Bacterial culturing with reused media: a sustainable laboratory practice

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ABSTRACT :

Microbial growth is an integrative outcome of utilization of available nutrients under a set of physiochemical conditions by microbial cells. While culture media provide the essential biochemical framework for proliferation, bacterial responses are strongly influenced by nutrient availability and the physicochemical properties of gelling agents like agar. Recently, concerns regarding environment sustainability have prompted interest in reused media for regular culturing of microbes in order to reduce waste generation making it cost effective. This review examines its practical implications. Existing literature indicates that while agar is chemically robust and recyclable through specific treatments, repeated reuse often compromises growth performance due to nutrient depletion or the accumulation of inhibitory metabolites. Studies demonstrate that recycled agar typically requires concentration adjustments to maintain necessary gelling properties. Furthermore, colony diameter can be identified as a critical parameter for accurately assessing growth dynamics on such modified substrates. By exploring the literature available on nutrient formulation, agar quality, and recycling protocols, this article highlights both the feasibility and biological limitations of microbiological media reuse. This review addresses fundamental questions regarding microbial adaptation to altered nutritional environments while supporting the advancement of sustainable laboratory practices.

Keywords: Microbial growth, Culture media, reuse, Sustainable microbiology

INTRODUCTION :

Understanding how microorganisms respond to changes in their nutritional and physical environment has been a fundamental question in microbiology since the early development of the discipline. Nutrient availability has a great influence on microbial growth and nature and extent of bacterial growth depends strongly on availability of available nutrient sources, Burrows (1936). This foundational perspective laid the groundwork for subsequent studies examining how culture media composition governs microbial physiology, metabolism, and growth dynamics. Ehrenberg et al. (2013) demonstrated that bacterial growth rate is dependent on nutrient concentration as well as macromolecular composition of the cell. Their work emphasizes the effect of prior exposure to specific growth media and current environmental

conditions on bacterial growth responses. This perspective is particularly relevant when evaluating modified, reused, or reconstituted culture media, where the nutritional profile and physicochemical properties may differ subtly from freshly prepared media. The aim of this review is to explore the possibilities of reusing the culture medium for bacterial growth by understanding its feasibility and sustainability.

THE PHYSICOCHEMICAL NICHE OF CULTURE MEDIA :

Culture media provide the biochemical and biophysical niche required for microbial growth. According to Hayek et al. (2019), essential nutrients such as carbon, nitrogen, vitamins, minerals, and growth factors in suitable proportions, are supplied by an appropriate culture medium, also maintaining favourable physical conditions for microbial proliferation. Differentiation, enumeration and physiological characterization of microorganisms are the most crucial standardizations which majorly depend on media formulation (Corry et al. 2012). Solid culture media additionally rely on a gelling agent, most commonly agar, to provide a stable surface for colony development and observation. Due to its chemical properties, agar holds an important position among culture media components. Extracted from red algae of the Rhodophyceae class, agar dissolves in boiling water and forms a firm gel upon cooling, without re-melting below approximately 85°C (Armisen and Galatas 1987). The property of agar being resistant to degradation by most bacterial enzymes proves it optimum to be used in the media. Different sources of algae used for extraction of agar, gives a variety of agar qualities varying in their chemical structures, gel strength, transparency, all of which can indirectly influence colony morphology and growth characteristics. According to Armisen and Galatas (1987), Agar purity and its interaction with other media components majorly affects its important application in microbiology. Therefore, checking its compatibility with nutrients and essential factors to avoid formation of inhibitors is a considerable fact when media containing agar are subjected to repeated dehydration, sterilization or reuse. Despite the long history of media development traced by Collard and Collard (1976) back to Koch's identification of agar as a solidifying agent in the late nineteenth century, standardized specifications for bacteriological agar remain limited. Quality assessment often depends on manufacturer-specific criteria and user requirements (Armisen and Galatas 1987). This lack of universal standards complicates the evaluation of recycled or reprocessed agar influencing microbial growth outcomes. Along with the agar, Culture media are formulated with carbon sources, nitrogenous compounds, vitamins, minerals, and growth factors that are required for microbial metabolism (Hayek et al. 2019). Once a media has supported microbial growth,

partial depletion of nutrients is inevitable.

MEDIA REUSE ATTEMPTS :

In parallel with recent advances in science, concerns regarding the economic and environmental sustainability of laboratory practices have intensified. Preparation and disposal of culture media by educational and diagnostic institutes as well as industries, contributes to the generation of biological waste. Recycling and reusing the media can be one of the solutions to approach sustainability. Arora et al. (2023) highlighted that residual nutrients often remain in spent or used media suggesting that growth media recycling can enable nutrient recovery. However, accumulation of inhibitory metabolites leads to changes in media chemistry thereby inhibiting growth on recycled media. This study focuses more on using recycled media, accumulation of inhibitory compounds and resulting growth of algae, which can also be applicable to bacterial systems.

