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INVESTIGATION OF ANTIMICROBIAL PROPERTIES OF GRAPHENE BASED MATERIALS IN LIQUID AND SOLID MEDIUM

Merve KAS[*1] & Sibel YIGITARSLAN[2]

[*1,2]Department of Chemical Engineering, SuleymanDemirel University, Isparta, Turkey

ABSTRACT

Graphene and its derivatives named as rising star because of its unique properties such as electrical and thermal conductivity, mechanical and physical properties. Graphene has a lot of application area such as biomedical, biotechnology, physic, engineering. In biotechnology and biomedical there is some important expectation from materials as antimicrobial effect. Definition of antimicrobial effect, materials has to be kill microorganisms cells or broke some life functions. In biotechnology area there are some studies about graphene's antimicrobial-antibacterial property. Graphene and graphene metarials called as antimicrobial material, in fact there is microorganism growth. In this study some experiments were done with solid graphene and different graphene oxide concentrations to eliminate the contradiction between the given definition and the work done.

Keywords*: Graphene, Graphene-oxide, Antimicrobial, EscherichiacoliNissle 1917, Lactobacillusacidophilus.*

I. INTRODUCTION

Graphene is firstisolatedfromgraphite as a twodimensionalsimplematerial**[1].**It is one of thecarbonalloltropesshows in Fig.1 which is occurssinglelayer of carbonatoms**[2].**

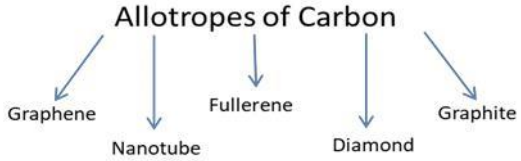

Figure1: Carbonallotropesexamples

In more detail; graphene occurs if carbon atoms are arranged in hexagonal structure in one sheet shows in Figure 2.

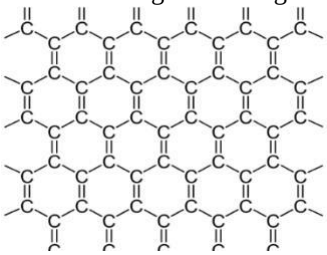

Figure 2: Carbon atoms hexagonal structure

After its isolation, many research groups started to investigate graphene properties. With its unique properties graphene has a large of application area, despite its short history. When graphene properties checked over, their thermal conductivity (5000 W/m.K) can compete with graphite [3-10]. Because of its high electron mobility and remarkable electrical conductivity (1738 siemens/m) it can be defined as semimetal semiconductor [4-8]. It has perfect mechanical strength (1100 gPa) and mechanical performance as know so far for the engineering and physic area [5-6-9].

Also newly produced graphene based materials especially tried in biotechnology due to their physicochemical matchless properties [11-12]. Graphene oxide (GO)(used in this study) is modified graphene sheets. Its consist of hydroxyl, epoxy and carbonyl groups obtained from oxidizing agents [13].

In biotechnology area, materials antimicrobial activity is important to use in sterilization process. Antimicrobial agents exists in different classes and property of molecules. In order for a material to be named antimicrobial, it must be able to disrupt the functioning of the microorganism and causes its death. Some agents effect mechanisms is so sensitive and still unknown but its predicted that it should be close to cell membrane its operation. To assign that cell damage is very difficult depend on cell membrane complexity [14]. In these days, research groups investigate graphene based materials antimicrobial effect.

In these days, research groups investigate graphene based materials antimicrobial effect. One of these studies, 5 wt% graphene that is produced by Hummers method was used. After production, characterization was done by using XRD, FTIR analysis. XRD analysis shows that graphite powder has a strong sharp peak at 26 o and interlayer spacing about 0.335 nm. Graphene oxide samples shows that witch oxidation a formation of a new board peak at 10.92 o with interlayer spacing about 0.809 nm. FTIR used for finding bound types. According to the FTIR analysis results observed there is hydrophobic side in grapheme oxide samples. That further explains the most important property of grapheme oxide in biotechnology area is hydrophobicity. As a result of that study, SEM images shows that there is survived microorganism on grapheme oxide plack and they said GO has perfect antimicrobial effect and its more effective on gram-positive bacteria. Although they saw microorganism growth both solid and liquid phases [15].

In another study research group investigated antibacterial effect of GO nanowalls with one gram-negative and one gram-positve bacteria. They produced that nanowalls by using electrophoretic deposition method. After characterization SEM images shows that, these GO nanowalls has so sharp edges. As a result of that study, it has been stated that these surfaces are antibacterial due to fact that the cells contacting with sharp surfaces of the nanowalls. Very sharp edges of walls killed the bacteria [16].

Given the complications of the meaning in the literature, the purpose of study determined as microorganism growth on solid surfaces produced by chemical etching method and in liquid medium with graphene oxide was examined.

II. METHOD & MATERIAL

Two different bacteria used in this study. Gram positive bacteria representative Lactobacillus acidophilus and gram negative bacteria representative *Escherichia coli Nissle 1917*. In order to examine the antimicrobial properties in liquid medium, 6 different concentrations graphene-oxide solutions (5 µg GO/ml, 40 µg GO/ml, 50 µg GO/ml, 100 µg GO/ml, 200 µg GO/ml, 400 µg GO/ml) were used. For solid medium experiments two solid graphene surfaces which are created by Chemical Etching method were used in this study.

Escherichia coli Nissle 1917 is mesophilic bacteria and growth in Tryptic Soy Broth (TSB). For prepare 1 liter TSB, 17 gram pepton, 3 gram peptone from soy meal, 2.5 gram D-Glucose, 5 gram NaCl and 2.5 gram K_2HPO_4 were added to 1 liter pure water and mixed well. After mixing process medium maintained at 37^oC temperature in incubator.

Lactobacillus acidophilus is mesophilic bacteria too and growth in MRS Broth. 10 gram peptone, 8 gram meat extract, 5 gram D-glucose, 4 gram yeast extract, 2 gram K_2HPO_4 and 1 mL Tween-80 were mixed with 1 liter pure water and left to rest in incubator.

Firstly, the optimum growth time was determined. Each microorganism put into to 200 ml liquid medium. At the same time, hourly measurements were taken by spectrophotometric analysis at 600 nm wave length.
S-plot graphs were drawn (Figure 3 and Figure 4) for each microorganism by using Abs values obtained as a result of analysis (Table 1. and Table 2.).

