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RESEARCH PAPER

# Uranium (VI) Biosorption on Marine Sponge, *Sarcotragus foetidus* (Schmidt, 1862) and Its Statistical Investigation Using Central Composite Design

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## Abstract

The biosorption of heavy metals, radionuclides, uranium and thorium for the recovery or removal from aqueous systems using various micro-organisms and biological substances has been investigated by several groups of workers. In this paper, the uptake of uranium (VI) from aqueous systems by a marine sponge, Sarcotragus foetidus (Schmidt, 1862) has been investigated. S. foetidus samples were collected from Babakale/Ayvacık/Canakkale in Turkey. Uranium (VI) uptake experiments were carried out by the batch technique. The affecting parameters were analyzed using Central Composite Design (CCD) as the experimental method. Four independent variables such - pH, temperature, contact time, and initial uranium concentration were selected for this study. The influences of initial pH, contact time, and initial uranium concentration on the biosorption process were considered statistically significant as main effects. On the other hand, interaction effects of pH and contact time, as well as pH and initial uranium concentration, were considered as significant parameters on the biosorption process. The optimum condition of biosorption process was found to be at init”ial pH: 4, temperature: 35°C, initial uranium concentration: 20 mg/L, and contact time: 31 minutes. At these conditions, the biosorption yield of uranium (VI) ions was obtained to be 93.29±2%.

*Keywords*: Marine sponge, Statistical design, Experimental Design, Analysis of variance (ANOVA), biological sorption.

## *Sarcotragus foetidus* (Schmidt, 1862) Denizel Süngeri ile Uranyum (VI) Biyosorpsiyonunun Merkezi Kompozit Dizayn ile İstatistiksel Olarak Araştırılması

## Özet

Mikroorganizmalar ve biyolojik materyaller kullanarak radyonüklitlerin, ağır metallerin, toryum ve uranyum gibi radyoaktif metallerin sulu çözeltilerden geri alınması veya uzaklaştırılması üzerine birçok çalışma vardır. Bu çalışmada, *Sarcotragus foetidus (Schmidt, 1862)* olarak adlandırılan deniz süngerinin sulu çözeltilerden uranyum alımı istatistiksel olarak araştırıldı. *S. foetidus* (*Schmidt, 1862)* deniz süngeri örnekleri Babakale (Ayvacık / Çanakkale) açıklarından toplandı. Uranyum (VI) geri alım denemeleri kesikli yöntem ile gerçekleştirildi. Biyosorpsiyonu etkileyen parametreler Merkezi Kompozit Deneysel Tasarımı (CCD) kullanılarak analiz edildi. Bu çalışma için; pH, sıcaklık, temas süresi ve uranyum başlangıç konsantrasyonu gibi dört bağımsız değişken seçildi. Ana parametrelerden pH, temas süresi ve uranyum başlangıç konsantrasyonu biyosorpsiyon prosesinde istatistiksel olarak anlamlı olarak kabul edildi. Diğer bir yandan; biyosorpsiyon işlemi üzerinde, pH- temas süresi, pH - uranyum başlangıç konsantrasyonu gibi ikili etkileşimler önemli parametreler olarak belirlendi. Biyosorbsiyon prosesinin optimum koşulları; pH:4, sıcaklık: 38 , uranyum başlangıç konsantrasyonu: 20 mg/L ve temas süresi: 31 dakika olarak hesaplandı. Bu koşullar altında biyosorpsiyon verimi % 93.29±2 olarak elde edildi.

*Anahtar Kelimeler*: Denizel sünger, İstatistiksel dizayn, Deneysel tasarım, Varyans analizi (ANOVA), Biyolojik sorpsiyon

## Introduction

Sponges are multicellular invertebrate organisms and over 5000 sponge species have been discovered worldwide. Among these, 15–20 species have commercial value. *Sarcotragus foetidus (*Schmidt, 1862*)*, described in detail by Vacelet (Vacelet 1959), is a species that belong to the family of *Spongiidea* of the order of *Karatosa* of the class of *Demospongia* of the *Porifera* phylum and distributed in Mediterranean, West African shores, and Australia. Its outer surface is very irregular and large, its body is wide, and the height of its conules is 2 – 3 mm and the distance between these conules is 8 – 15 mm. The diameter of the species is 20 cm in Marseille region, but could reach 50 cm in Eastern Mediterranean. Primary fibrils are hard with a reticular structure, and the secondary fibrils are slim, lamellar and 0.5 – 1.5 microns thick. Body walls of sponges contain several pores, which are called porocytes. These pores are distributed quite unevenly. The water intake through a series of inflow pores and dermal ostia found on sponge body wall reaches the gastral cavity and flows out of the sponge through the wide oscula on top. There are channels between the pores and the gastral cavity. Continuous water flow that enters through the pores and leaves the body through osculum is maintained by the flagellum of the collar cells called the *Choanocytes*. Sponges could feed on all organic particles between 0.1 and 50 μm equally (phytoplankton, heterotrophic bacteria, heterotrophic eukaryotes, and organic matter in soil). Thus, they could contain diverse types of microorganisms in their structure. Due to the ability of sponges to process great volumes of sea water and obtain the particles they need, it is considered that they have a great advantage against other organisms that feed via suspension. According to Vacelet (1985), large sponges could filter water that is equal to their volume in 10 seconds (Vacelet 1985). Thus, they have the potential to collect heavy metals or other pollutants in the environment (Selvin *et al*. 2009). Furthermore, since marine sponges could stay at one location for one year without moving, they are also being used as potential indicator organisms to monitor heavy metal pollution in these regions (Berthet *et al*. 2005; Rao *et al*. 2006). The biosorption of metals by various marine sponges has been reported by different research groups. Aslani at al. characterized soption of thorium (IV) from aqueous medium on ignited *Sarcotragus muscarum* in terms of equilibrium metal loading, thermodynamics, kinetics, and effect of sorption parameters (Aslani, Akyil, and Eral 2001). A new type of sponge belonging to the family *Demospongiae* that has nanoholes and is native to the Persian Gulf was investigated for the first time in the present study for use as an adsorbent to remove calcium, magnesium, cobalt, cadmium, and lead ions from water and adsorption in sponges of different aggregate sizes, contact time, particle size, and ambient pH were measured (Ghafourian, Khodadad, and Rabbani 2015). *Callyspongia aerizusa***,** a marine sponge was used as metal pollution indicator because this sponge can accumulate heavy metal Zn in high level (Melawaty *et al*. 2014). Tarbaoui *at al*. (2015) studied the biosorption and desorption of lead, copper, and cadmium ions by a new material prepared from the marine sponge *Cinachyrella tarentina* (Tarbaoui *et al*. 2015). Lindino *at al*. (2014) investigated the adsorption of cadmium in vegetable marine sponge, *Luffa cylindrica* (Lindino *et al*. 2014). Araujo *at al.* (2003) reported that ability of marine sponge in accummulating heavy metals depends on the metal types (Mn, Fe, Ni, Cu, Zn, As, Sr, Zr and Pb, etc.) (Araujo *et al*. 2003). Olesen *at al.* (1994) researched the effect of cadmium upon sponge filtration rate and accumulation of cadmium by marine sponge, Halichondria panicea Pallas (Olesen and Weeks 1994). Also, Norman *at al.* (2015) reported adsorption of C. I. natural red 4 onto spongin skeleton of marine demosponge, *Hippospongia communis* (Norman M. *et al*. 2015.). Furthermore; Marine sponges were successfully used for monitoring of heavy metals by research groups. Marine sponge, *Haliclona tenuiramosa*, was used as bioindicator to monitor heavy metal pollution by Rao *et al*. (Rao *et al*. 2009). Marine sponges, *Cliona viridis, Cacospongia scalaris, Chondrosia reniformis, Spongia officinalis, Spongia agaricina* and *Agelas oroides*, was used for monitoring of heavy metals (Pb, Cd, Cu, Zn, Hg, Fe,…) by Perez *et al*. (Perez, Vacelet, and Rebouillon 2004). In addition, sponges could hold 80% of the organic substances suspended and 70% of the bacteria in the water they filter (Pronzato 1999). As a result, they could lower the concentration of bacteria that are hazardous to human health in the seas such as *Vibrio* and control the total bacteria density (Fu *et al*. 2006). The colors of *Sarcotragus* species sponges are variable and they could be black-yellow or hazel (Figure 1). Found in deep seas, *S. foetidus*, known also as rough sponge, is the most prevalent sponge

