JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2021, *27* (2), 133-139

*Journal of Agroalimentary Processes and Technologies*

# Bacterial growth on recycled agar medium media used in different cultures

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***Abstract***

Treating the biological waste used in bacterial culture is so important to save the environment and this can be useful for laboratories in countries with limited resources, which means financial benefit. The investigation of the bacterial growth on the used media recycled was studied in this work. The general media (plate count agar) can be reused twice after the recycling to cultivate and identify *E. coli*, *S. aureus*, *Salmonella sp*, and *B. subtilis* by the spread method on the plate count agar medium. The size of bacterial colonies decreased in the second step of the recycling from -30 to -60% in comparison with the control. The processes to recycle the used media help transform it to new powder that could be stored for a long time (up to six months). Three methods of additional sterilization were tested on the used media: (1) the sterilization by gamma irradiation at 5 kGy, (2) the microwave for 8 min, and (3) the hot drying in oven at 80 °C. Using the microwave radiation during the processes of recycling allowed having new media, with color and clarity similar of the fresh media and available in laboratories.

***Keywords***: Recycling , Media, *E. coli*, *B. subtilis*, *S. aureus*, *Salmonella sp.*

## 1. Introduction

The scientists have used the bacterial culture media to study the microorganisms as visual colony to distinguish one bacterial type from another, bacteria are everywhere [1]. The media consists of agar, salts, vitamins, colorants, different necessary nutrients and some texture enhancers [2,3]. Agar is a kind of polysaccharide that is obtained from marine red algae with good solubility in water [4.5]. The used media could be considered as environmental and economic problems. So, the important question is: Can media be reused in some laboratories for economic reasons? Scientific literatures have indicated that limited number of studies have addressed the utilization of microbial culture media previously used. Ahmed and Khan [6] relied on the process of re-dissolving the media in autoclaves after removing the colonies from the Nutrient Agar media. In another work, the scientists transformed the used agar from microbial and plant tissues to a new one [5] without testing the bacterial growth. Some tried to replace the traditional microbial culture media with cheaper natural materials such as cereals and vegetables to develop bacteria and fungi growth [7-10]. Xinyi [11] were able to recycle an algae culture environment up to four times only through the post-growth and no additives [12-14]. Nowadays, the solid and industrial wastes management becomes more important on the governmental and non-governmental scale especially with the increase of the population and the industry that are related to the new type of life [15,16]. In the developing countries the media could be impose problems related to the high cost [10]. The safe reuse maybe contribute in saving the environment [5,17,18]. Therefore, the researches - of methods to recycle the used media - give the ability to reduce the biological waste. The aim of this present work was to have a response of this question: Can media be reused for economic reasons to contribute in finding the environmental and economical solutions of the quantity of biological wastes?

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## 2. Materials and Method

### 2.1. Bacterial strains

Four strains of bacteria were chosen: Gram-negative (*E. coli, Salmonella sp*) and Gram-positive (*S. aureus, B. subtilis*). All strains were supplied by Syrian Atomic Energy (Biotechnology department). In each tube, 10 mL of nutrient broth were inoculated by one colony (colony from each strain/ tube). All tubes were incubated overnight at 37 °C. Serial dilutions were realized then 100 microliters were spread on the medium (plate count agar: Himedia-India) [19]. This media contains casein enzymatic hydrolysate, yeast extract, dextrose, and the major component is agar. It was already autoclaved at 121 °C / 15 min / 1 Bar (Autoclave-Italian-PBI). Then, it was poured in Petri dishes. After that, it was inoculated before the incubation for 24 h at 37 °C [10,20] (Cabolit-Type: PIF120-England). This step was called R0. This work was done by using only general medium and in future other media will be tested.