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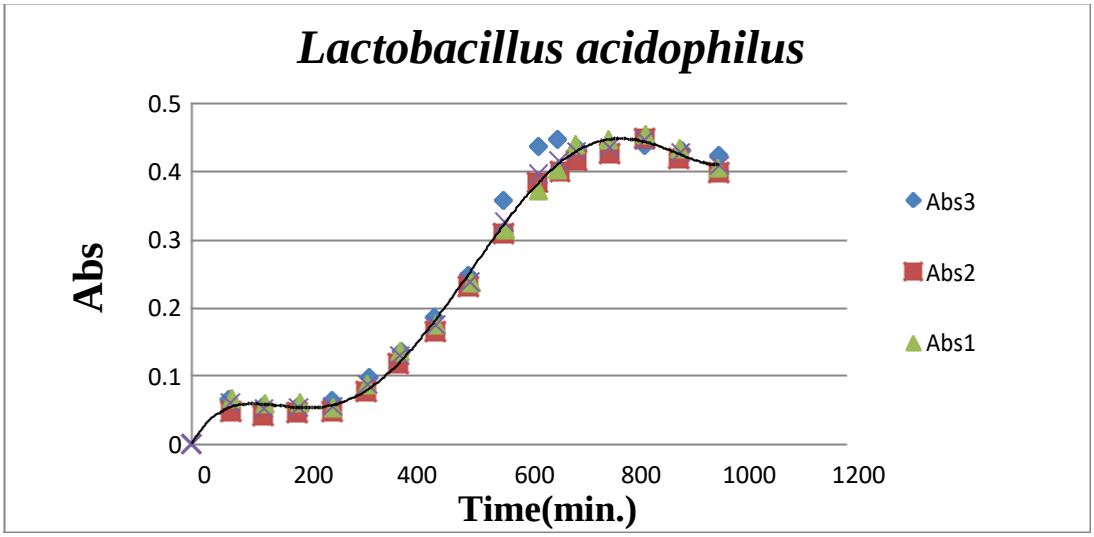

Figure 3: Lactobacillus acidophilus S-plot

Table 1. Lactobacillus acidophilus spectrophotometric results for optimum time

Time(min.)	Abs1	Abs2	Abs3
69	0,0657	0,0476	0,0675
131	0,0559	0,041	0,06
195	0,0554	0,0455	0,0617
257	0,0638	0,0464	0,0535
325	0,0979	0,0768	0,0882
384	0,1367	0,117	0,1354
445	0,1864	0,164	0,1755
507	0,2475	0,2309	0,2372
572	0,3572	0,3072	0,3151
636	0,4359	0,3833	0,3721
671	0,4464	0,3986	0,4031
707	0,4334	0,4159	0,44
767	0,4347	0,4247	0,4463

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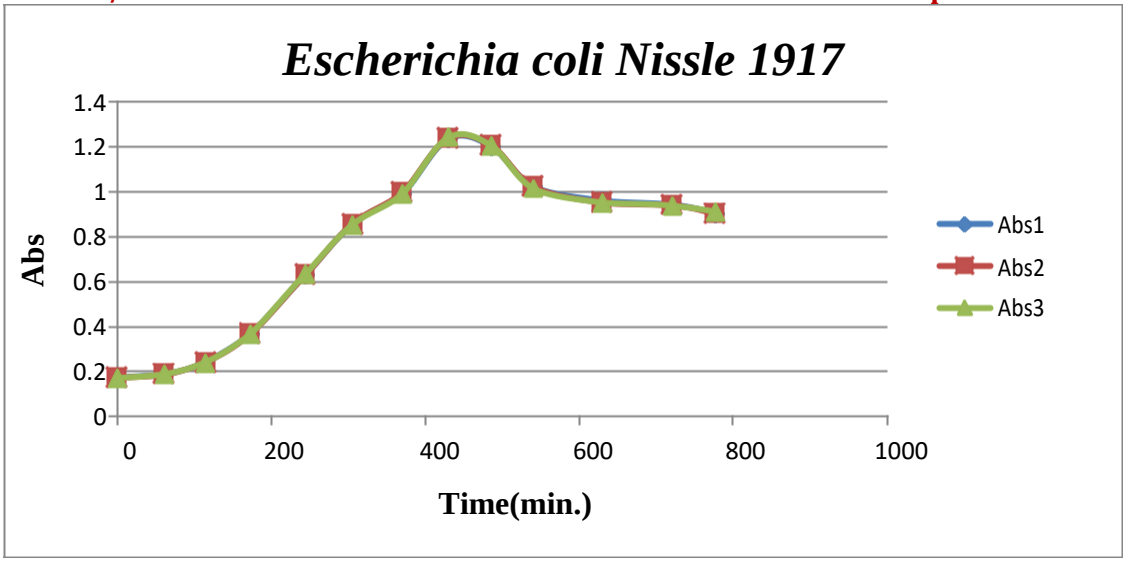

Figure 4: Escherichia coli Nissle 1917 S-plot

Table 2. Escherichia coli Nissle 1917 spectrophotometric results for optimum time

Time(min.)	Abs1	Abs2	Abs3
0	0,1728	0,1721	0,171
61	0,187	0,186	0,1862
115	0,2426	0,2398	0,2398
173	0,3672	0,366	0,3682
245	0,629	0,6295	0,6332
306	0,8531	0,8555	0,8526
370	0,9916	0,9974	0,9908
430	1,2343	1,2372	1,2422
486	1,2011	1,2051	1,2032
541	1,0215	1,0217	1,015
631	0,9583	0,9507	0,9527
721	0,9425	0,9392	0,9374
777	0,9018	0,9008	0,9093

Then according to S-plot graphs optimum time were calculated by using Equation 1.
$T=(G+D)/2$ (1)

At this equation T is represent optimum time, G is the point where the microorganism begins to grow and D is the point where the microorganism begins to die. The minimum time required for this experiment is predicted by the values obtained from this equation.