**Figure 1.** *Sarcotragus foetidus* (Schmidt, 1862) from Aegean Sea.

species found in the Aegean Sea and Mediterranean shores that surround Turkey. *S. foetidus* is observed almost in all regions with rocky and stony substratum seabed types.

Uranium is an element that could be found everywhere in nature. Uranium concentrations in natural waters could vary within a wide spectrum. There are three main uranium isotopes; $^{234}U$ (0.0055%, $t_{1/2}$=4.47.10$^{9}$), $^{235}U$ (0.72%, $t_{1/2}$=7.04.10$^{8}$), and $^{238}U$ (99.27%, $t_{1/2}$=2.46.10$^{5}$), and their half-lives are quite long. This fact demonstrates that uranium is rather chemotoxic than being radiotoxic. Uranium could be found either in inert state with low oxidation U (IV) form or in quite mobile U (VI) form with high solubility in water. Its concentration in seawater is 2 – 4 mg/L on average. In other waters, it could vary between 0.1 and 1 mg/L. The highest uranium concentration is found in underground waters per mg liter (Lehto and Hou 2011). Uranium is found in the form of 200 different minerals in lithosphere with a mean concentration of 2.7 ppm. Uranium is also detected in certain air samples as fly ash and suspended solids (Eisenbud and Gesell 1997). Uranium could reach the top level in the food chain, and when that happens, it could be easily swallowed by humans, which in turn would concentrate in lungs and kidneys, causing serious harm (Dursun 2006; Samson *et al*. 2002). Removal of uranium or other toxic metals from waters is possible via a few chemical (neutralization, precipitation) and physical (evaporation, ion exchange, membrane technology) methods. But, these technologies require high capital investment and construction costs, and are ineffective in low-resistance waters. Therefore, researchers have focused on finding easy, effective, economic, and environment-friendly techniques to remove heavy metals in aqueous solutions (Bashardoost *et al*. 2010; Kalantari *et al*. 2014; Muhamad, Doan, and Lohi 2010; Naddafi *et al*. 2007). Biosorption process is increasingly accepted as an alternative method to remove metal pollution from natural and industrial wastewater. Removal of heavy metals, and radionuclides such as thorium and uranium from aqueous solutions using biological materials and various microorganisms was investigated in several studies. Natural bio-adsorbents such as seaweeds (Yang and Volesky 1999), bacteria (Li *et al*. 2016), yeast (Breierova *et al*. 2002), fungi (Hou *et al*. 2016), algae (Al-Qunaibit, Khalil, and Al-Wassil 2005) and marine sponges (Hansen, Weeks, and Depledge 1995; Maloubier *et al*. 2015) were used in these studies. Biosorption process could be considered as a combined method of passive fusion (coagulation) process independent from the metabolism, and physical and/or chemical adsorption, ion exchange, coordination, complexation, chelate formation and micro-precipitation processes. Cellular wall of the biomass contains polysaccharides, proteins, and fats. It also includes certain functional groups that could bond with metal ions such as carboxyl, hydroxyl, sulfate, phosphate and amino groups. Metal ions could be inhibited by forming physical and/or chemical bonds with these groups or via ion exchange that occurs on the cell membrane (Hou *et al*. 2016). Biosorption process is affected by the ambient temperature and pH, used buffer type, other chemicals, pretreatments applied to bio-sorbents, the age of bio-sorbent biomass, ionic forces and other chemical-physical factors.

Statistical methods are increasingly used in advancing and competitive fields of industries, agriculture, food, chemistry, biology industries, and many others more effectively with every passing day. Today, experimental design and use of optimization methods became necessary in the studies conducted to increase product quality and to lower the costs. In optimization studies, it is important to obtain the best results with the lowest cost and in the shortest time. For the target set, experimental design methods are utilized. Response surface methodology (RSM), one of the experimental design methods, is used to determine the effect of the values of certain factors on the response variable and to find the value(s) among the factor value combinations that result in a maximum (or minimum) response variable. This methodology includes a series of mathematical and statistical techniques used to define the relationship between response and independent variables. These techniques inquire the effects of the independent variables (factors) in the process on the response alone or in combination. The first step in response surface methodology is to determine the factors that are supposed to affect the response variable, i.e. the independent variables. After this step is completed, techniques of experimental design, regression modeling and optimization are used in a nested manner in response surface methodology (Bas and Boyaci 2007).