### 2.2. Recycling of bacterial used media

All the formed colonies were removed gently by cotton swap. All the used media were removed from Petri dishes and were classified depending on strains. The used media were divided into three groups, and each one was treated by only one additional sterilization before transform it to a new powder media: (1) by gamma irradiation (IR) at different doses (5, 10, 15) kGy using $^{60}$Co facility (Russian Type: 87 ROBO). The dose rate was 25.6 kGy/h. The use of irradiation in bacterial culture sterilization was noted in some studies [21-25]; (2) by microwave (wa), the used media were placed in a flask (500 mL) in open glass for 8 min approximately (Wattar Lux, MI42D, 1250 W, 2450 MHZ, Syria) to be sterilized. During this short period of boiling, we stopped the microwave 2-3 times for 2-3 s to avoid the overflow of the medium. After this procedure, the reused liquefied media were poured in shallow and large dishes to transform it to dried slices. The use of microwave in laboratory for sterilization the culture vessels to reuse them was noted by researchers [26-28]; (3) by hot temperature (T): the used media have been removed from Petri dishes. Then, they have been dried in an oven at 80 °C for 8 h (Memmert-looding Model 100-800. Germany). They were transformed by consequence to dried slices (Figure 1). All the used media that sterilized by gamma irradiation or by microwave (for microbial decontamination) were dried for overnight at 35 °C, and were transformed to new slices (Figure 1). All the dried slices were crushed (Starway, SW-012-SS, 150W, China) and transformed to powder (Figure 2). This new material was used to reconstitute the media for bacterial growth. The pH was measured by pH-meter (HANNA, HI8314, Romania). The different strains were spread - as we mentioned before - this step was called R1. After the incubation of all Petri dishes for 24 h at 37 °C, the formed colonies were removed again gently. All the used media in this step were treated and transformed to powder, then the strains were spread for the third time, this step was called R2. Each strain was grown on the same media during the three steps of recycling.

### 2.3. Bacterial growth

The bacterial growth was estimated by the microbial load (expressed by CFU: Colony Forming Unit) and by the strength of bacterial growth which was measured using Eq. 1, based only on colony size for the same time of incubation.

$$S\% \text{ (Strength of bacterial growth)} = = (x*100) / X-100 \quad \text{(Eq. 1)}$$

Where:

$x$ – Average of diameter of CFU in recycling media by different treatment

$X$ – Average of diameter of CFU in controlled media.

## 3. Results and discussion

### 3.1. Comparing the different methods of recycling

The different applied doses of gamma irradiation to sterilize the used media had the good effect of sterilization. However, this procedure takes a long time and needs the existence of gamma facility that costs a lot, so we used the dose of (5 kGy) for economical cases. In the second way of additional sterilization before recycling the used media, we used the microwave to melt and to sterilize the used media. The duration of (8 min) was enough to have good results, and the new media that are prepared from dried slices had the clarity and the color as the control media. The third one, drying in hot temperature (80 °C) for a long time in the oven, gave the dried slices a little dark color, and slightly affected the color of the recycled media and maybe affected the media composition. So the short time needed before the drying gives the microwave more

**Figure 1.** The slices produced after drying the different media


**Figure 2.** The new media transformed as powder after recycle the used media

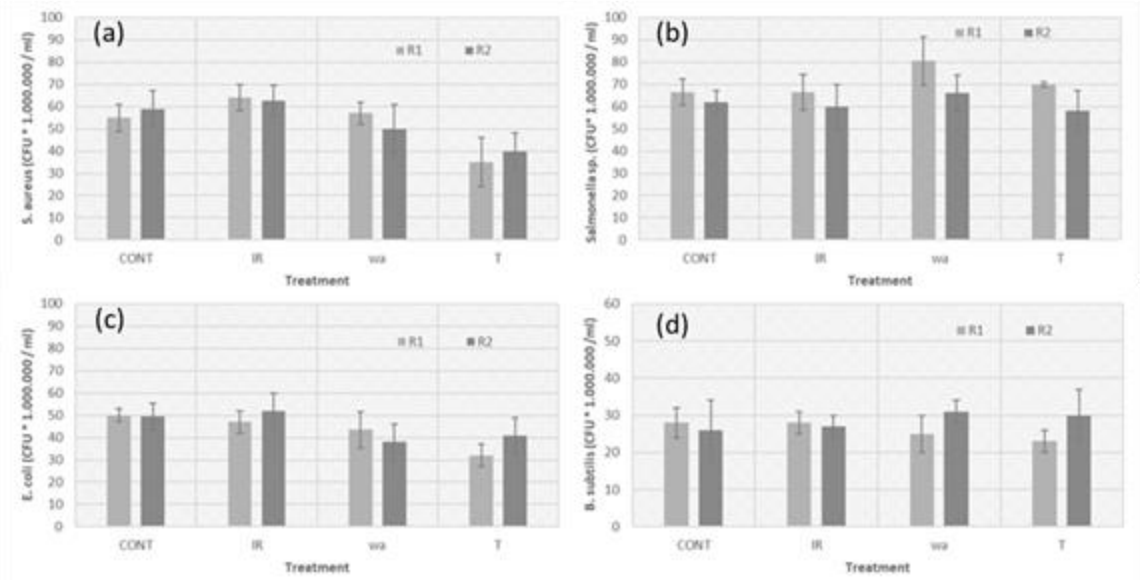


**Figure 3.** Comparison in bacteria load in different steps of recycling. R1: the first recycling, R2: the second recycling in the different treatment of sterilization (CONT: Control; IR: gamma irradiation treatment; wa: microwave treatment; T: hot temperature treatment). (a) *S. aureus*; (b) *Salmonella* sp.; (c) *E. coli*; (d) *B. subtilis*