According to Figure 3.*Lactobacillus acidophilus* optimum time requirement found as 930 minutes. When Figure 4. was examined *Escherichia coli Nissle 1917* bacteria optimum time requirement is 340 minutes. For liquid medium experiments 1 ml microorganisms were added to 200 ml mediums and 2 ml graphene oxide solutions. For comparison, the control group consisted of only medium and microorganisms were used. All medium left to rest along minimum optimum growth time and every hour spectrophotometric analysis were noted. After incubation time with spectrophotometric results s-plot graphs were draw for every concentrations graphene oxide and control group. For solid medium experiment, graphene placks were used which is produced by chemical entching method. Added mediums with grown microorganism over the entire surface of solid surfaces. For *Escherichia coli Nissle 1917* bacteria surfaces incubated 340 minutes and for *Lactobacillus acidophilus* bacteria solid surfaces incubated 930 minutes. After optimum time, surfaces were examined with colony counter device.

III. RESULT & DISCUSSION

When the experiments were done, s-plot graphs were drawn according to spectrophotometric results.*Lactobacillus acidophilus* trails were complete for liquid medium, 6 different grapheme oxide experiments results compared with control group which is shows in Figure 5.

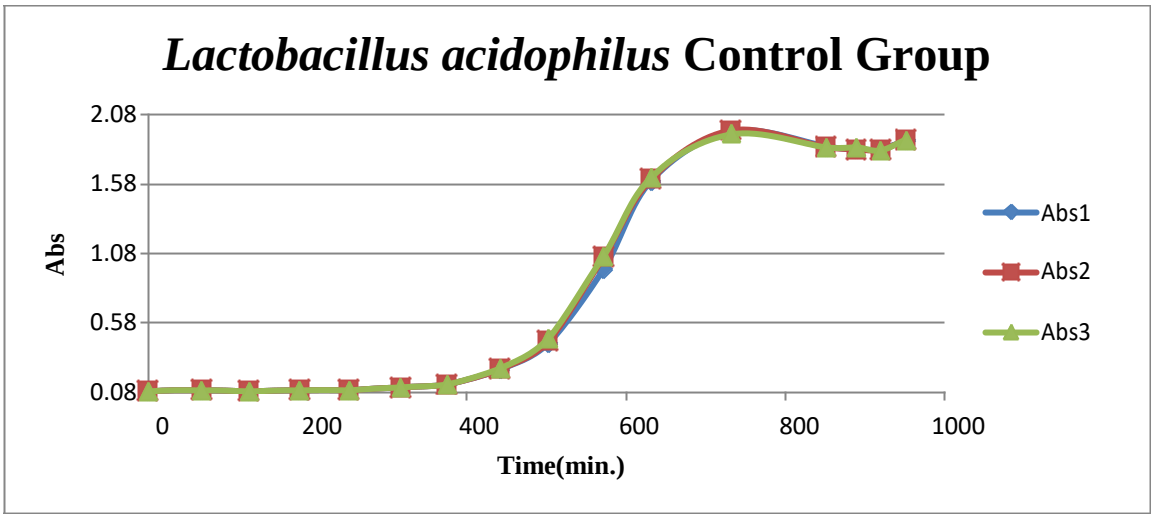

Figure 5: Lactobacillus acidophilus control group

When 5 µg GO/ml (Figure 6) trail compared with control group, their Lag phase nearly similar but control group lag phase bigger than 400 µg GO/ml (Figure 7) concentration trail.

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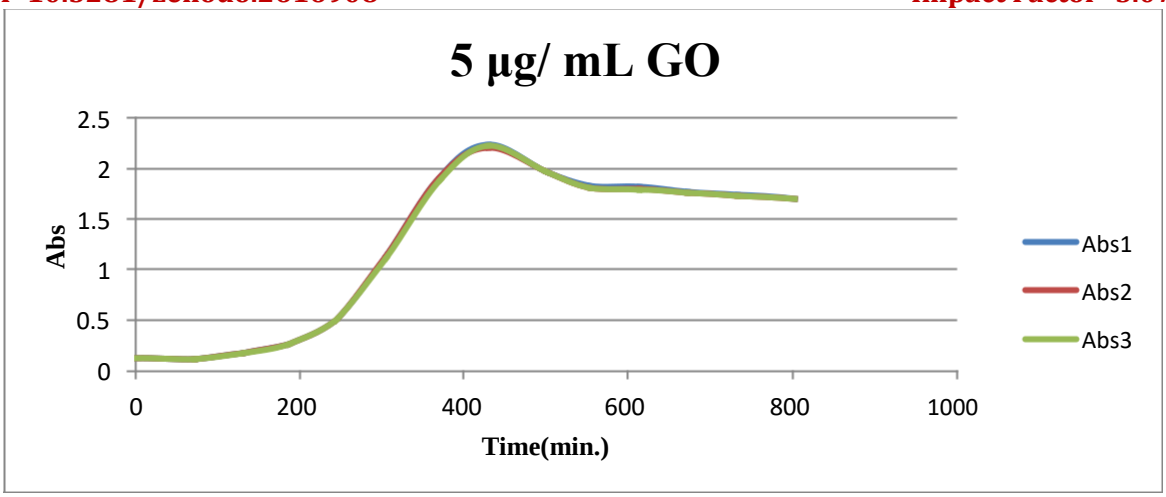

Figure 6: Lactobacillus acidophilus 5 µg GO/ml trail

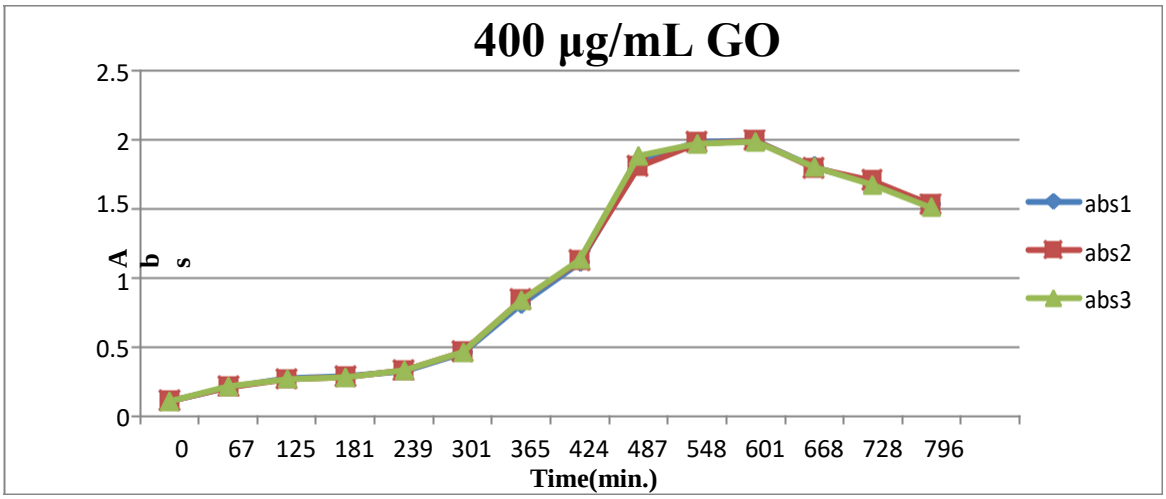

Fig.7 Lactobacillus acidophilus 400 µg GO/ml trail

When 50 µg GO/ml (Figure 8) and 40 µg GO/ml (Figure 9) compered about getting maximum microorganism concentration, clearly seen 40 µg GO/ml bacteria concentration bigger than 50 µg GO/ml.