In the present study, *S. foetidus* (Schmidt, 1862) marine sponges were collected from its natural habitat at Aegean Sea Babakale shores and its uranium intake capacity from aqueous solutions was investigated using statistical methods. Central Composite Design (CCD), a response surface method was used as statistical experimental method to study the parameters (temperature, time, pH, etc.) that affected biosorption. Adsorption isotherm parameters (Langmuir, Freundlich, Dubinin-Radushkevitch (D-R), etc.) were identified, and thermodynamic calculations ($\Delta H$, $\Delta G$ and $\Delta S$) were conducted.

## Materials and Methods

### Materials

All chemicals used in the study were in analytical purity. Ultra-pure water was used in the preparation of solutions and throughout the experiments. The uranium stock solution with 1000 mg/L uranium metal ($^{238}U$) concentration was prepared with uranyl acetate hexahydrate $UO_2(CH_3COO)_2.6H_2O$ (Merck, Germany). Later on, this stock solution was used to obtain uranium solutions with desired metal concentrations. pH adjustments of the solutions were conducted using 0.1 M ammonia and acetic acid solutions and with a pH-meter (EZDO PL-700 PC). pH values of the solutions were measured before and after the contact and it was observed that the variation was below 0.1. pH variations were not measured during the contact.

## Collection of Marine Sponges and Their Preparation as Bio-Sorbent

Marine sponge *S. foetidus* (Schmidt, 1862) was collected by scuba divers offshore Aegean Sea waters (Babakale/Canakkale/Turkey) at an average depth of 25 m on July 23, 2015 (Figure 1). Collected sponges were carried into the laboratory in sterile containers. The contaminants in the sponges were cleaned by running nitric acid solution through the sponges. Later on, they were decontaminated from parasites by lavage in sterile ultra-pure water for 2 hours under 60°C temperature for three times. They were separated into pieces in a sterile environment and dried in freeze-driers (Labconco FreeZone® Plus 2.5 Liter Cascade Console Freeze Dry System model) for 24 hours. Dried marine sponges were grinded in Tekmar A-10 model water-cooling mill, sifted in a 250 µm (-120 mesh) sieve, and obtained powder samples were stored in a desiccator at room temperature.

## Characterization

FTIR analyses were performed with metal free and metal-loaded biomass. The samples were allowed to dry in vacuum oven at 25°C and analyzed with ATR-FTIR (Thermo Scientific Nicolet iS50) spectrometer.

## Batch Biosorption Experiments

Batch method biosorption experiments were conducted with a temperature controlled thermostatic shaker (GLF-1086 model). For the experiments, 0.01 g *S. foetidus* (Schmidt, 1862) marine sponge powder was placed in a 15 ml propylene plastic tube. 10 ml $UO_2(CH_3COO)_2.6H_2O$ solutions in concentrations determined by the experimental design were added and contact process was conducted at 150 rpm shaking speed, for the duration and under the temperature identified in the experimental design. After the supernatants were filtered by a filter paper (Whatman No. 41, circles, cotton filters, diameter:125 mm, thickness: 220µm, pore size: 20-25 µm), $UO_2^{2+}$ concentration in the supernatant was determined with Arsenazo III method before the contact and with spectrophotometric method using an Optima SP-3000 nano model spectrometer after the contact. In a 10 mL volumetric flask, 2 mL buffer solution (pH:1.7, acetic acid, phosphoric acid, boric acid) and appropriate volumes of uranium were added and made up to the mark with triply distilled water. The final concentrations of uranium must be between 0.10-21.00. For each measurement, about 2 mL of the above solution was transferred to a spectrophotometric cell and absorbencies were measured at 653 nm, against a reagent blank, for uranium ions (Niazi *et al*. 2007).

$UO_2^{2+}$ amount adsorbed by *S. foetidus* (Schmidt, 1862) marine sponge was calculated using Equation (1):

$$Q_e = (C_0 - C_e)x\frac{V}{m} \text{ …………………… ( 1 )}$$

Where $Q_e$ depicts metal amount received per unit adsorbent (mg/g), $C_0$ and $C_e$ identify pre-contact and post-contact $UO_2^{2+}$ concentration in the solution, respectively (mg/L), $V$ is the solution volume (L), and $m$ depicts the sorbent mass (g).

Distribution coefficient of metal ion between the aqueous phase and solid phase, Kd (mg/L) could be obtained with Equation (2):

$$K_d = \left(\frac{C_0 - C_e}{C_e}\right)x\frac{V}{m} \text{ ……………………. ( 2 )}$$

In the equation, $\frac{V}{m}$ (ml/g) shows the ratio of the volume of metal solution (ml) to sorbent mass (g) in the batch biosorption process.

## Statistical Experiment Design for Biosorption

The most suitable condition for biosorption of $UO_2^{2+}$ by *S. foetidus* (Schmidt, 1862) marine sponge was determined with Central Composite Design (CCD), one of the response surface methods (RSM). Secondary methods such as Central Composite Design (CCD) and Box-Behnken Design (BBD) are especially preferred when precise predictions, low costs, and quality products according to predetermined specifications are required in the studies. The operation process of Central Composite Design includes analysis of obtained valid and objective findings and assessment of suitable data using statistical methods (Montgomery 1996). Response Surface Methodology (RSM) is a combination of mathematical and statistical techniques that determine the behavior of a dataset to identify statistical predictions based on the transformation of empirical data into a polynomial equation (Bezerra *et al*. 2008). Central Composite Design (CCD) is a design that could utilize and rotate each factor on five levels (-α -1, 0, +1, +α). Rotatability is a basic reason to pick a response level design (Myers and C. 1995).

The effects of four active independent parameters such as pH (X1), temperature (X2), contact time (X3), and initial uranium concentration (X4) on $UO_2^{2+}$ ion biosorption with *S. foetidus* (Schmidt, 1862) marine sponge were analyzed in five different levels (Table 1) and optimized with Central Composite Design (CCD). Twenty-nine experiments were conducted with the design prepared based on determined factors and levels in the study. In all experiments, dependent variable and % *adsorption* (*Y*) – accepted as the response – values were calculated and presented in Table 2.