The reuse and recycling of microbial culture media have received comparatively limited attention in bacteriology. Abboudi (2021) investigated the growth of bacteria on recycled agar media by removing the colonies followed by subjecting the media to various sterilization methods including gamma irradiation, microwave treatment and heat.Along with the bacterial growth in first recycled media, decrease in colony diameter and gel strength was observed. The decline became more pronounced in subsequent recycling cycles, suggesting progressive nutrient depletion or accumulation of inhibitory compounds. These findings align with earlier observations that nutrients in culture media are not fully exhausted during initial use, allowing limited reuse, but that repeated recycling compromises growth performance.

Detailed and systemic approach presented by Yesudas et al. (2019), focuses on recycling and reusing agar from Murashige & Skoog (MS) media. Considering decrease in nutrient availability, accumulation of inhibitory compounds in reused media, they employed a multi step treatment to restore agar quality. To remove soluble metabolites, freeze thaw cycles were performed along with decontamination. Activated charcoal treatments to adsorb carbon-based impurities, acid and alkali treatments to remove minerals and free ions, bleaching with sodium hypochlorite, and repeated washing with double-distilled water to neutralize pH and remove residual chemicals were a few techniques mentioned in the article. In the same attempt it was demonstrated that using slightly higher concentration of agar compared to regular agar would help in reusing the recycle medium. For nutrient agar, 2 g/100 ml of recycled agar was

required compared to 1.5 g/100 ml of regular agar, while MS media required 1 g/100 ml instead of 0.8 g/100 ml. These studies shows that the polysaccharide matrix provided by agar remain structirally intact even after the procedures for microbial growth followed by sterilization were performed suggesting laboratory waste and cost can be reduced simultaneously without the functionality getting affected. Also, pre treating the agar chemically might help in maintaining its robustness.

Above mentioned studies focus more on the effect of reusing on agar but effect on nutrients upon reusing is also important criteria to be considered. Culture media are formulated to provide balanced proportions of carbon sources, nitrogenous compounds, vitamins, minerals, and growth factors required for microbial metabolism (Hayek et al. 2019). During bacterial cultivation, these nutrients are actively assimilated for biomass formation, energy generation, and synthesis of macromolecules. Repeated dehydration and reconstitution do not replenish nutrients that were metabolically consumed. Instead, the residual medium represents a nutritionally modified environment shaped by prior microbial activity. Ehrenberg et al. (2013) demonstrated that bacterial growth rate is dependent on nutrient concentration and macromolecular composition of the cell, indicating that even subtle reductions in nutrient levels can influence growth kinetics. Therefore, if carbon or nitrogen sources become limiting after initial use, subsequent growth on reconstituted media may exhibit reduced colony diameter, delayed lag phase adjustment, or decreased biomass accumulation.

APPROACHES FOR BACTERIAL GROWTH MEASUREMENT :

Upon inoculation, bacteria would utilise all the available components in the medium and grow in colonies. In fresh medium, which is not reused, optimum bacterial growth can be observed. As compared to the fresh medium, bacterial cultivation on reused medium might be achieved for which analytical studies should be performed. Traditional approach for includes observing the colony characteristics and any particular growth pattern, if present. Palumbo et al. (1971) evaluated colony height, diameter, viable cell count. Among these, colony diameter emerged as a particularly valuable parameter because it allows repeated measurements over time without disturbing the colony. Results from studies of Abboudi (2021) also mentions about change in colony size upon cultivation on reused medium. Along with these approaches, more detailed approach for analysing the nutritional value in treated media to be reused for further