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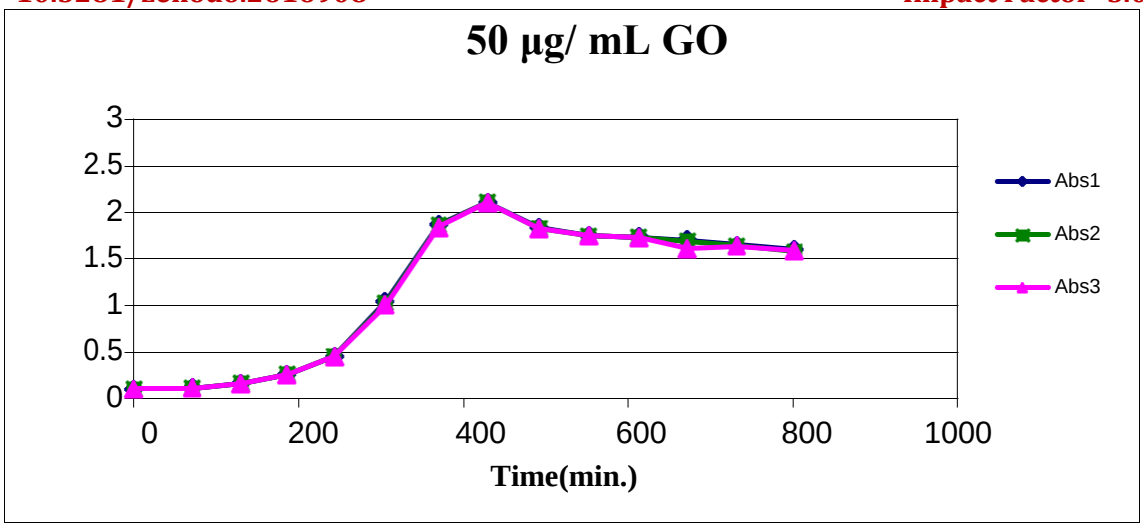

Figure 8: Lactobacillus acidophilus 50 µg GO/ml trail

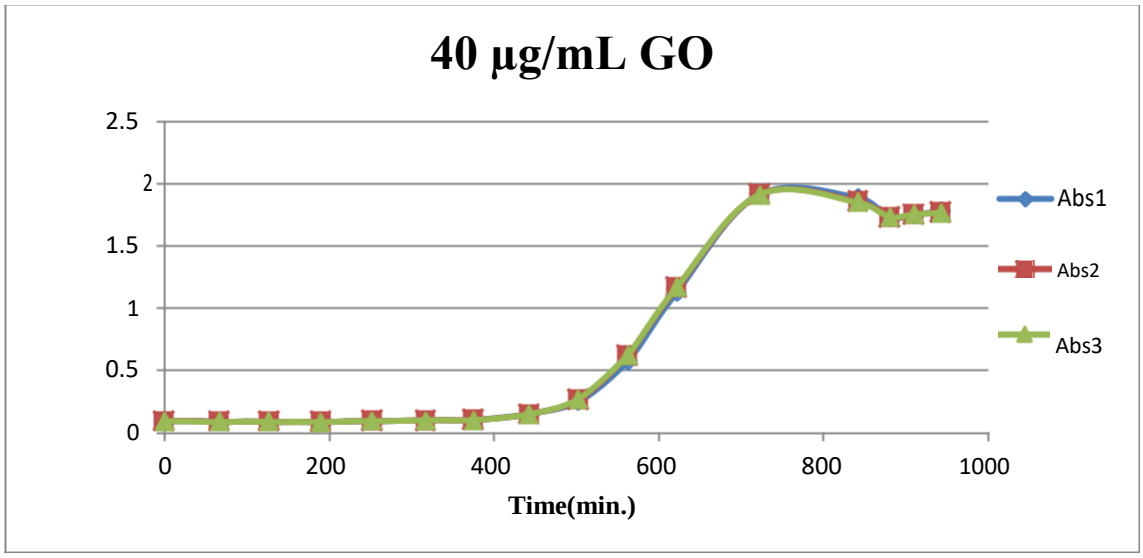

Fig.9 Lactobacillus acidophilus 40 µg GO/ml trail

According to control group graph, 200 µg GO/ml (Figure 10) and 100 µg GO/ml (Figure 11) they have lag phase too and 100 µg GO/ml lag phase time bigger than 200 µg GO/ml.