The second degree polynomial equation (3), which is a mathematical expression of the method, is used to determine linear, quadratic and cross effects of above mentioned independent variables on the result and to approach the real answer.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j + \varepsilon \quad (3)$$

In this equation; $X_1$, $X_2$, $X_3$……….,$X_k$ depict the factors affecting Y response, $\beta_0$, $\beta_i$, $\beta_{ii}$ (i= 1,2,3,……….,k), $\beta_{ij}$ (i=1,2,3,……….,k; j=1,2,3,……….,k) represent the regression coefficients, and ε depicts the random error term. To be used in statistical calculations, four independent factors that affect the result were coded as $X_1$, $X_2$, $X_3$ and $X_4$, and the resulting second degree polynomial equation (4) is given below:

$$Y_i = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_{11}X_1^2 + \beta_{22}X_2^2 +$$

**Table 1.** Independent biosorption parameters and levels in empirical design

| Factors | Factor code | Level and range (coded) | | | | |
|---|---|---|---|---|---|---|
| | | -α | -1 | 0 | 1 | +α |
| pH | $X_1$ | 1 | 2 | 3 | 4 | 5 |
| Temperature (°C) | $X_2$ | 20 | 25 | 30 | 35 | 40 |
| Contact time (min.) | $X_3$ | 10 | 30 | 50 | 70 | 90 |
| Initial uranium concentration (mg/L) | $X_4$ | 10 | 20 | 30 | 40 | 50 |

**Table 2.** Experimental design created based on CCD model with five independent variables and obtained results

| Run no. | Coded values of independent variables | | | | Responses (% adsorption) | | Residuals values |
|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | X4 | Experimental values | Predicted values | |
| 1 | 0 | -α | 0 | 0 | 11,59 | 23,09 | -11,50 |
| 2 | 0 | 0 | +α | 0 | 13,77 | 0,37 | 13,40 |
| 3 | 1 | -1 | 1 | 1 | 18,27 | 16,38 | 1,89 |
| 4 | -1 | 1 | -1 | -1 | 22,52 | 27,90 | -5,38 |
| 5 | -α | 0 | 0 | 0 | 15,33 | 15,57 | -0,24 |
| 6 | 0 | 0 | 0 | -α | 89,91 | 77,19 | 12,72 |
| 7 | 0 | 0 | 0 | 0 | 34,64 | 19,56 | 15,08 |
| 8 | 0 | 0 | -α | 0 | 12,21 | 20,92 | -8,71 |
| 9 | 1 | -1 | -1 | 1 | 44,67 | 37,31 | 7,36 |
| 10 | 0 | 0 | 0 | 0 | 18,44 | 19,56 | -1,12 |
| 11 | -1 | 1 | -1 | 1 | 13,13 | 8,18 | 4,95 |
| 12 | 0 | 0 | 0 | +α | 15,18 | 23,21 | -8,03 |
| 13 | 0 | 0 | 0 | 0 | 13,77 | 19,56 | -5,79 |
| 14 | +α | 0 | 0 | 0 | 88,22 | 83,29 | 4,93 |
| 15 | 0 | 0 | 0 | 0 | 15,95 | 19,56 | -3,61 |
| 16 | -1 | -1 | -1 | 1 | 14,53 | 3,07 | 11,46 |
| 17 | 0 | 0 | 0 | 0 | 15,02 | 19,56 | -4,54 |
| 18 | 1 | 1 | -1 | 1 | 39,07 | 46,10 | -7,03 |
| 19 | 1 | 1 | -1 | -1 | 94,72 | 94,73 | -0,01 |
| 20 | 1 | 1 | 1 | 1 | 27,38 | 26,51 | 0,87 |
| 21 | 0 | +α | 0 | 0 | 55,51 | 39,33 | 16,18 |
| 22 | -1 | -1 | -1 | -1 | 19,72 | 21,80 | -2,08 |
| 23 | -1 | -1 | 1 | 1 | 14,30 | 15,49 | -1,19 |
| 24 | -1 | 1 | 1 | -1 | 19,72 | 28,29 | -8,57 |
| 25 | 1 | 1 | 1 | -1 | 46,82 | 61,77 | -14,95 |
| 26 | 1 | -1 | -1 | -1 | 88,41 | 84,95 | 3,46 |
| 27 | 1 | -1 | 1 | -1 | 44,49 | 50,64 | -6,15 |
| 28 | -1 | -1 | 1 | -1 | 24,39 | 20,84 | 3,55 |
| 29 | -1 | 1 | 1 | 1 | 15,00 | 21,95 | -6,95 |

$$\beta_{33}X_3^2 + \beta_{44}X_4^2 + \beta_{12} X_1X_2 + \beta_{13} X_1X_3 + \beta_{14} X_1X_4 + \beta_{23} X_2X_3 + \beta_{24}X_2X_4 + \beta_{34}X_3X_4 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .. (4)$$

The model developed above was assessed with statistical analyses including Fisher's F-test and analysis of variance (ANOVA). Goodness of fit of the model equation was identified with the stability factor, $R^2$. Later on, spherical and surface graphs were plotted to demonstrate the relationship between the responses in fitted model. Best expected combination effects were determined with the statistical software package, Design Expert 9.0.6. Finally, to evidence the fitness of Central Composite Design (CCD) model, $UO_2^{2+}$ biosorptions were conducted under predetermined optimal conditions and biosorption process was characterized via isothermal and thermodynamic studies.

## Results and Discussion

### The Results of The Fourier Transform Infrared Spectroscopy (FTIR-ATR)

Fourier transform infrared spectroscopy (FTIR-ATR) was performed by using the Thermo Scientific Nicolet iS50 FTIR spectrometer. The FTIR spectra of *S. foetidus* (Schmidt, 1862) marine sponge biomass before and after adsorption of uranium (VI) were used to clarify the vibrational frequency changes of the functional groups in the biosorbent. The spectra of adsorbents were measured within the range of 4000–600 $cm^{-1}$ wavenumbers (Figure 2).

