

## Antibacterial and Chemical Properties of Sr-BDC<sub>∞</sub> Metal-Organic Framework (MOF)

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### Abstract

**Introduction.** In this work, Sr-BDC MOFs were obtained by a simple solvothermal process without the use of elevated pressure. This method is easily scalable and does not require any special equipment. In this work, the crystals obtained from the synthesis were studied by Raman spectroscopy. In addition, the obtained materials were analysed for antibacterial activity against Gram-positive and against Gram-negative bacteria.

**Aim.** During this work, the main objective was to comparatively evaluate the antibacterial properties of Sr-BDC MOFs activated by different methods (and without activation).

**Materials and methods.** In this work we used a solvothermal process using terephthalic acid, strontium nitrate and dimethylformamide. The peculiarity of this method is the absence of autoclaving in the synthesis process. Optical microscopy and Raman spectroscopy were used for characterization. Also, to study the antibacterial properties, a medium diffusion test was performed. The combination of these methods will help to establish the relationship between the method of activation and the biological activity of the resulting materials.

**Results and discussion.** In this work, the chemical structure of Sr-BDC MOFs was studied by Raman spectroscopy. The influence of the activation method on the chemical structure of MOFs was studied. It was found that the characteristic peaks of Raman spectroscopy can be used to confirm the removal of solvent (DMFA) from the crystal structure. In addition, tests on the manifestation of antibacterial activity were carried out for MOFs with different activation method. The MIC and MBC were established for each sample.

**Conclusion.** In the course of the work the effect of the activation method on the chemical structure of Sr-BDC MOFs was shown. We also found that the activation method could affect the biological activity of the obtained MOFs. It was also demonstrated that MOFs exhibit different antibacterial activities depending on the type of bacteria, which can be primarily related to the composition of the cell wall of microorganisms.

**Keywords:** metal-organic framework (MOF), antibacterial properties, MOFs' characterization, Raman spectroscopy, Strontium, Sr-BDC MOFs, solvothermal method, terephthalic acid

**Conflict of interest.** The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

**Contribution of the authors.** Andrey A. Vodyashkin designed the course of the experiment. Andrey A. Vodyashkin, Joseph Arsene A. Mbarga and Maria Y. Putirskaya conducted the experiments (Andrey A. Vodyashkin – synthesis and post-synthetic modification. Maria Y. Putirskaya Raman spectroscopy. Joseph Arsene A. Mbarga – antibacterial activity). Andrey A. Vodyashkin and Parfait Kezimana prepared the manuscript and author Y. M. Stanishevskiy proofread it. All the authors participated in the discussion of the results.

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## Изучение антибактериальных и химических свойств металл-органических координационных полимеров Sr-BDC<sub>∞</sub>

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## Резюме

**Введение.** В ходе данной работы был получен МОФ Sr-BDC с помощью простого сольвотермального процесса без использования повышенного давления. Данный способ является легко масштабируемым и не требует специального оборудования. В ходе этой работы кристаллы, полученные при синтезе, изучаются с помощью Рамановской спектроскопии. Кроме того, полученные материалы были проанализированы на антибактериальную активность по отношению к грамположительным и к грамотрицательным бактериям.

**Цель.** В ходе данной работы основной целью являлись сравнительная оценка антибактериальных свойств металл-органического соединения Sr-BDC, активированных различными способами (и без активации).

**Материалы и методы.** В данной работе применяется сольвотермальный процесс с использованием терефталевой кислоты, нитрата стронция и диметилформамида. Особенностью данного метода является отсутствие автоклавирования в процессе синтеза. Для характеристики использовали оптическая микроскопия и Рамановская спектроскопия. Также для изучения антибактериальных свойств, был проведен тест на диффузию в среду. Совокупность данных методов поможет установить взаимосвязь, между методом активации и биологической активностью получаемых материалов.