advantages. The changes in bacterial load were investigated. Figure 3 shows changes in the number of bacteria that is expressed by CFU for each of the four studied strains during the steps of the experiments (R1, R2). This number was compared with the control corresponding to each step by different treatments. There was no significant difference in microbial load between all treatments and their control at (R1) and (R2) for each strain, that was spread on the surface of the recycled media in the same dilution. Except the condition of (T) in the case of *S. aureus*, the CFU was slightly less than the other treatments in both R1 and R2 (Figure 3a). The results approved that there was no significant differences in microbial load for *Salmonella* sp., *E. coli* and *B. subtitls* (Figures 3b, 3c and 3d), respectively, neither in R1, nor in R2.

*3.2. Strength of bacterial growth*

Despite of the bacterial growth in microbial load for all bacterial strains in R1 and R2, the colonies had the same morphological form and the strength growth in the R1 except *B. subtilis*, whereas the diameter of colonies decreased in all methods of sterilization comparing to the control. The growth strength decreased to 50% based on colony diameter. The nutritive elements in the first recycling were not completely utilized, so the bacteria could grow without problems [6]. In R2, under all conditions the diameter of bacteria was small comparing to the control condition for all strains. Figures 4a, 4b, 4c and 4d clearly shows the decrease in diameter of bacteria for *S. aureus*, *Salmonella* sp., *E. coli*, and *B. subtilis*, in different treatments of sterilization by gamma irradiation (IR), microwave (wa) and hot temperature (T) comparing by the control (CONT) for the same dilution of microbial suspension spread on the media during the recycling experiments. The strength of bacterial growth in R2 was affected during the same time of incubation. That appears clearly via the measure of colonies diameter used in Eq. 1. The S % was -60, -50 and -30% in *B. subtilis*, *Salmonella* sp., *S. aureus* and *E. coli*, respectively (Figure 5). It seems that *B. subtilis* is more susceptible to the change in composition of media, whereas the average of the colony diameter decreased from 10 mm to 4 mm in the R2 after 24 h at 37 C° (Figure 4). The decrease in bacterial growth strength in the recycled used media maybe related either to the noted slight change in pH of new reconstitute media or to the loss of some nutrients as dextrose, yeast extract, casein or even agar. Trace elements may need to be analyzed because of its major role in the growth of bacteria [6]. When the third time R3 was tested, the results showed that despite of the bacterial growth as CFU, the stability of the gel media had decreased considerably and became less solid (filtered water and cracked at any effort or any movement in the Petri dish). This was due to the repeated heat stress during the repeated recycling operations and the effect of re-melting [19]. The weight loss of the recycled used media in the first time was 10±2 % comparing to the quantity used to prepare the basic non-recycled media. The percentage of losses between the first and the second step reached 20±3 %, and the loss at the end of the third step amount reached 50±5 %. This loss was due to the drying and grinding operations. Different chemical devices could analyze soluble nutrients in both control and recycled media as ion chromatography [11]. Other experiments will be considering to obtain more information about the possibility to make mutation in bacteria that grow in reused media. These new experiments will be done by PCR (Polymerase Chain Reaction) analysis and by SEM (Scanning Electron Microscope).

The results presented in this work approve the ability to recycle the used plate count agar to save the environment and to reduce the biological waste. Different methods to sterilize the used media were tested (gamma irradiation, microwave radiation and hot temperature) before transforming it into a new powder by drying. The general media (plate count agar) can be used twice after the recycling to cultivate and identify *S. aureus*, *Salmonella* sp., *E. coli* and *B. subtilis* by the spread method. The microwave method has more advantage by the simplicity of the use and the clarity of new recycling media. The microbial load on the recycled media was not affected, but the strength of microbial growth -the diameter of CFU - in the second recycling decreased up to -60%.