To determine the functional groups of loaded and unloaded *S. foeditus*, biomass was analyzed by FTIR spectroscopy. In unloaded spectrum, the very strong adsorption band around 3200-3400 cm-1 found in these samples may be due to the presence of N-H stretching of amines and amides and polymeric association which was normally found in hydroxyl compounds. Alkenes and aromatics show a C-H stretch slightly higher than 3000 cm-1. The peak near 2925 cm-1 is characteristic of the presence of aliphatic (-CH) groups in these compounds. The adsorption band around 2900-2850 cm-1 correspond to C-H stretching of CH2 groups. The higher frequency adsorption (around 1630 cm-1) is called the Amide I band. The lower frequency Amide II band (around 1530± 30 cm-1) is largely due to N-H bending trans to the carbonyl oxygen (Li *et al*. 2008). The adsorption bands at around 1450 cm-1 and 3080 cm-1 were attributed to the C=C-H stretching and scissoring peaks of Alkenes. The peak near 1250 cm-1 which is related to C-O stretching in COOH group, again strengthens the hypothesis that a carboxyl group was involved in metal biosorption (Yee *et al*. 2004). C–N and C–O functional groups stretching bands overlapped at around 1,020 cm-1. In loaded spectrum, no considerable differences were observed except the peak at 950 cm-1 wavenumber. The presence of this new peak in loaded biomass was attributed to heavy metal ion–oxygen bond (Chhikara *et al*. 2010).

### Optimization of Ions with *Sarcotragus foetidus* (Schmidt, 1862) Marine Sponge Using Box Behnken Design (BBD)

To conduct biosorption under best conditions and to obtain the highest yield, Central Composite Design (CCD), one of response surface methods (RSM), was utilized, and regression and analysis of variance analyses were conducted with the least squares method on the data obtained as a result of 29 different experimental designs created with various combinations of different parameters (Table 1) such as pH, temperature, contact time, and initial metal concentration.

The results of the analysis conducted to fit a mathematical model to % yield of $UO_2^{2+}$ ion biosorption with *S. foetidus* (Schmidt, 1862) marine sponge and sequential total of the squares for linear, quadratic and cubic terms that belong to the obtained model are presented in Tables 3 and 4. Analysis of the empirical data demonstrated that the quadratic model was suitable for use in achieving the appropriate regression equation. Fitness of the model was assessed based on P-value statistically and in addition, the $R^2$ values (Tables 3 and 4). Since P-values were small for the selected quadratic model (P<0.05),

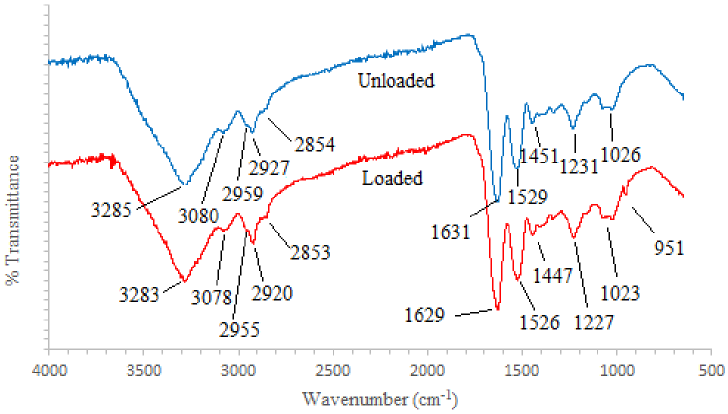

**Figure 2.** The effect of primary parameters: (a) pH, (b) Contact time, (c) Initial U(VI) concentration.

**Table 3.** Sequential total data for squares related to the model

| Source | *SS* | *df* | *MS* | *F value* | *P value*<br>*Prob > F* | |
|---|---|---|---|---|---|---|
| Mean vs Total | 30904.11 | 1 | 30904.11 | | | |
| Linear vs Mean | 12278.67 | 4 | 3069.67 | 10.27 | < 0.0001 | |
| 2FI vs Linear | 2143.66 | 6 | 357.28 | 1.28 | 0.3156 | |
| Quadratic vs 2FI | 3126.08 | 4 | 781.52 | 5.75 | 0.0059 | Suggested |
| Cubic vs Quadratic | 1535.07 | 8 | 191.88 | 3.14 | 0.0905 | |
| Residual | 367.12 | 6 | 61.19 | | | |
| Total | 50354.71 | 29 | 1736.37 | | | |

**Table 4.** Statistical summary for the models

| Source | Std. Dev. | $R^2$ | Adjusted $R^2$ | Predicted $R^2$ | Multiple R | |
|---|---|---|---|---|---|---|
| Linear | 17.29 | 0.6313 | 0.5698 | 0.4515 | 0.7945 | |
| 2FI | 16.71 | 0.7415 | 0.5979 | 0.4756 | 0.8611 | |
| Quadratic | 11.66 | 0.9022 | 0.8044 | 0.5006 | 0.9498 | Suggested |
| Cubic | 7.82 | 0.9811 | 0.9119 | 0.4491 | | |

second degree model was considered fit for % yield responses of $UO_2^{2+}$ ion biosorption with *S. foetidus* (Schmidt, 1862) marine sponge.

Determination of the $R^2$ value as 0.9022 reflects that there was a high fit between empirical and estimated values. This value is always between 0 and 1. Values close to 1 reflect the closest estimate of the response and the strength of the applied model (El-Naggar and Abdelwahed 2014). A very high correlation coefficient (0.9498) demonstrates a perfect relationship between independent values and a good correlation between empirical and estimated values (Box, Hunter, and Hunter 1978).

According to the polynomial equation (5) obtained as a result of the analysis on the good for fit model for the results of the experiments based on central composite design, the following equation provided the response surface graphs:

$$Y = +19.56 + 16.93\,X_1 + 4.06\,X_2 - 5.14\,X_3 - 13.50\,X_4 + 7.47\,X_1^2 + 2.91\,X_2^2 - 2.23\,X_3^2 + 7.66\,X_4^2 + 0.92\,X_1X_2 - 8.34\,X_1X_3 - 7.23\,X_1X_4 + 0.34\,X_2X_3 - 0.25\,X_2X_4 + 3.34\,X_3X_4 \ldots\ldots\ldots\ldots(5)$$

The second degree polynomial equation explains the effects of each variable on % yield of $UO_2^{2+}$ ion biosorption with *S. foetidus* (Schmidt, 1862) marine sponge and the relationships between the variables. When the effect of pH, temperature, contact time, and initial U (VI) concentration on % yield of $UO_2^{2+}$ ion biosorption is analyzed based on the second degree polynomial equation, analysis of variance (ANOVA) was applied on the implemented model within 95% confidence interval and the fit between the applied model and empirical findings was tested. ANOVA is necessary to test the significance and sufficiency of the model. The fact that the calculated Fisher rate of variance (F-value) was lower than 0.05 within 95% confidence interval and also Fisher F-test (F) value for the model was found as 9.23 indicated that the model was highly statistically significant (Table 5) (Garg *et al*. 2008).