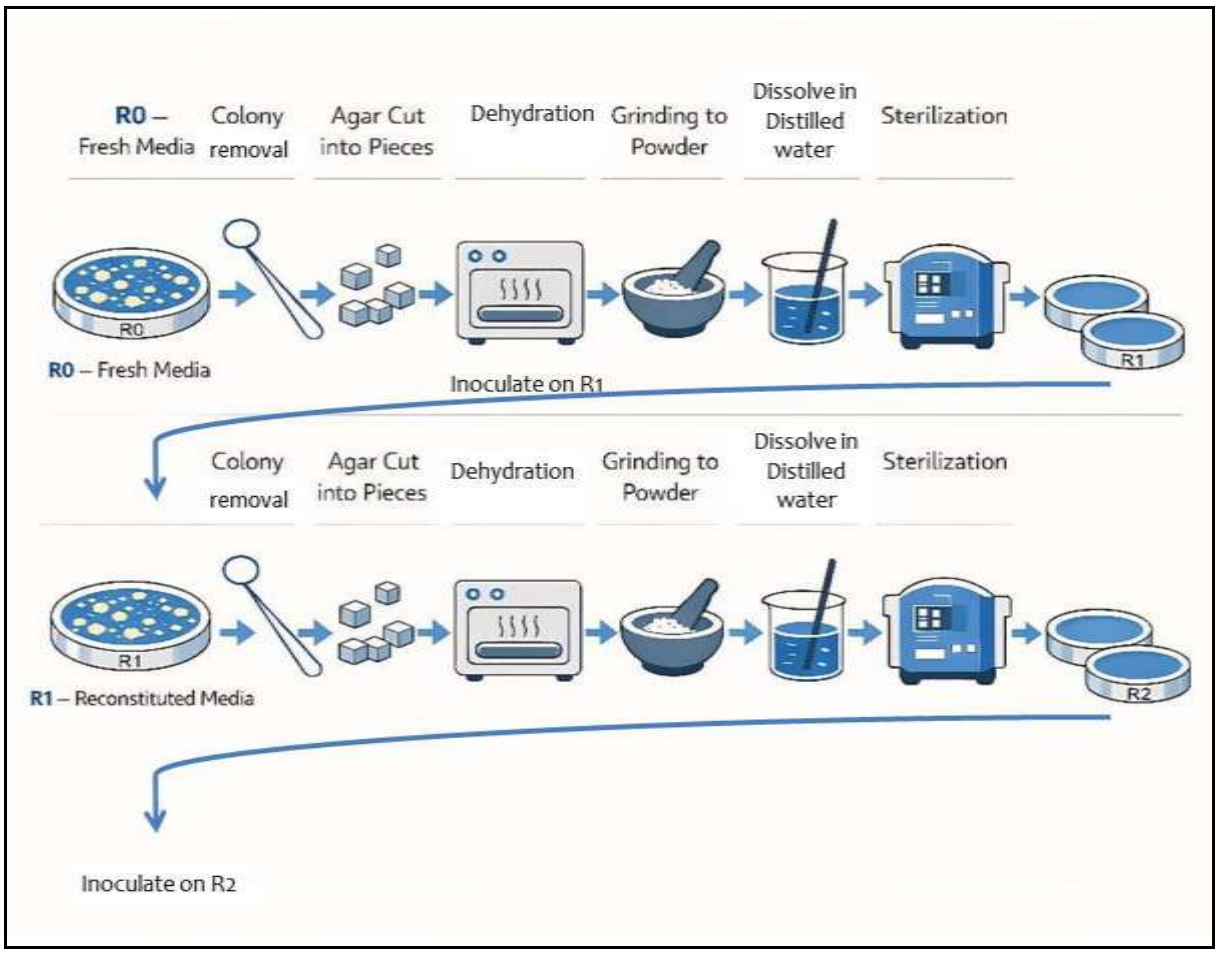
bacterial cultivation can be performed. Using

CONCLUSION :

Collectively, existing literature demonstrates that bacterial growth is intricately linked to media composition, agar properties, and the history of media usage. While extensive work has addressed nutrient formulation and agar quality, fewer studies have systematically examined the effects of repeated media recycling on microbial growth. The work of Yesudas et al. (2019) provides strong evidence that agar recycling is feasible with appropriate treatment, while studies such as Abboudi (2021) highlight the limitations associated with nutrient depletion and growth inhibition during repeated reuse by dehydrating the medium after colony clearance. (Arora et al. 2023) gives relation between bacterial growth and economic sustainability. Such studies addresses practical concerns related to laboratory sustainability which can be resolved by understanding microbial adaptation to the fresh and repeated cycles of reused medium environment. By integrating physiological, physicochemical, and environmental perspectives, research on media reuse contributes to a more comprehensive understanding of how microorganisms interact with their growth substrates over repeated cycles of use.

FUTURE ASPECTS :

According to the earlier discussion about the preliminary study on bacterial growth upon reused media, study focusing on secondary metabolite production like antibiotics, toxins,, inhibitors, pigments by bacteria on reused medium by the bacteria on reused media can also be performed. This assessment might help us to get a comprehensive understanding of the effect of reused media on bacterial cultivation. Changes in colony pigmentation observed on recycled media may influence interpretation, therefore confirmatory biochemical and molecular tests should be performed to ensure accurate identification. Similar experiments on reusing selective media with suitable cultures would further strengthen validation of reuse strategies. Nutrient loss over recycling the medium can also be compared and analysed between complex and synthetic media. Such investigations would provide a deeper insight into limitations and optimization potential of media reuse for sustainable microbial cultivation.



The above mentioned image is the proposed schematic representation of media reuse. R2 inoculated medium can be further processed to get R3 media by following the same procedure.

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