## 4. Conclusions

The general bacterial media can be reused two times - after the processes of recycling - to cultivate and identify *E. coli*, *S. aureus*, *Salmonella* sp., and *B. subtilis* by the spread method on the plate count agar medium. These processes help to transform the used media to a new powder that could be stored for six months with good cultivable potential. Another

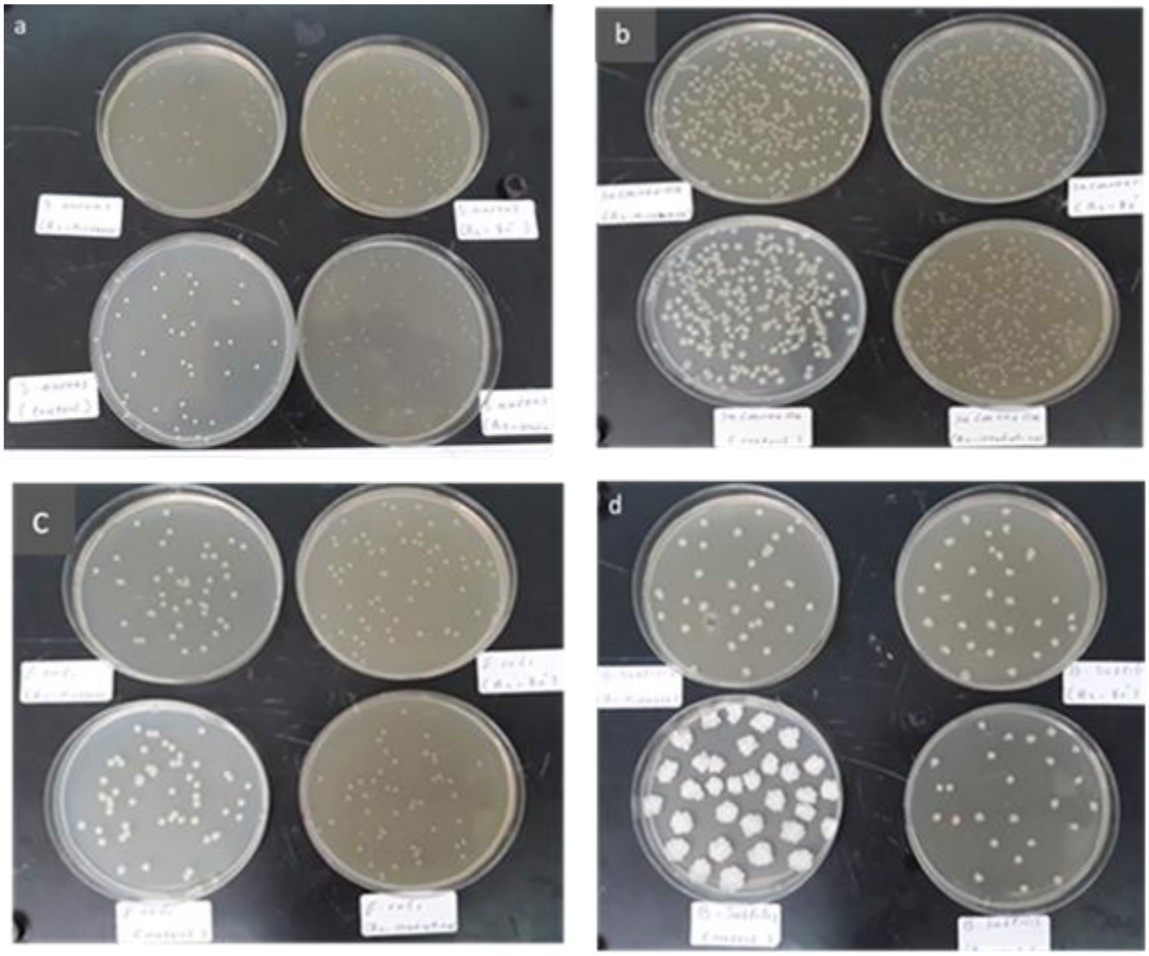


**Figure 4.** Growth in the second recycling (R2) in the different treatment of sterilization: (CONT: Control; IR: gamma irradiation treatment; wa: microwave treatment; T: hot temperature treatment). (a) *S. aureus*; (b) *Salmonella* sp.; (c) *E. coli*; (d) *B. subtilis*.