As a result of statistical analyses, F value was taken as $F_{0.05;14.4} = 5.86$ from the statistics table. The fact that empirical F values were greater and P values were smaller than that value increase the significance of related coefficients and the stand alone parameters or the relationships among the parameters (Rodrigues and Lemma 2015).

ANOVA findings for the model demonstrated that, while‘‘Prob>F’’ values were insignificant when they were greater than 0.1, those that were smaller than 0.05 were quite significant (Erick and Padmanabhan 2015). Thus, $X_1$, $X_3$, $X_4$, $X_1X_3$, $X_1X_4$, $X_1^2$ and $X_4^2$ were significant terms for the model.

## Effect of Primary Parameters on Biosorption

The effect of the primary parameters such as pH ($X_1$), temperature ($X_2$), contact time ($X_3$), and initial U (VI) concentration ($X_4$) on $UO_2^{2+}$ ion biosorption with *S. foetidus* (Schmidt, 1862) marine sponge was analyzed. It was accepted that initial pH, temperature, contact time, and initial U (VI) concentration were significant parameters for $UO_2^{2+}$ ion biosorption. Initial solution pH ($X_1$), contact time ($X_3$), and initial U (VI) concentration ($X_4$) were found to be statistically significant ($P<0.05$). Furthermore, squares of pH and initial U (VI) concentration primary factors ($X_1^2$ and $X_4^2$) were found to be statistically significant ($P<0.05$). When the second degree polynomial equation is analyzed (Equation 5), it is known that the parameter with a greater coefficient has a greater effect on the biosorption. A positive sign shows that parameters and dependent variables are directly related (Can and Yildiz 2006).

Thus, it was observed that, when the value of a factor is increased from a lower level to a higher level, metal retaining effect increased as well. On the other hand, when the coefficient is negative, it is observed that when the value of the factor is raised from a lower value to a higher value, metal retention effect decreased (Carmona, da Silva, and Leite 2005). As is seen in polynomial equation (Equation 5), the positive pH coefficient ($X_1$=+16.93) reflects that as pH increases, its effect on % biosorption yield is positive. As the active parameter pH increases, % metal biosorption increases as well (Figure 3a), however, biosorption of U(VI) ions on bio-sorbent was studied between pH = 1 and 5, because a precipitation effect was observed at pH>6 (Hafez *et al*. 2002). In addition, negative values for contact time ($X_3$=-5.14) and initial U (VI) concentration ($X_4$=-13.50) demonstrated that increasing the values of these parameters would affect the cumulative yield of biosorption negatively proportional to the coefficient located at the beginning of the equation (Figure 3b, c).

## Response Surface Method Plots

Dual contacts between independent variables were determined by anchoring one of the independent variables at the optimum level and plotting three-dimensional response surface and two-dimensional contour lines using Design Expert (9.6.0) software. Elliptical or circular contour lines that demonstrate the effects between pH-contact time ($X_1X_3$ = -8.34, P = 0.0126) dual contact are statistically significant (P<0.05). Similarly, pH-initial U (VI) concentration ($X_1X_4$ = -7.23, P = 0.0264) dual contact is statistically significant (P<0.05). Each contour line (Figures 4b and 5b) gives the indefinite number of combinations for the other two variables while one variable is at the center. Circular, elliptic or saddle type contour lines based on the coefficients could give maximum point, minimum point, saddle point, stationary peak or increasing/decreasing peak conditions (Luo 2008). Response surface graphs and contour lines for maximum point, minimum point, and saddle point are presented in Figures 4a, b and 5a, b. A circular

**Table 5.** ANOVA for Response Surface Quadratic model

| Source | *df* | SS | MS | F-value | P-value Prop>F | |
|---|---|---|---|---|---|---|
| Model | 14 | 17548.41 | 1253.46 | 9.23 | < 0.0001 | significant |
| $X_1$-pH | 1 | 6878.60 | 6878.60 | 50.63 | < 0.0001 | |
| $X_2$-Tempereture (ºC) | 1 | 395.52 | 395.52 | 2.91 | 0.1101 | |
| $X_3$-Contact time (min.) | 1 | 633.32 | 633.32 | 4.66 | 0.0487 | |
| $X_4$-Initial U(VI) concentration (mg/L) | 1 | 4371.22 | 4371.22 | 32.17 | < 0.0001 | |
| $X_1X_2$ | 1 | 13.54 | 13.54 | 0.100 | 0.7569 | |
| $X_1X_3$ | 1 | 1112.41 | 1112.41 | 8.19 | 0.0126 | |
| $X_1X_4$ | 1 | 835.99 | 835.99 | 6.15 | 0.0264 | |
| $X_2X_3$ | 1 | 1.80 | 1.80 | 0.013 | 0.9099 | |
| $X_2X_4$ | 1 | 0.99 | 0.99 | 7.257E-003 | 0.9333 | |
| $X_3X_4$ | 1 | 178.92 | 178.92 | 1.32 | 0.2704 | |
| $X_1^2$ | 1 | 1446.49 | 1446.49 | 10.65 | 0.0057 | |
| $X_2^2$ | 1 | 219.79 | 219.79 | 1.62 | 0.2241 | |
| $X_3^2$ | 1 | 128.99 | 128.99 | 0.95 | 0.3464 | |
| $X_4^2$ | 1 | 1521.68 | 1521.68 | 11.20 | 0.0048 | |
| Residual | 14 | 1902.20 | 135.87 | | | |
| Lack of Fit | 10 | 1606.27 | 160.63 | 2.17 | 0.2367 | not significant |
| Pure Error | 4 | 295.92 | 73.98 | | | |
| Cor Total | 28 | 19450.61 | | | | |