**Результаты и обсуждение.** В ходе этой работы изучено химическое строение МОФ Sr-BDC с помощью Рамановской спектроскопии. Проведено изучение влияния способа активации на химическое строение МОФ. Установлено, что с помощью характеристичных пиков Рамановской спектроскопии можно подтвердить удаление растворителя (ДМФА) из структуры кристалла. Кроме того, проведены тесты по проявлению антибактериальной активности для МОФ с различным способом активации. Для каждого образца установлены МИК и МБК.

**Заключение.** В ходе работы изучено влияние способа активации на химическую структуру металл-органической конструкции Sr-BDC. Установлено, что способ активации может влиять на биологическую активность получаемых кристаллов. Также продемонстрировано что к различным типам бактерий МОФ проявляют различную антибактериальную активность, что в первую очередь может быть связано с составом клеточной стенки микроорганизмов.

**Ключевые слова:** металл-органические соединения (МОФ), антибактериальные свойства, характеристика МОФ, Рамановская спектроскопия, стронций, Sr-BDC МОФ, сольвотермический метод, терефталевая кислота

**Конфликт интересов.** Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

**Вклад авторов.** А. А. Водяшкин разработал ход проведения эксперимента. А. А. Водяшкин, М. Дж. А. Мбарга и М. Ю. Путирская, провели эксперимент (А. А. Водяшкин – синтез и пост-синтетическая модификация. М. Ю. Путирская – рамановская спектроскопия. М. Дж. А. Мбарга – антибактериальная активность). А. А. Водяшкин и П. Кезимана подготовили статью, и автор Я. М. Станишевский вычитал ее. Все авторы участвовали в обсуждении результатов.

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## INTRODUCTION

Currently, there are actively developing directions for the development of various materials [1, 2]. It is especially worth mentioning the possibility of creating functional materials that can be applied to various spheres of life activities [3, 4]. The biological activities of various materials can be actively used in medicine, biology, and the creation of hybrid devices [5].

Various polymeric materials have been actively used in the biomedical field [6]. It can be used to create devices, modify surfaces, as implants, and for other purposes [7]. In recent years, methods for applying metal nanoparticles [8], intermetallides, and polymers [9] to solve the problems of various spectra in the biomedical industry have become relevant.

It is also worth noting that in recent years, the topic of creating various metal-organic coordination polymers (MOFs) [10, 11] has intensified. Currently, these methods allow for a variety of systems that use different ligands and metals [12]. Of particular importance are MOFs that use biocompatible metals in their structure [13]. Such

MOFs can be successfully used in the near future to treat and diagnose various diseases, as well as to create hybrid devices [14, 15]. MOFs have a relatively high specific surface area, which suggests high potential for their use as sorbents for *in vivo* and *in vitro* applications. In addition, there is have potential applications as carriers of biologically active substances [16].

Bacterial diseases are among the most common [17]. Simultaneously, new and increasingly dangerous microorganisms with resistance to antibiotics have emerged owing to evolution [18]. MOFs can be used both as antibacterial agents and carriers in hybrid systems [19]. Therefore, studying the effects of different materials on microorganisms is an important and urgent task.

## MATERIALS AND METHODS

### Research materials

All reagents used for the preparation of Sr-BDC MOFs were of analytical grade. During the synthesis we used N,N-dimethylformamide (DMFA) solvent imp.

(C.P.) JSC "VEKTON"; strontium chloride 6-water (P) ( $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ ), "LenReactiv", mass fraction not less than 99.7 %; terephthalic acid, 98 %, "Acros Organics"; ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ), mass fraction not less than 98 %; dimethyl sulfoxide imp. (C.P.) JSC "VEKTON", Doxorubicin (DOX) produced by "BRYNZALOV-A" (Russia).

### Synthesis of Sr-BDC MOFs

The organometallic compound Sr-BDC was prepared according to the following procedure: in the solvothermal synthesis of Sr-BDC, strontium nitrate (0.038 g, 0.180 mmol), terephthalic acid (0.05 g, 0.301 mmol) and 7.2 ml of N,N-dimethylformamide solvent were used as precursors, placed in heat resistant glass tubes, and stirred, then incubated at 120 °C for 30 h. After cooling to room temperature, the synthesized transparent crystals were washed repeatedly with DMF (5 mL) and dried at 120 °C for 24 h.