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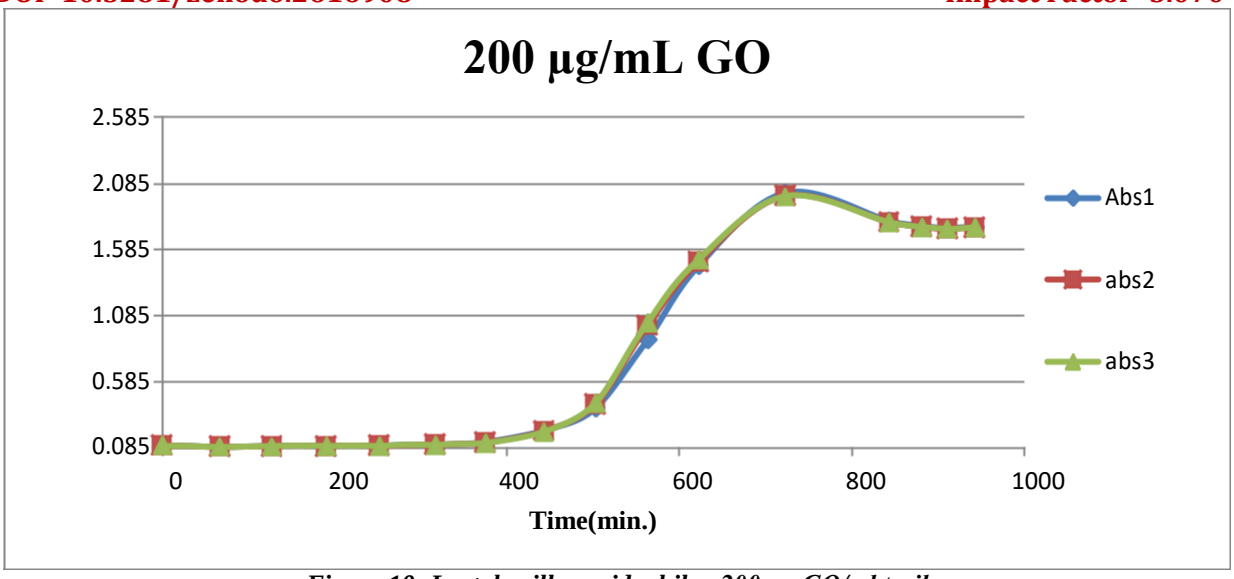

Figure 10: Lactobacillus acidophilus 200 µg GO/ml trail

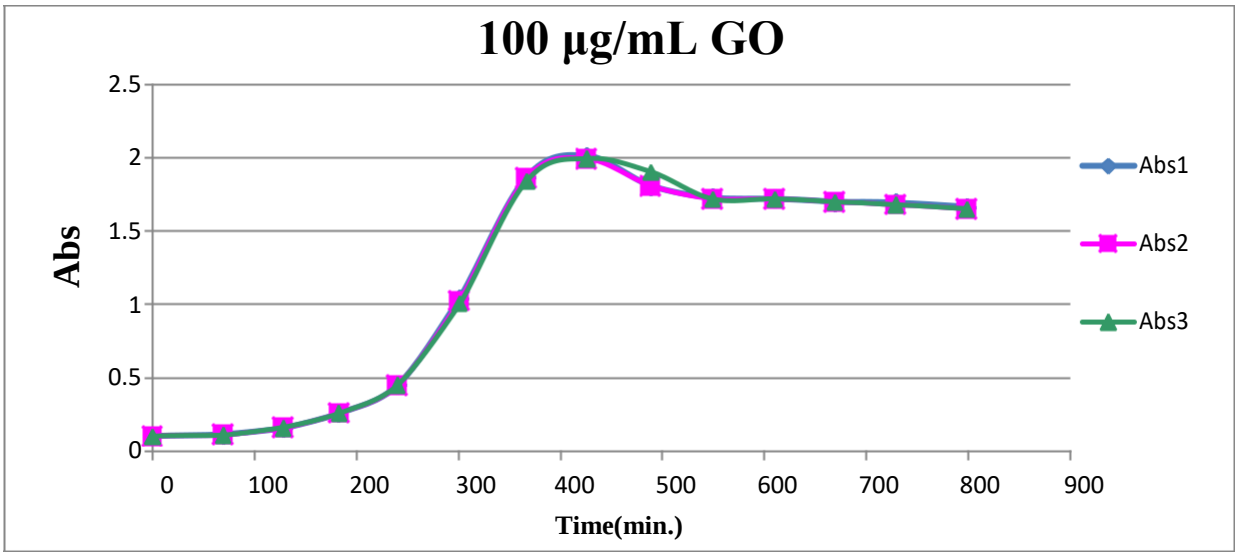

Figure 11: Lactobacillus acidophilus 100 µg GO/ml trail

When *Escherichia coli Nissle 1917* liquid medium control group (Figure 12) were evaluated, maximum bacteria concentrations was 1.2588 Abs and maximum microorganism concentration time requirement was 651 minutes.

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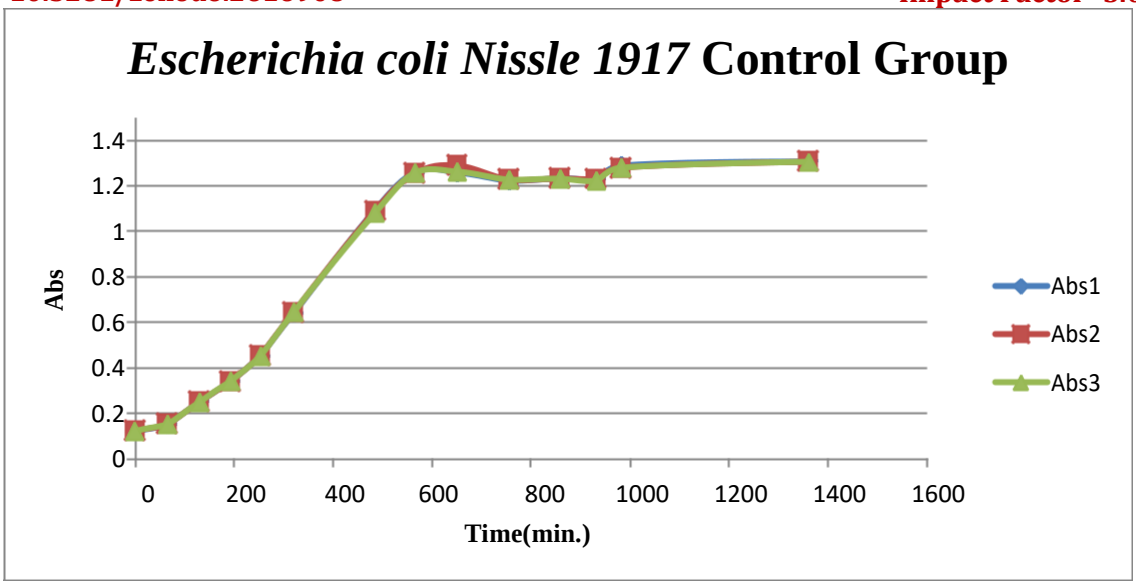

Figure 12: Escherichia coli Nissle 1917 Control group s-plot graph

200 µg GO/ml (Figure 13) and 400 µg GO/ml (Figure 14) concentrations have same maximum microorganism time requirement but 200 µg GO/ml solutions has bigger concentration than which is 0.8695 Abs value.