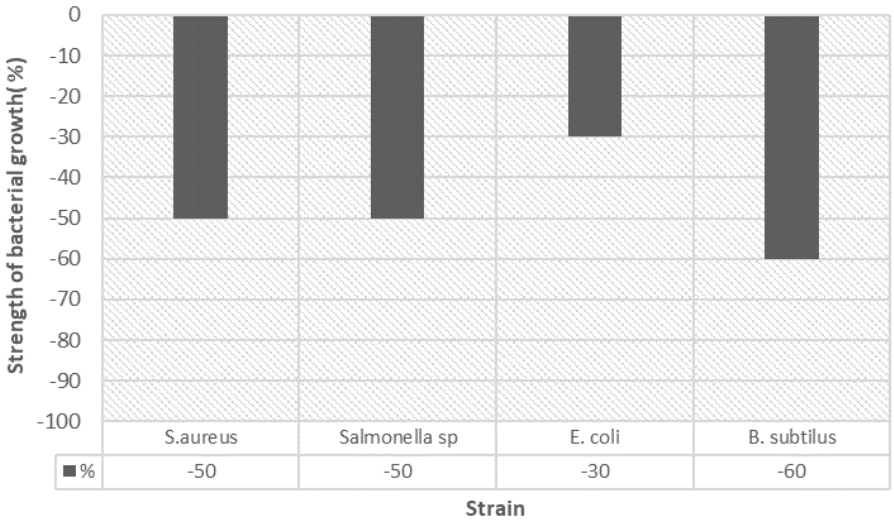


**Figure 5.** Strength of bacterial growth estimated by only the comparison between the diameter of Colony Forming Unit (CFU) of different conditions and the control in the R2

work is going to recompense this decrease by adding more time of incubation or by adding some nutrients. Transforming the used media to powder makes the long storage possible up to 6 months and saves the environment by decreasing the biological waste and reutilization of Petri dish after the sterilization by gamma irradiation.

**Acknowledgements.** The author would like to express his deep appreciation to Professor Othman (D.G. of AECS) for his encouragement, Dr. Al-Bachir, the head of Irradiation Technology Department, Mr. Saoud for his help in microbiological experiments, M.S. Rihawy who performed the English review, and finally thanks to Dr. Al-Marirri for supplying us the bacterial strains.

**Compliance with Ethics Requirements.** The author declares that he respect the journal's ethics requirements. The author declares that he has no conflict of interest.