The synthesis was carried out using DMFA solvent in closed tubes, and this system is naturally not a closed system, but the loss of solvent (about 40 %) in the process did not have a significant impact on the synthesis. It should also be noted that this is a heterogeneous process and Sr-BDC MOFs, after formation, "fall out" of the system into a precipitate, and the process of crystal formation ends before the solvent evaporates.

The obtained Sr-BDC samples were further processed to remove the residual DMFA solvent from the pores of the obtained crystals.

### Post-Synthetic Modification

Post-synthetic modification is a special step in the production of Sr-BDC. This step made it possible to treat the obtained MOFs and provide them with the necessary properties. In this study, Sr-BDC MOFs are planned for use in biomedical applications, namely, for the delivery of drug molecules. The ability to incorporate the maximum amount of biologically active substances is an important factor in the delivery of drug compounds. After solvothermal synthesis, due to the forces of adsorption, pressure, and non-covalent interactions, traces of solvent can remain in the pores of MOFs, which reduces the loading capacity of MOFs and can be unsafe for biomedical applications. Elevated temperatures can be used to remove solvents, which are not always available and do not allow all solvents to be removed. Selecting special solutions into which the solvent will be desorbed is an easy and safe way to remove it from the pores.

For the developed system, a method for removing DMF using ethanol was proposed. After solvothermal synthesis, the obtained samples of Sr-BDC(DMF) dried at 120 °C for 4 h were immersed in 10 ml of ethanol and incubated under constant stirring. After 4 h the solution was decanted, the white crystals were dried at 60 °C for 3 h to remove the ethanol (Sr-BDC)

Heating to a temperature approximately equal to the boiling point of the solvent is often used for such systems, so we used heating to 260 °C for 4 hours as an alternative heating method (Sr-BDC (260 °C))

### Raman spectroscopy

Raman spectroscopic studies were recorded using a specially made Raman spectrometer with 150/500 grating, solid-state laser model LM473 with wavelength 473 nm, accumulation time 10 seconds, setup of signal registration by CCD camera.

### Antibacterial activity

**Well diffusion method** – The well diffusion method was used to assess the antimicrobial activity of the three solutions (Sr-BDC, Sr-BDC(DMF), and Sr-BDC (260 °C)). First, concentrations of 1 mg/ml were prepared by dissolving the MOFs samples in distilled water. The 3 solutions were sterilized by microfiltration with sterile filter membranes of 0.22 μm. Subsequently, three cultures of microorganisms were prepared in Brain Heart infusion broth (for *E. coli* ATCC 25922 and *S. aureus* ATCC 6538) and Sabouraud Dextrose broth (for *C. albicans* ATCC 10231). After 24 h of incubation at 37 °C, the strains were centrifuged (6000 rpm for 10 min) and then re-dissolved in sterile Phosphate Buffer Saline (PBS) at 0.5 McFarland scale (approximately  $1.5 \times 10^8$  CFU/ml). The strains were plated in Petri dishes containing sterile Muller–Hinton agar (for *E. coli* ATCC 25922i and *S. aureus* ATCC 6538) and Sabouraud Dextrose Agar (for *C. albicans* ATCC 10231). Wells with a capacity of 20 μL were then perforated on agar, and 20 μL of each solution was added. One antibiotic disk and one antifungal disk were used as positive controls, while distilled water used to prepare the solutions was used as a negative control. Inhibition diameters were observed after 24 h of incubation at 37 °C.

**Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination:** MIC and MBC were determined by microbroth dilution method in a 96-well microplate. Sabouraud dextrose broth (SDB) (*C. albicans* ATCC 10231) was used for the fungus, whereas brain Heart Infusion broth (BHIB) was used for the bacteria (*E. coli* ATCC 25922 and *S. aureus* ATCC 6538). The method used was that used in our previous study, without any modifications. Briefly, the culture broths (100 μL) were introduced into all wells of the microplates, and 100 μL of the test solutions (Sr-BDC, Sr-BDC(DMF), and Sr-BDC (260 °C)) was introduced into the first line according to previously described labelling. A two-fold dilution was performed, and the excess of the last line was discarded to obtain an identical volume (100 μL) in each well. Each microorganism was added to three columns, each containing the culture medium and one of the test solutions. The plates were then covered and incubated for 24 h at 37 °C. Moving downwards for each test solution, the

last dilution at which no visible growth was observed was defined as the MIC. Subsequently, the lines on which no growth was observed were further inoculated into the solid culture medium after labelling the Petri dishes. The final concentration at which no growth was observed after further incubation of the Petri dishes at 37 °C for 24 h was considered as the MBC.

## RESULTS AND DISCUSSION

### Synthesis of Sr-BDC MOFs

Strontium metal-organic frameworks were synthesized using DMF. During the solvothermal synthesis, a 6-water strontium chloride salt was used in the reaction; the water reacting chemically with DMFA formed strong heteromolecular associations with the help of hydrogen bonds. After cooling to room temperature, the synthesized transparent crystals were washed repeatedly with DMF (5 mL) and dried at 120 °C for 24 h (Figure 1).



**Figure 1.** The obtained Sr-BDC MOF crystals dried at 120 °C for 24 h

### Raman spectroscopy

Figure 2 shows the Raman spectra of Sr-BDC MOFs with different activation modes.

Peaks in the region of 1000-1500  $\text{cm}^{-1}$  can be associated with C—C or C—H bending oscillations as well as stretching and bending oscillations of carboxylates in the terephthalic acid molecule. The peaks around 350–420  $\text{cm}^{-1}$  can be related either to lattice vibrations or to vibrations of the metal-ligand coordination bond. C—H valence oscillations around 3100  $\text{cm}^{-1}$  were observed in all samples, regardless of the activation method. In addition, the peak at approximately 1610  $\text{cm}^{-1}$  indicates the presence of an aromatic group in the crystal structure, which confirms the stability of the MOFs, regardless of the activation method. The peak at approximately 280  $\text{cm}^{-1}$  observed in the samples after activation confirms the successful removal of the solvent from the crystal structure, as previously stated [20].

Notably, the wave-like structures of the spectra indicate a high level of fluorescence within the MOFs structure, which is associated with the presence of organic groups in the structure [21]. It is worth noting that when

using activation with ethanol, this effect is seen to a lesser extent, which may also indicate the most successful activation of MOFs.