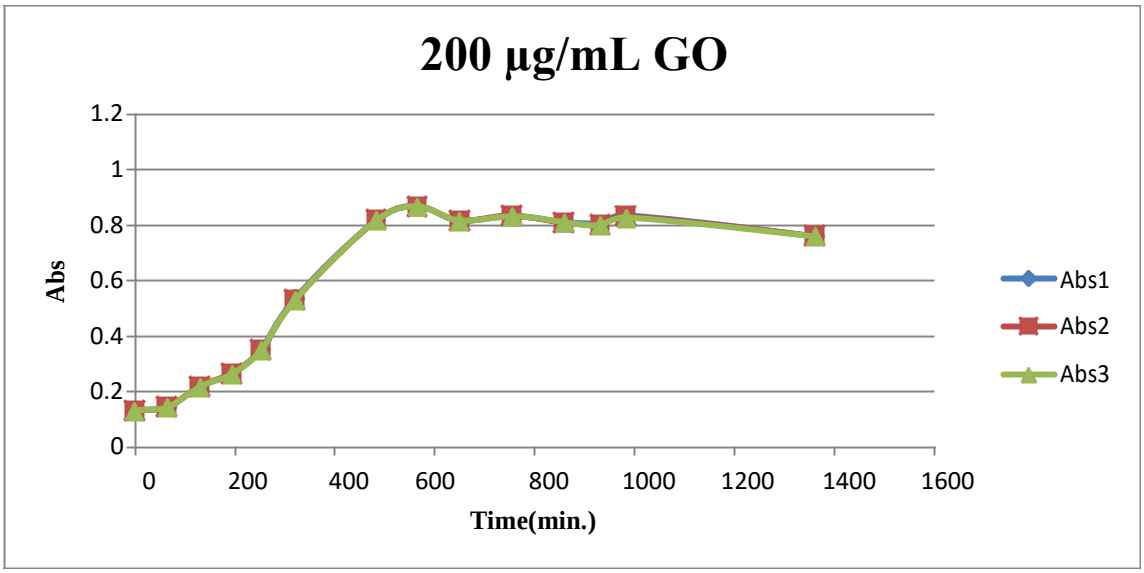

Figure 13: Escherichia coli Nissle 1917, 200 µg GO/ml trail

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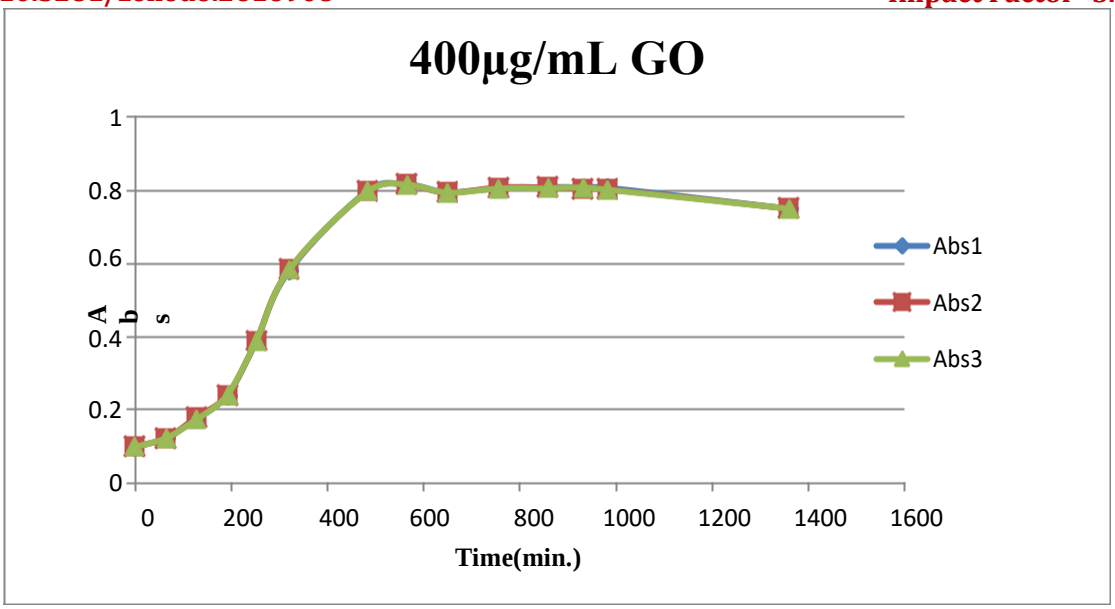

Figure 14: Escherichia coli Nissle 1917, 400 µg GO/ml trail

Highest concentration after control group observed as 5 µg GO/ml (Figure 15) concentration which is 1.0969 Abs value. 100 µg GO/ml (Figure 16) and 50 µg GO/ml (Figure 17) samples have same time requirement and maximum concentration basically same value.

