Recycling and Reuse of Agar from Used Media

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Abstract

Recycling of agar and then reusing it is of great importance since the agar waste creates issues in environment. In the present study we will recycle and reuse the agar from the used media. Various agar media will be collected, decontaminated and subjected to freeze thawing to remove nutrients and metabolic wastes. The activated charcoal will be then used to remove the carbon based impurities as well other impurities like chlorine. It was treated with acid and alkali respectively such that the minerals and free ions presented reacts with them and get removed. Bleaching was done by sodium hypochlorite solution. The wash treatment with double distilled water will remove the activated charcoal as well as neutralized the pH. The recycled agar will be used to prepare MS media and nutrient agar media. In the proposed study it was found that the amount of recycled agar to be used for nutrient media was 2g/100ml (1.5g/ 100ml when regular agar used) while 1g/100ml was added to MS media (0.8g/ 100ml when regular agar used).

Keywords

Recycle, agar, decontamination, environment

Introduction

Louis Pasteur was the first person to use culture medium for cultivating bacteria in the laboratory in the year 1860. The medium used sugar, yeast ash and ammonium salts. Later in 1887, petri dish was used in microbiology and this led to the increased focus on media formulations for culture growth (Diagnostics, 2009; Koch, 1882; Arulanantham et al, 2012). In 1658 agar was discovered by Minora Tarazaemon in Japan. Agar is water soluble polysaccharide obtained from Rhodophyceae (marine red purple algae) (Murano, 1995). *Gelidium*, microalgae is the preferred source for agars among *Pterocladia*, *Gracilaria* and *Gelidium* (Hitchens and Leikind, 1939).

Agar is a cell wall component of various species of red algae. It is chemically polymer with subunits of galactose (Ahmed and Khan, 2014). It becomes gelatinous when dissolved in boiling water and solidifies when cooled. Agar tends to be resistant to microbial digestion and liquefaction. It is not only used for laboratory purposes but also for thickening of soups, sauces, ice creams and jellies.

Agar is classically used in a final concentration of 1-2% for hardening of culture media. Lesser amounts (0.05-0.5%) are added to media to study motility (0.5% w/v) and for anaerobes growth (0.1%) and microaerophiles. There are varieties of agar media that are used in microbiological culture development. Some of them are blood agar, tryptic soy agar, chocolate agar, Luria Bertani agar, MacConkey agar and so on.

Recycling of agar and then reusing it is of great importance since the agar waste creates issues in environment (Armisen and Galatas, 1987). Decontamination of the media used in the experiments, will help to remove any hazardous matter present in it but the agar remains the unaffected. Since it is resistant to microbial digestion agar pertains in the environment as such and hinders the other life forms. Bigger industries carrying out micro propagation generate large quantity of agar waste. Hence lies a need to develop a method that can lead to recycling of the agar in the used media and re use it for the laboratory purposes. Recycling and reusing of the agar obtained from the used media will help in saving environment as well as will be cost effective.

Materials and methods

Collection and decontamination of various agar media

Various used agar media from plant tissue culture and microbiological laboratory were collected. They were taken together in the conical flask and autoclaved. Used media were thus decontaminated.

Removal of solid wastes

The decontaminated media was then filtered through the mesh cloth. The solid wastes like plant residues, seeds, microbial debris and so on were removed through the mesh cloth. The media when poured though the mesh cloth should be warm enough so that agar present in the media does not solidify and clog the mesh cloth.

Freezing and thawing

The filtrate was poured into plastic boxes and made to cool at room temperature for gel formation. It was then frozen slowly by keeping them in freezer. The frozen gel was thawed. The process was repeated for thrice.

Purification treatment

The so obtained treated agar was then added on with activated charcoal (2%) in order to remove the carbon based impurities as well as impurities such as chlorine. The obtained agar to be treated was then kept on shaker for 24 hrs.

Acid Alkali treatment

The filtered agar media was subjected to acid-alkali treatment such that minerals and ions present shall be removed for it. Hydrochloric acid (0.1 N) was prepared and poured into the media. It was then kept on the shaker at 50 rpm for 24 hrs. After the acid treatment the media was filtered out. It was then followed with alkali treatment. Sodium hydroxide (0.1 N) solution was added to the media and left overnight on shaker at 50 rpm. The treated media was then filtered out using mesh cloth.

Bleaching

The obtained agar was then subjected to bleaching using 0.4% sodium hypochlorite solution (pH 5-6) for overnight. The solution will act as surfactant as well as decolorizing agent. The agar was then filtered out. The obtained agar media was then filtered and the washed with double distilled water till the excess sodium hypochlorite solution is removed. The treated agar was then filtered out and shade dried in aseptic conditions.

Assessment of recycled agar

The so obtained agar was powder using mortar and pestle. It was then used as regular agar for the media preparation in plant tissue culture and microbiological laboratory. The amount of agar needed for the perfect media consistency was quantified.

Result

Various agar media obtained were collected, decontaminated and subjected to freeze thawing leading to removal of nutrients and metabolic wastes present. It was then treated with hydrochloric acid and sodium hydroxide respectively such that the minerals and free ions presented reacts with them and get removed. Most of the nutrients used while media preparations were used up by the explants and microbes grown in them. The activated charcoal used helped to remove the carbon based impurities as well other impurities like chlorine. The sodium hypochlorite solution helps to decolorize the obtained agar. The wash treatment with double distilled water removed the excess sodium hypochlorite solution as well as neutralized the pH. The recycled agar filtered out, dried was used to prepare media used in both plant tissue culture (MS media) and microbiological laboratory (nutrient agar media). It was found that the amount of recycled agar to be used for nutrient media was 2g/100ml (1.5g/ 100ml when regular agar used) while 1g/100ml was added to MS media (0.8g/ 100ml when regular agar used).

Conclusion

The recycled agar used showed no harmful effect in the growth of microbes or plant explants. The growth was observed same as that in the control plates using the regular fresh agar. The purity or porosity of agar needs to be tested since it may get decreased due to re-melting (Meinita

et al, 2017). If the agar is recycled and reused from the used media it can prevent environmental hazards and save money as well.

References

1. *Diagnostics, B.D., 2009. Difco™ & BBL™ Manual, Manual of Microbiological Culture Media, 3-4.*
2. *Koch, R., 1882. Die Atiologie der Tuberculose. Berl. Klin. Wochenschr, 19, pp.221-230.*
3. *Arulanantham, R., Pathmanathan, S., Ravimannan, N. and Niranjan, K., 2012. Alternative culture media for bacterial growth using different formulation of protein sources. J Nat Prod Plant Resour, 2(6), pp.697-700.*
4. *Murano, E., 1995. Chemical structure and quality of agars from Gracilaria. Journal of Applied Phycology, 7(3), p.245.*
5. *Hitchens, A.P. and Leikind, M.C., 1939. The introduction of agar-agar into bacteriology. Journal of bacteriology, 37(5), p.485.*
6. *Ahmed, S.Z. and Khan, M., 2014. Recycling of Culture Media by Re-melt & Re-plating Method. International Journal of Scientific & Engineering Research, 5(1), p.2159-2162.*
7. *Armisen, R. and Galatas, F., 1987. Production, properties and uses of agar. Production and utilization of products from commercial seaweeds. FAO Fish. Tech. Pap, 288, pp.1-57.*
8. *Meinita, M.D.N., Marhaeni, B., Hong, Y.K. and Jeong, G.T., 2017. Enzymatic saccharification of agar waste from Gracilaria verrucosa and Gelidium latifolium for bioethanol production. Journal of applied phycology, 29(6), pp.3201-3209.*