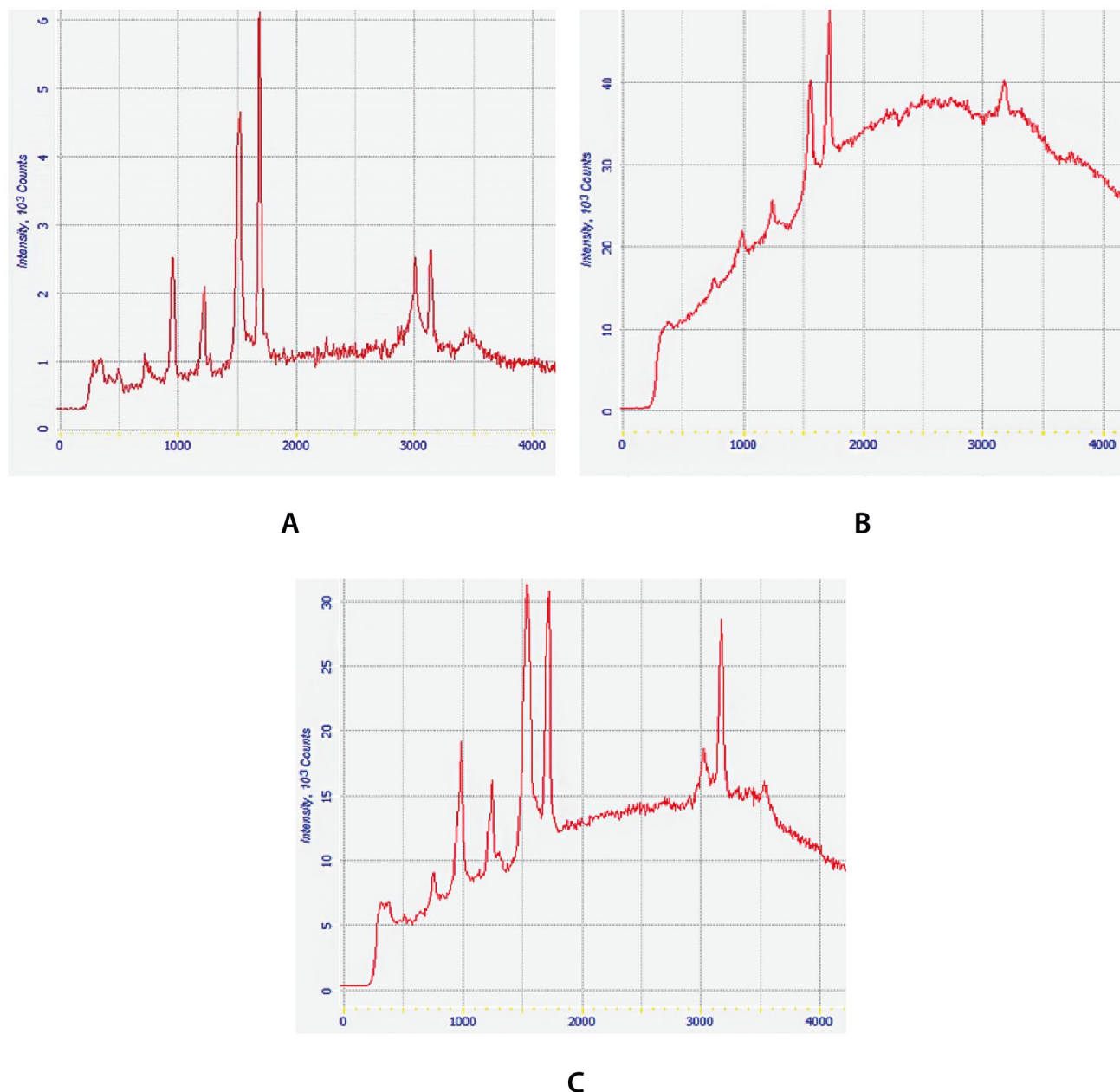
The oscillations observed in the peaks are related to the stretching of the CH— in the acid molecule and are encompassed by a broad peak around 3100  $\text{cm}^{-1}$ , which reflects similar bonds resulting from the inter-frame interactions. The peak at 3000  $\text{cm}^{-1}$  appears due to the activation process and the MOFs interacting with temperature and ethanol. As a result of this interaction, a vibrational or electronic band splitting is observed in the molecular crystals, which is related to the difference in the strength of the C—H bonds.

In addition to Raman spectroscopy, we intend to use other methods of physico-chemical analysis, such as IR spectroscopy, X-ray diffraction, thermal analysis for a complete description of our obtained MOFs.

### Antibacterial Activity

In the present study, the antimicrobial activity evaluation of the three solutions of MOFs – Sr-BDC, Sr-BDC(DMF), and Sr-BDC (260 °C) at 1mg/ml (obtained by diluting the crystals in distilled water) by the well diffusion method revealed that none of these solutions exhibited an inhibition zone against *E. coli* ATCC 25922, *S. aureus* ATCC 6538 and *C. albicans* ATCC 10231. This result was clearly observed, given that the inhibition diameters exhibited by the positive controls consisting of tetracycline were very large (25 mm and 32 mm, respectively, against *E. coli* ATCC 25922 and *S. aureus* ATCC 6538). The same observation was made with fluconazole as a positive control against *C. albicans* ATCC 10231, in which an inhibition diameter of 22 mm was observed. As expected, no inhibition zone was observed in the test with distilled water (negative control). However, as suggested by Choyam et al. [22], the absence of an inhibition zone does not automatically mean that the tested molecules have no antimicrobial activity. Choyam et al also explained that the results of such indirect methods (well diffusion method) do not necessarily correlate with antimicrobial activity [22]. Thus, we determined the minimum inhibitory concentration (MIC) and minimum bactericidal (fungicidal) concentrations (MBC or MFC). Interestingly, it was found that the 3 test solutions inhibited the growth of all the three microorganisms at the first or second dilution, which indicated that the MICs for all of them were 0.25 mg/ml or 0.5 mg/ml. More specifically, as shown in Table 1, against *E. coli* ATCC 25922 the MICs were 0.5, 0.25 and 0.25 mg/ml for Sr-BDC, Sr-BDC(DMF), and Sr-BDC (260 °C) respectively. *S. aureus* ATCC 6538 was the most susceptible strain against all the test solutions, since all of them showed an MIC equal to 0.25 mg/ml; this was confirmed by the MBC, since the only MBC determined was against this strain (MBC = 0.5 mg/ml), while the other MBCs were higher than the highest dilution that was tested (MBC > 0.5 mg/ml).