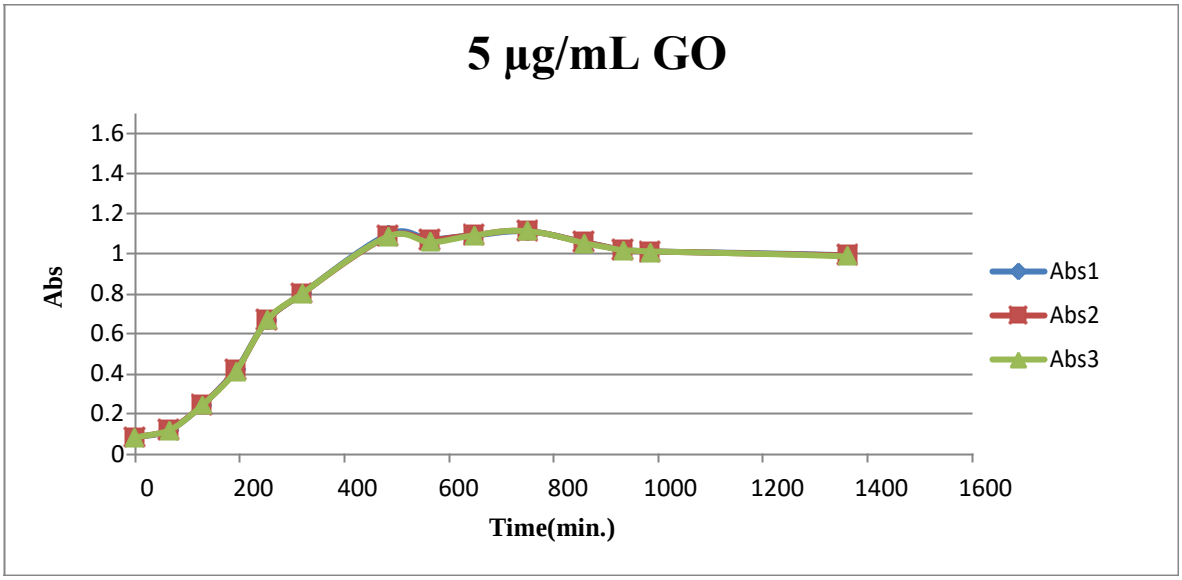

Figure 15: Escherichia coli Nissle 1917, 5 µg GO/ml trail

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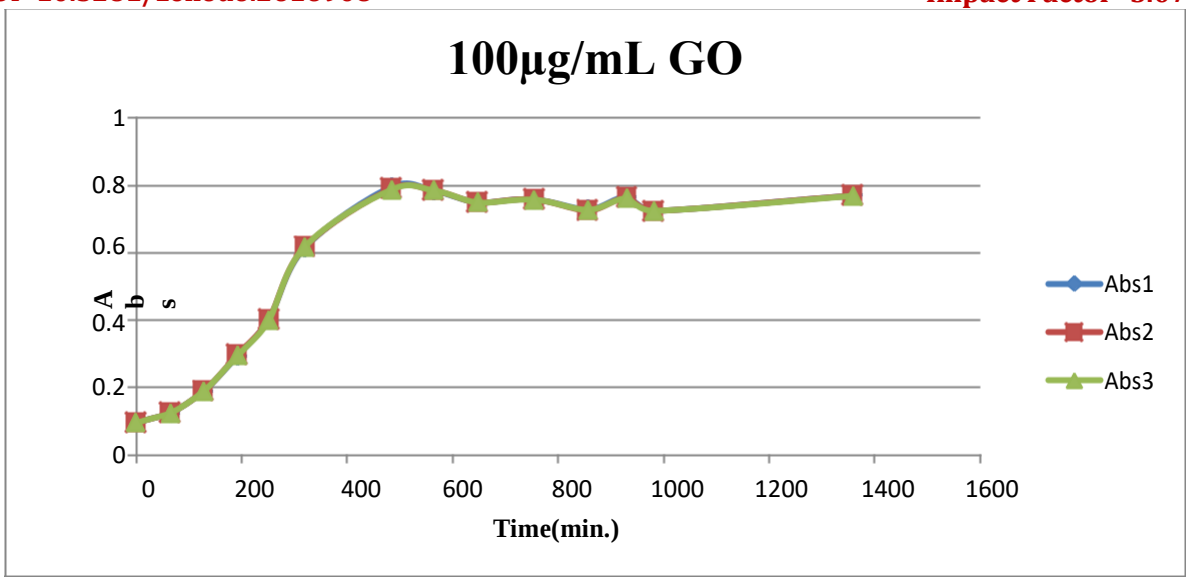

Figure 16: Escherichia coli Nissle 1917, 100 µg GO/ml trail

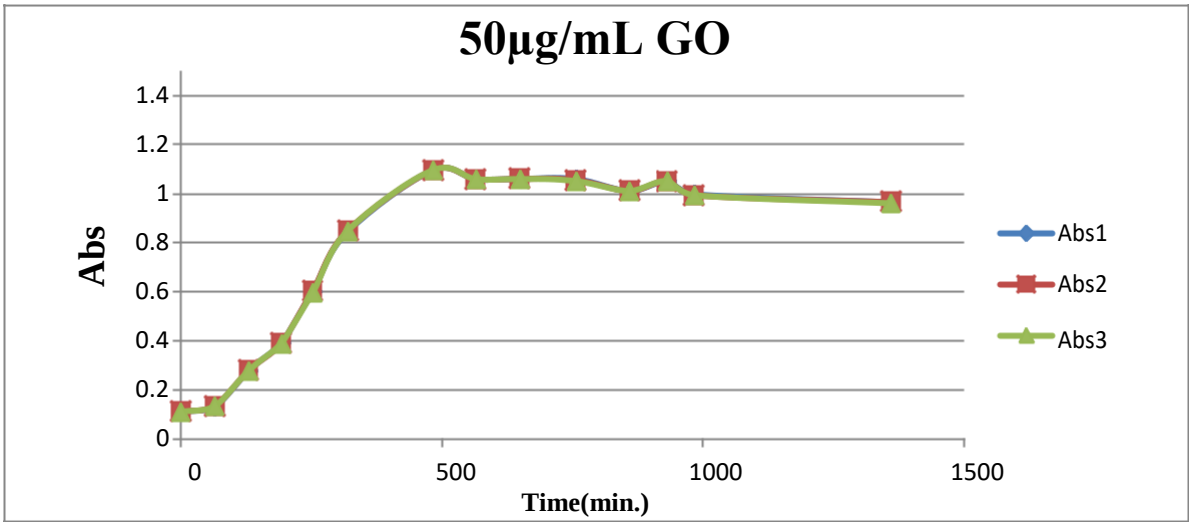

Figure 17: Escherichia coli Nissle 1917, 50 µg GO/ml trail

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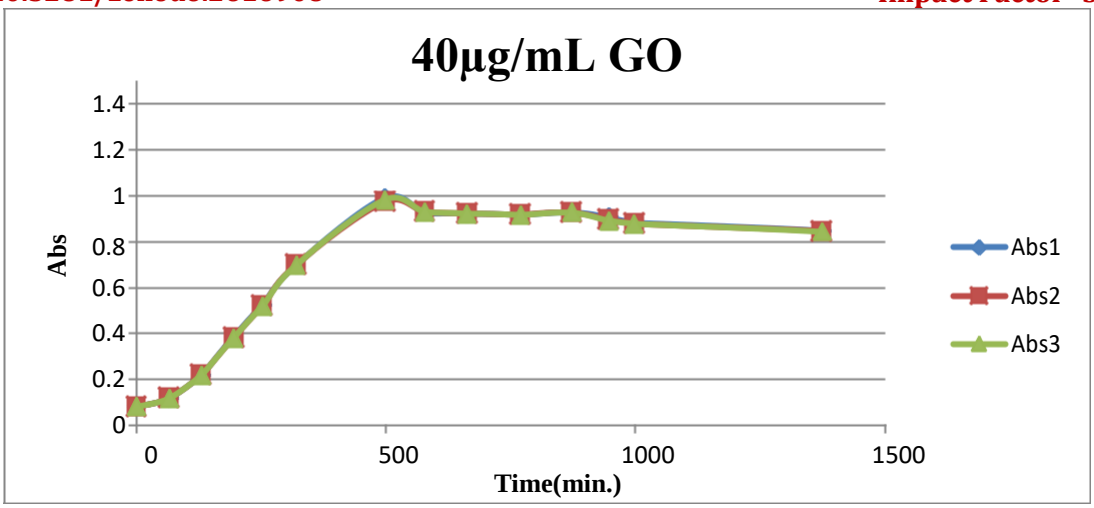