**References**

1. Kaláb, M., Microscopy and Higene, *Infocus* **2011**, *23*, 5
2. Srimongkon, T.; Ishida, T.; Garashi, K.; Enomae, T., Development of a bacterial culture system using a paper platform to accommodate media and an ink-jet printing to dispense bacteria, *American Journal of Biochemistry and Biotechnology* **2014**, *10*(1), 81-87
3. Zimbro, M., History microbiology and culture media. Manual of microbiological culture media Difco laboratories second edition page no 3-4, **2009**
4. Murano, E., Chemical structure and quality of agars from *Gracilaria*, *Journal of Applied Phycology* **1995**, *7*(3), 245-245
5. Yesudas, R.; Rajput, R. S.; Sharma, V., Recycling and Reuse of Agar from Used Media, *Pramana Research Journal* **2019**, *9*(4), 385-388
6. Ahmed, S.Z.; Khan, M., Recycling of culture media by re-melt and re-plating method, *International Journal of Scientific & Engineering Research* **2014**, *5*(1), 2159-2161
7. Adesemoye, A.O.; Adedire, C.O., Use of cereals as basal medium for the formulation of alternative culture media for fungi, *World Journal of Microbiology and Biotechnology* **2005**, *21*, 329-336
8. Ravathie, A.; Sevvel, P.; Nirmala, R.; Kularajany, N., Alternative culture media for bacterial growth using different formulation of protein sources, *The Journal of Natural Product and Plant Resources* **2012**, *2*(6), 697-700
9. Ravimannan, N.; Arulanantham, R.; Pathmanathan, S.; Niranjan, K., Alternative culture media for fungal growth using different formulation of protein sources, *Annals of Biological Research* **2014**, *5*(1), 36-39
10. Uthayasooriyan, M.; Pathmanathan, S.; Ravimannan, N.; Sathyaruban, S., Formulation of alternative culture media for bacterial and fungal growth, *Der Pharmacia Lettre* **2016**, *8*(1), 431-436
11. Xinyi, E.; Crofcheck, C.; Crocker, M., Application of recycled media and algae based anaerobic digestate in *Scenedesmus* cultivation, *Journal of Renewable and Sustainable Energy* **2019**, *8*(1), 01316/1-01316/14
12. Moller, K.; Muller, T., Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review, *Engineering in Life Sciences* **2012**, *12*(3), 242-257
13. Shin, H.Y.; Ryu, J.H.; Bae, S.Y.; Crofcheck C.; Crocker, M., Lipid extraction from *Scenedesmus* sp. microalgae for biodiesel production using hot compressed hexane, *Fuel* **2014**, *130*, 66-69.
14. Wilson, M. H.; Groppo, J.; Placido, A.; Graham, S.; Morton, S. A.; Santillan-Jimenez, E.; Shea, A.; Crocker, M.; Crofcheck, C.; Andrews, R., $CO_2$ recycling using microalgae for the production of fuels, *Applied Petrochemical Research* **2014**, (4), 41-53
15. Brotosusilo, A.; Nabila, S.H.; Negoro, H.A.; Utari, D., Individual participation of community in implementing effective solid waste management policies, *Global Journal of Environmental Science and Management* **2020**, *6*(3), 341-354
16. Shayesteh, A.A.; Koohshekan, O.; Khadivpour, F.; Kian, M.; Ghasemzadeh, R.; Pazoki, M., Industrial waste management using the rapid impact assessment matrix method for an industrial park, *Global Journal of Environmental Science and Management* **2020**, *6*(2), 261-274
17. Zhang, M.; Wang, Y.; Song, Y.; Zhang. T.; Wang, J., Manifest system for management of non-hazardous industrial solid wastes: results from a Tianjin industrial park, *The Journal of Cleaner Production* **2016**, *133*, 252-261
18. Armisen, R.; Galatas, F., Production, properties and uses of agar. **1987**, pp. 1-57. In: McHugh, D.J. (ed.), Production and Utilization of Products from Commercial Seaweeds, FAO Fisheries Technical Pap. 288
19. Atlas, R.M., Handbook. Microbiological Media for the Examination of Food, 2nd ed., CRC Taylor and Francis, **2006**, pp. 278
20. Gulsun, A.E.; Zhang, Q.H.; Richter, E.R., Inactivation of *Escherichia coli* 0157:h7 and *Echerichia coli* 8739 in apple juice by pulsed electric fields, *Journal of Food Protection* **1999**, *62*(7), 793-796
21. Aquino, K. A. da S., Sterilization by Gamma Irradiation, Gamma adiation, Feriz Adrovic (Ed.), **2012**, ISBN: 978-953-51-0316-5
22. IAEA, Trends in radiation sterilization of health care products, International Atomic Energy Agency, Vienna, **2008**, 1-260
23. Rawat, K.P.; Arjun, C.; Khade, S. A.; Padmanabham D.; Samuel G.; Sarma K.S.S., Sterilization of Bacteriological Culture Media by High Energy Electron Beam Machine, NICSTAR **2015**

24. Hewitt, P.; Leelawardana, D.S., Gamma irradiation as a treatment to address pathogens of animal biosecurity concern, Department of Agriculture, Final policy review, November **2014,** http://iiaglobal.com/uploads/documents/24.pdf
25. Tittabutr, P.; Teamthisong, K.; Buranabanyat, B.; Teaumroong, N.; Boonkerd, N., Gamma Irradiation and Autoclave Sterilization Peat and Compost as the Carrier for Rhizobial Inoculant Production, *The Journal of Agricultural Science* **2012**, *4*(12), 59-67
26. Kothari, V.; Patadia, M.; Trivedi, N., Microwave sterilized media supports better microbial growth than autoclaved media, *Research in Biotechnology* **2011**, *2*(5), 63-72
27. Sanborn, M.R.; Wan, S.K.; Bular, R., Microwave Sterilization of Plastic Tissue Culture Vessels for Reuse, *Applied and Environmental Microbiology* **1982**, *44*(4), 960-964
28. Shareef, S.A.; Hamasaeed, P.A.; Ismaeil, A.S., Sterilization of Culture Media for Microorganisms Using a Microwave Oven Instead of Autoclave, *Rafidain Journal of Science* **2019**, *28*(1), 1-6, https://doi.org/10.33899/rjs.2019.159390
29. Meinita, M.D.N.; Marhaeni, B.; Hong, Y.K.; Jeong, G.T., Enzymatic saccharification of agar waste from *Gracilaria verrucosa* and *Gelidium latifolium* for bioethanol production, *Journal of Applied Phycology* **2017**, *29*(6), 3201-3209