1	650	WV*	2,16	910	1,50
RH-2	650	absent	0,97	630	1,05
Remark: * WV – water vapour					

The adsorption of a nonpolar solute will be higher on a nonpolar adsorbent. But since there is competition between the solute and the solvent, the solvent should be polar in nature for the solute to be adsorbed preferentially. The other factor that also determines the adsorption from solutions is the steric arrangement or the chemical structure of the adsorbate molecule. Lipopolysaccharides combine hydrophobic and charged groups in their structure. The core region close to lipid A and lipid A itself are partially phosphorylated; thus endotoxin molecules exhibit a net negative charge in common protein solutions. This peculiarity can be used as a starting point in optimization and improving efficacy and specificity of carbonized adsorbents. Two main strategies in surface modification can be suggested in order to stimulate LPS adsorption (Figure 2).

*LPS***.** The lipopolysaccharide (LPS) used in this study was derived from *Escherichia coli* isolate 0111:B4. The powder samples purchased from Sigma Co. were resuspended (1µg/mL) in 30 ml of sterile 0.2 M phosphate-buffered saline (PBS) at pH 7.3 and stored at −80°C. The initial concentration used in adsorption experiments was 1.0 ng/mL

Adsorption experiments. Time-course batch experiments were performed to establish the dynamics of sorption for our material exposed to different adsorbates. In this set of experiments, 100 mL of adsorbate solution were added to 5 g of adsorbing material. The concentration of adsorbate was measured after certain periods of time. Afterwards, these data were used to calculate the amount of adsorbed material.

The experiment was carried out at room temperature using the platform shaker with a rate of 110 cycles/minutes. This was done to allow a uniform distribution of the adsorbate over the adsorbent. The rate of shaking was important as its proven later on, the rate of adsorption was vastly improved when agitation was present, it will be explained on detail once the LPS batch experiments are discussed.

Endotoxin detection: LAL test. The Limulus Amebocyte Lysate (LAL) test is a quantitative test for gram-negative endotoxin. The use of LAL for the detection of endotoxin evolved from the observation by Bang, that a gram-negative bacterial infection of *Limulus polyphemus*, the horseshoe crab, resulted in a fatal intra-vascular coagulation. Levin and Bang later demonstrated that this clotting was the result of a reaction between endotoxins and a clottable protein in the circulating amebocytes of the *Limulus.* We used a commercially available LAL-based kit QCL-1000® from Lonza Co. The method utilizes the initial part of the LAL endotoxin reaction to activate an enzyme which in turn releases p-nitroaniline (pNA) from a synthetic substrate, producing a yellow color.

Results and discussion

LPS Removal. The LPS adsorption experiments performed on unmodified rice husks (MRH) showed very high efficiency of these native materials in LPS adsorption from buffer solutions (Fig.3). The time-course curve in the Figure 3 demonstrates that most of LPS was eliminated from the solution within the first 40 minutes. This is possibly governed by relatively higher specific area of the unmodified rice shells (see table above). The studied materials showed very high adsorption capacity in respect of pure solutions of endotoxins.

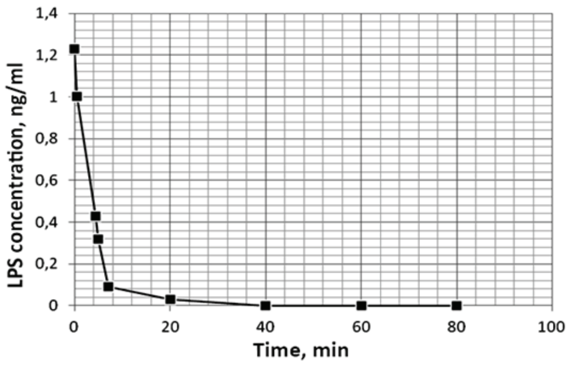

Figure 3 – Endotoxin adsorption from 1 ng/mL solution in PBS buffer as a function of time

Summarizing, the present work described the adsorption process using carboneous materials samples for the removal of endotoxins and protein adsorption from aqueous solutions. During the batch experiment setup, several techniques were used and improved for the handling of the material during the experiments; from it those the results obtained had an improvement from previous experimental work. The dust contents were significantly reduced and autoclaving allowed the elimination of the air interface in the carboneous material before interaction with the endotoxins and proteins.

From the data obtained after the different experiments were done it was possible to analyze the characteristics of carboneus material. The amount of endotoxin removed from the solution in comparison to the amount of proteins gives is encouraging further studies and suggests possible ways to improve the performance of LPS-elimination setups. Together with batch experiments, it is important to proceed into direction of column-based flow-through setups, as very promising from the point of view of efficiency.

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