Figure 18: Escherichia coli Nissle 1917, 40 µg GO/ml trail

Solid medium experiments results show in Figure 19, left side *Escherichia coli Nissle 1917* bacteria results and right side is *Lactobacillus acidophilus* bacteria results. As it appears medium can't hold onto the surface because of the graphene oxide hydrophic forces.

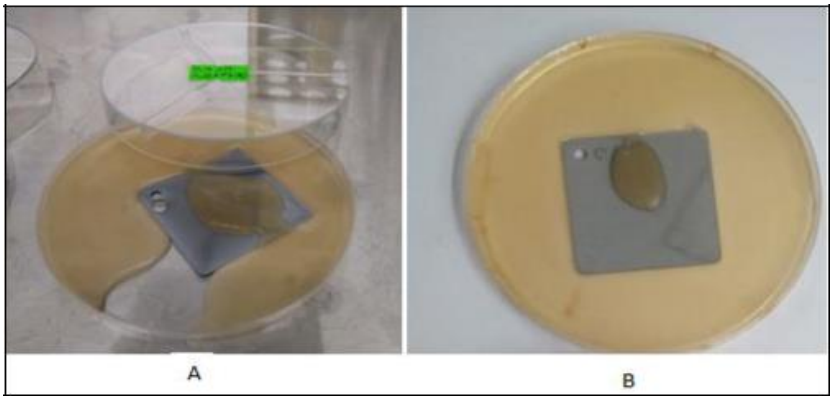

Figure 19: Escherichia coli Nissle 1917(A), Lactobacillus acidophilus(B) solid surface trail

IV. CONCLUSION

As a conclusion of the study, that is observed at all graphene oxide concentrations, the microorganisms can be growth. Graphene and graphene oxide materials were not antimicrobial but due to their hydrophobic site, the graphene solid surfaces produced by special methods, did not allow the development of microorganisms.

V. ACKNOWLEDGEMENTS

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REFERENCES

1. *R.M. Balleste, C. G. Navarro, J. G. Herrero and F. Zamora, "2D Materials to Graphene and Beyond", Journal of Nanoscale, 3(2011), 20.*
2. *A.C. Ferrari, J.C. Meyer, V. Scaraci, C. Casiraghi, M. Lazzeri, F. Mauri, S. Piscanec, D. Jiang, K.S. Novaselov, S. Roth and A. K. Geim, "Raman Spectrum of Graphene and Graphene Layers", Physical Review Letters, 97(2006), 187401.*
3. *S. Stankovich, D. A. Dikin, G. H. B. Domment, K. M. Kohlhass, E. J. Zimney, E. A. Stach, R. D. Piner, S. T. Nguyen and R. S. Ruoff, "Graphene Based Composite Materials", Nature Int. Journal of Science, 442(2006), 282.*
4. *S. Kaciulis, A. Mezzi, P. Calvani and M. Trucchi, "Electron spectroscopy of the main alloltropes of carbon", Surface and interface analysis, 46(2014), 966.*
5. *G. Odahara, T.Ishikawa, K. Fukase, S. Otani, C. Oshima, M. Suzuki, "Self-standing Graphene sheets prepared with Chemical Vapor Deposition and Chemical Etching",e-Journal of Surface Science and Nanotechnology, p.837-840(2009).*
6. *Y. Liu, B. Xie, Z. Zhang, Q. Zheng and Z. Xu,"Mechanical properties of Graphene papers", Journal of the Mechanics and Physics of Solids, 60(2011), 4.*
7. *Y. Yong, A. M. Asiri, Z.Tong, D. Du and Y. Lin, "Graphene based material for biomedical applications", Materials today, 16(2013), 10.*
8. *N. O. Wiss, H. Zhou, L.Liao, Y. Liu, S. Jiang, Y. Huang, X. Duan, "Graphene: An Emerging Electronic Material", Advanced Materials, 24(2012), 43.*
9. *X. An, T. W. Butler, M. Washington, S. K. Nayak and S. Kar, "Optical and Sensing Properties 1-Pyrenecarboxylic Acid-Functionalized Graphene Films Laminated on Polydimethylsiloxane Membranes", American Chemical Society Nano 5(2011), 2.*
10. *A. A. Balandin, S. Ghosh, W. Bao, I. Calizo, D. Teweldebrhan, F. Miao and C. N. Lau, "Superior Thermal Conductivity of Single-Layer Graphene", Nano Letters, 8(2008), 3.*
11. *Y. Wang, Z. Li, J. Wang, J. Li and Y. Li, "Graphene and graphene oxide: biofunctionalization and applications in biotechnology", Trends in Biotechnology, 29(2011), 5.*
12. *Z. Liu, J. T. Robinson, X. Sun and H. Dai, "PEGylated Nanographene Oxide for Delivery of Water-Insoluble Cancer Drugs", Journal of the American Chemical Society, 130(2008), 33.*
13. *C.M. Ana de Moraes, M.B. Simoes, O. L., Alves, et.al., "Nanomedicine and nanotoxicology", New York, p.1-29(2014).*
14. *T. Musumeci, G. Puglisi, Antimicrobial Agent, Drug-Biomembrane Interaction Studies, 2013.*
15. *R. F. Al-Thani, N. Patan and M. A. Al-Maadeed, "Graphene Oxide as Antimicrobial Against Two Gram-positive and Two Gram-negative Bacteria in Addition to One Fungus", Online Journal of Biological Science, 14(2014), 3.*
16. *O. Akhavan, E.Ghaderi, "Toxicity of Graphene and Graphene Oxide Nanowalls Against Bacteria", American Chemical Society, 4(2010), 10.*