**Figure 2.** Raman spectroscopy:  
A – Sr-BDC; B – Sr-BDC (DMFA); C – Sr-BDC (260 °C)

**Table 1.** MIC, MBC, and MFC of MOFs' solutions against *E. coli* ATCC 25922, *S. aureus* ATCC 6538, and *C. albicans* ATCC 10231.

	Sr-BDC		Sr-BDC(DMF)		Sr-BDC (260°C)	
	MIC	MBC (MFC)	MIC	MBC (MFC)	MIC	MBC (MFC)
<i>E. coli</i> ATCC 25922	0.5	>0.5	0.5	>0.5	0.25	>0.5
<i>S. aureus</i> ATCC 6538	0.25	0.5	0.25	>0.5	0.25	>0.5
<i>C. albicans</i> ATCC 10231	0.5	>0.5	0.5	>0.5	0.5	>0.5

As a comparison (Table 2), we also looked at different antibacterial activity of other MOFs against Gram-negative and Gram-positive bacteria by well diffusion method and their MIC are provided in the following table.

It should be noted that the MOFs used in our comparison were mixed with the antibacterial agent (the first name in the compound), which has an influence on their antibacterial activity. As we can see in the table, the Zn-based MOFs, IRMOF-3 and MOF-5, and the silver-based Ag-MOF, when mixed with the

antibacterial agents, ampicillin or kanamycin or graphene oxide, show a high antibacterial efficiency compared to our Sr-BDC MOFs.

**Table 2.** Comparison of MIC value of Sr-BDC MOFs with other compounds

Compound	MIC (µg/mL)		References
	<i>E. coli</i>	<i>S. aureus</i>	
Sr-BDC	500	250	This work
Sr-BDC(DMF)	500	250	
Sr-BDC (260 °C)	250	250	
Kanamycin/MOF-5	100	100	[23]
Kanamycin/IRMOF-3	25	50	[23]
Graphene-oxide/Ag-MOF	50	–	[24]
Ampicillin/IRMOF-3	50	100	[23]
Ampicillin/MOF-5	100	100	[23]

In the work we are trying to demonstrate the biological activity of our system against bacteria. At the same time, we want to highlight the possibility of loading the MOF with different drugs that can increase the antibacterial activity, as shown in the comparison in Table 2.

## CONCLUSIONS

In this study, without the use of mechanochemical treatment or elevated pressure, the metal-organic framework Sr-BDC has been successfully synthesized. The presence of an aromatic group in the crystal structure, indicating that the strontium ions are coordinated by the terephthalic acid molecule, was confirmed by Raman spectroscopy. In order to remove the solvent residues from the structure, a post-synthetic modification synthesis using ethanol and heating was proposed for these crystals. In addition, the antibacterial properties were investigated for all samples. MIC and MBC were determined for *E. coli*, *S. aureus* and *C. albicans*. It is noteworthy that the antibacterial assay demonstrates the need for customization of conditions for each biological object to optimize exposure (lack of exposure). This study shows the importance of the post-synthetic modification and, in particular, of the activation steps for different materials used in biomedical applications.

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