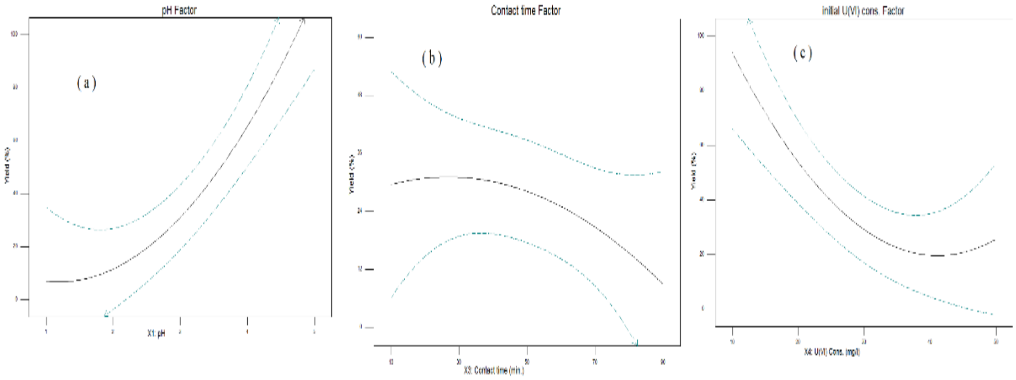

**Figure 3.** (a) Response surface graph for pH-Contact time, (b) contour line.

response surface shows that contact between the variables is negligible, while elliptical or saddle type surfaces demonstrate that internal contact was significant. The surface limited by the least elliptical or circular shape identify the highest or lowest estimated response (Khuri and Cornell 1996).

When all obtained data were analyzed with Design Expert v. 9.0.6 computer software, optimum biosorption conditions with *S. foetidus (*Schmidt, 1862*)* were determined as pH = 4, temperature = 35 °C, contact time = 31 minutes, and initial U (VI) concentration = 20 mg/L. Sorption operation was conducted with the bio-sorbent under these conditions and U (VI) retention yield was found as 93 ± 2%.

## Biosorption Isotherms

To determine adsorption capacity of the bio-sorbent, equilibrium data were assessed based on Freundlich, Langmuir, and Dubinin–Radushkevich (D-R) isotherms. These isotherm equations are the most common isotherms used to determine adsorption equilibrium in water and wastewater treatment applications (Yusan and Akyil 2008). Langmuir equation (6) applied successfully to several biosorption processes is given below:

$$\frac{C_e}{Q_e} = \frac{1}{bn_m} + \frac{C_e}{n_m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

In this equation, $C_e$ is equilibrium concentration (mg/l), $Q_e$ is adsorbed substance amount per unit mass at equilibrium(mg/g), $n_m$ and $b$ depict Langmuir constants for single layer sorption capacity (mg/g) and sorption energy (L/mg), respectively. Langmuir isotherm identifies the adsorption surface as single layer. This model is based on the assumption that adsorbent is structurally homogenous and all adsorption areas are the same based on energy (Kutahyali and Eral 2010). Freundlich isotherm equation (7) provides information about the biosorption of U (VI) on a biosorbent with a heterogeneous surface.

$$Q_e = K_F . C_e^{1/n} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

When the equation (7) is transformed into a linear form, Equation (8) is obtained:

$$logQ_e = logK_F + \frac{1}{n} logC_e \quad \ldots\ldots\ldots (8)$$

In this equation, $Q_e$ shows adsorbed substance amount per unit bio-sorbent mass (mg/g), $C_e$ depicts solution equilibrium concentration (mg/l), and $K_F$ and $n$ are the characteristic constants of Freundlich isotherm. These constants are identified via the slope

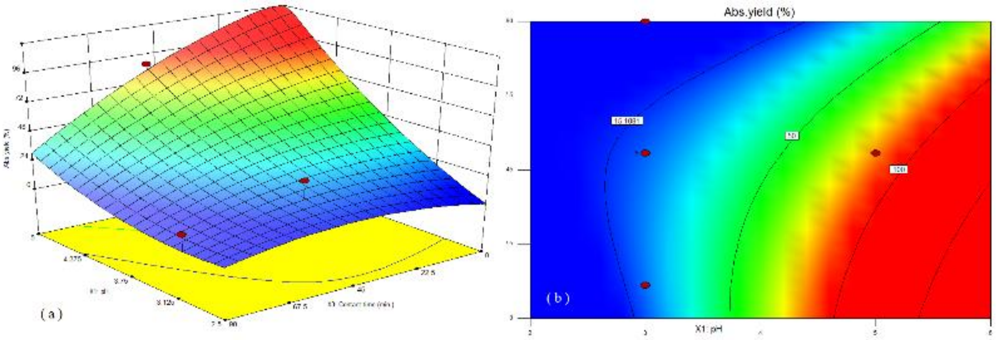

**Figure 4.** (a) Response surface graph for pH- Initial U (VI) concentration, (b) contour line.

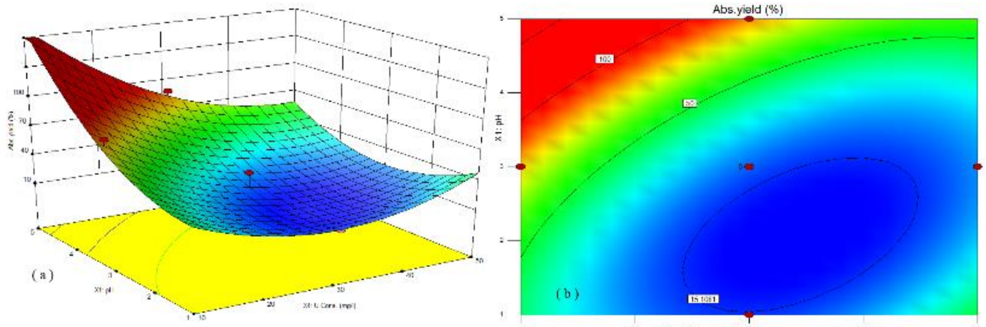

**Figure 5.** (a) Response surface graph for pH- Initial U (VI) concentration, (b) contour line

of the graph plotted between $Q_e$ and $C_e$, *n*, and the intersection point, $K_F$.

D-R adsorption isotherm is defined as the energy released while 1 mol ion is adsorbed from the solution by the adsorbent. D-R adsorption isotherms are used to calculate adsorption energy. Adsorption energy is drawn from the slope of these isotherms. Furthermore, D-R isotherm model could be used to define the adsorption on homogenous and heterogeneous surfaces in low concentration ranges. It assumes adsorption within regions close to the surface. D-R adsorption isotherm equation (9) is expressed as follows:

$$lnX = lnX_m - K\varepsilon^2 \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (9)$$

$\varepsilon$ in the equation depicts the Polanyi potential and found by the following equation (10):

$$\varepsilon = RT\ (1 + \frac{1}{C_e}) \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (10)$$

where, $X$ depicts adsorbed substance amount per unit adsorbent mass (mg/g), $X_m$ is theoretical adsorption capacity (mmol/g), $C_e$ is metal concentration at the equilibrium (mg/l), $K$ is the constant connected to the adsorption energy, $R$ is the general gas constant and $T$ is the temperature in Kelvin. A linear graph is plotted between $lnX$ and $\varepsilon^2$. The values are found using the slope ($K$) and the intersection ($X_m$). Average sorption energy (E) is the free energy change during one mole of the solid in the solution is transferred to the surface and calculated with equation (11):

$$E = -(2K)^{-1/2} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (11)$$

$E$ value is used to estimate the mechanism of generated reaction. If E value is between 8 and 16 kj/mol, then ion exchange mechanism is active. If $E$ is < 8 kj/mol, then physical forces could affect the reaction mechanism. When $E$ is>16 kj/mol, particle diffusion is dominant in the sorption. Data for Freundlich, Langmuir, and D–R isotherms are given in Table 6.

When adsorption models are assessed, it could be observed that the most suitable model is the Freundlich isotherm model ($R^2 = 0.9903$). In addition, EDR (kj/mol) > 16 means that particle population is dominant in this sorption study. The Freundlich isotherm constant (n=0.4423) of 0 < n < 10 shows that adsorption conditions are fulfilled.

## Thermodynamic Parameters for *Sarcotragus foetidus* (Schmidt, 1862) Biosorption

To determine the thermodynamic parameters (ΔH, ΔS ve ΔG) of U (VI) biosorption on *S. foetidus* (Schmidt, 1862), biosorption processes were conducted for the values of primary parameters (pH, contact time, and U (VI) concentration) under optimum conditions and the temperatures between 20-60 °C.

Adsorption enthalpy was calculated with the Van't Hoff graphical plotting method (Figure 6). $\Delta H^0$ and $\Delta S^0$ values were calculated with the slope of the graph ($R^2 = 0.9918$) plotted between Kd and 1/T, $\Delta H^0$ value was calculated with the breakpoint value, and $\Delta S^0$ values were calculated with reference to the equation (12) below (Sert and Eral 2010)

$$lnK_d = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (12)$$

In this equation (12), $\Delta H^0(\frac{kj}{mol})$ and $\Delta S^0(\frac{j}{mol.K})$ depict the change in standard enthalpy and standard entropy, respectively, $R$ reflects the ideal gas constant ($8.314\frac{j}{mol.K}$), and $T$ depicts the absolute temperature $(K)$. In a specific adsorption process, Standard Gibbs energy $(\Delta G^0)$ is calculated with the following equation (13):

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \qquad \ldots\ldots\ldots\ldots\ldots (13)$$

Thermodynamic parameters obtained with U (VI) biosorption are presented in Table 7.

In the measurement of Gibbs Free energy change $(\Delta G^0$ conducted under optimum conditions, ΔG>0 at the equilibrium point means that the reaction no longer moved towards the products and had a tendency to move towards the inputs. Generally, free

**Table 6.** Langmuir, Freundlich and D–R constants for the adsorption of U (VI) on *Sarcotragus foetidus* (Schmith,1862)

| Langmuir isotherm | | | Freundlich isotherm | | | Dubinin-Radushkevich (D-R) isotherm | | | |
|---|---|---|---|---|---|---|---|---|---|
| *R²* | $n_m$ (mg/g) | *b* (L/mg) | *R²* | *n* (L/mg) | $K_F$ (mg/g) | *R²* | *K* (mol2.kj-2) | Xm (mol/g) | EDR (kJ/mol) |
| 0.9887 | 26.67 | 0.7515 | 0.9986 | 0.4423 | 10.2188 | 0,9903 | 0.0000027 | 0.12 | 430.53 |

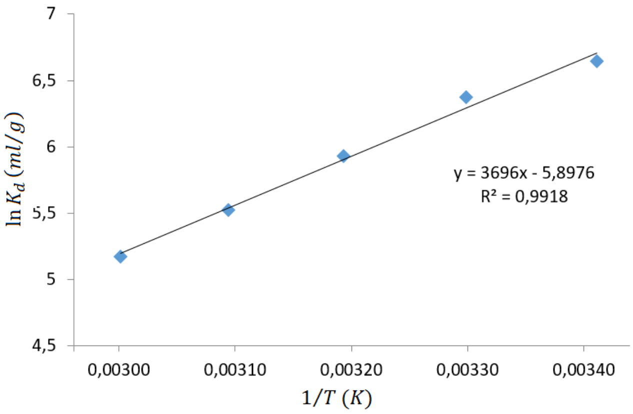

**Figure 6.** $lnK_d$ and $1/T$ curve for U (VI) biosorption on *Sarcotragus foetidus* (Schmidt,1862) ($X_1$:4, $X_3$:31, $X_4$:20, v:10 ml and m:0.01g).

**Table 7.** Thermodynamic parameters for U (VI) biosorption on *Sarcotragus foetidus* (Schmidt,1862)

| | ΔH° (kJ/mol) | ΔS° (kj/mol.K) | ΔG° (kj/mol) 298K | 303K | 308K | 313K | 323K |
|---|---|---|---|---|---|---|---|
| *Sarcotragus foetidus* (Schmidt,1862) | 30.73 | -0,49 | 174.39 | 179.29 | 184.20 | 189.10 | 194.01 |

energy change varies between -20 and 0 kj/mol in physisorption and between -80 and -400 in chemisorption. A negative enthalpy change shows a physical sorption that includes low gravitational forces and the sorption is exothermic. This demonstrates that the sorption process has stable energy. Negative entropy change $(\Delta S^{o} = -0.49\frac{kj}{mol.K})$ corresponds to a reduction in the degree of freedom of adsorbed types (Kavak 2009).

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