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Microbial Utilization of Ramie Gum: A Potential Avenue for Biotechnological Applications

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Abstract
Ramie (*Boehmeria nivea*) contains approximately 30–35% gum, composed of cellulose, hemicellulose, lignin, pectin, and waxes. This gum is a rich source of carbon and can be utilized as a medium component for microbial growth. The current study was conducted to investigate the mechanism by which Ramie gum stimulates the growth of various bioremediating bacterial strains. The high concentration of total carbohydrate in the Ramie gum substituted Luria–Bertani (LB) broth enhanced the log phase of growth substantially for *Bacillus* sp. (MCC0008), *Micrococcus luteus* (SRCHD08), and *Pseudomonas* sp. (SRCOD5), being cellulase, amylase, and pectinase producing strains. Bacterial strains *Brevundimonas diminuta* (SRCHD03), *Brucella pseudintermedia* (SRCHD05), and *Ochrobactrum* sp. (SRCHD06), which do not produce cellulase, amylase, or pectinase, showed no growth enhancement when Ramie gum was substituted in LB broth. These enzymes (cellulase, amylase, and pectinase) degrade cellulose, hemicellulose, and pectin into sugar compounds that can be utilized by bacteria. The stimulation of biofilm formation may be attributed to the presence of pectin and xylan in the gum, potentially mediated through the Spo0A gene. The strains showing higher growth and biofilm stimulation demonstrated higher bioremediation ability. Thus, the gum discarded during fiber spinning can be effectively valorized as a substrate for bacterial growth.

Keywords:
Ramie gum; microbial growth enhancement; natural carbon source; growth phase; cellulose; amylase; pectinase

1. Introduction

Microorganisms are a major reservoir of biodiversity, exhibiting diverse metabolic capabilities. They play essential roles in biogeochemical cycles, such as the carbon, sulfur, phosphorus, and nitrogen cycles, facilitating the circulation of chemical elements between biotic and abiotic components of the ecosystem [1–3]. Microorganisms also play a critical role in bioremediation through bioaugmentation, biostimulation, and natural attenuation. Beneficial microbes are involved in plant growth promotion and increasing productivity, either by supplying minerals and nutrients [4], secreting plant growth-promoting phytohormones [5] for plant growth, or by inhibiting the plant pathogens.

Six macronutrients—carbon, hydrogen, oxygen, nitrogen, sulfur, and phosphorus—serve as the primary building blocks of life [6]. Microbial growth strongly depends on cellular metabolic activity and the synthesis of biomolecules, including nucleosides, nucleotides, essential sugars, amino acids, lipids, and organic phosphates. These biomolecules serve as the building blocks for DNA, RNA, proteins, cell membranes, and bioenergetic compounds such as ATP (adenosine triphosphate). Microorganisms are highly adaptive to changes in their surroundings, which allows them to thrive in diverse environments across the Earth's ecosystems [4,7]. Microbes can thrive under extreme environmental conditions, including nutritional and water scarcity [8]. The survival of microbes in extreme environments is supported by the functional or native conformation of proteins or enzymes, genetic plasticity, and the ability to form biofilms [8,9].

Microbes can be cultivated in culture media containing nutrients such as carbon, nitrogen, sulfur, inorganic

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phosphate, minerals, water, and vitamins [10]. Different enrichment media provide essential nutrients and growth factors for the microbial cells and play a regulatory role in microbial trade-off mechanisms and resource investment. Depending on the surrounding extracellular nutrient profiles, microbes adopt different strategies (resource acquisition, growth strategies, and maintenance) for adaptation to the environment [11]. Plant-origin compounds have been continuously used in microbiological and biotechnological applications. Agar is one of the most important plant-derived compounds in the life sciences, known for its clarity, stability, and minimal interference with microbial metabolism [12]. A study by Jain et al. (2005) on the potential application of guar gum as a gelling agent, in addition to agar, for 33 microbial cultures (11 bacteria and 12 fungal cultures) revealed normal differentiation and growth in guar gum, similar to agar [12,13].

Lignocellulosic biomass is the cheapest source of carbon containing glucose, xylose, and arabinose monomers [14]. Use of the plant-origin Ramie (*Boehmeria nivea*) gum as an additive to diluted LB broth enhances microbial growth compared to the recommended concentration of LB broth alone [15]. It was reported that Ramie gum is composed of cellulose, hemicellulose, lignin, and pectin [15]. Microbes producing cellulase and xylanase can degrade the most abundant components of Ramie gum cellulose and hemicellulose, respectively [16]. Lignin in Ramie gum can be broken down by enzymes such as laccases, peroxidases [17], and oxidases [18]. Pectin can be degraded by pectinase-producing bacteria [16]. Microbes having this enzyme-producing ability can degrade lignocellulosic materials into smaller units, which further helps to stimulate their growth [19]. Microbial utilization of lignocellulosic waste is a widely studied topic nowadays. It has been reported that agro-waste can be valorized through bioethanol production using microbial consortia (fungal and bacterial), followed by yeast-mediated fermentation [20]. Ray Chaudhuri et al. (2025) reported that lignocellulosic biomass of oil-extracted lemongrass can be valorised through mushroom cultivation [21]. Precomposed Ramie decorticated waste was reported to be used for handmade paper making [22].

The literature indicates that complex polysaccharides, such as cellulose and xylan, when used as carbon sources for bacterial growth, exhibit less microbial antagonism than growth on simple sugars like glucose [19]. Moreover, enriched medium, often used for growing parent cultures in case of biofilm reactor setup at pilot/industrial scale for bioremediation and biotechnology purposes, often discharging waste medium with high chemical oxygen demand (COD). This high COD is due to unutilized growth nutrients and the end products of microbial metabolism. The other alternative to minimizing this pollution would be to optimize the growth medium composition to stimulate bacterial growth and attachment while minimizing the discharge of unused growth nutrients. Hence, this study was conducted: (a) to determine how, and at which growth phase, Ramie gum-supplemented LB medium stimulates the growth of different bioremedially important bacterial strains; (b) to assess whether supplementation with 25% Ramie gum and 75% LB can enhance the biofilm-forming ability of the microbes; (c) to elucidate the possible mechanism of biofilm enhancement by the strains in response to gum polysaccharides; and (d) to evaluate the elevated performance of the strains in terms of bioremediation ability when supplemented with 25% Ramie gum and 75% LB.

2. Materials and Methods

2.1. Ramie Gum Extraction

Five millilitres (5 mL) of 1% Na_2CO_3 solution was added to 1 g of Ramie fiber and boiled at 100 °C for 20 min. After 20 min of boiling, the liquid was collected from the fibers. The total volume of the collected liquid was determined, and an equivalent volume of chilled acetone was added. The gum in the solution precipitated upon the addition of chilled acetone. Finally, the acetone was decanted, leaving the precipitate behind. The precipitated gum slurry was dried in a hot air oven.

2.2. Enzyme Production by the Isolates

The microbes selected for the study were potent bioremediants. While *Bacillus* sp. (MCC0008) removes nitrate [23], *Pseudomonas* sp. (SRCOD5) removes ammonia, and *Micrococcus luteus* (SRCHD08), *Brevundimonas diminuta* (SRCHD03), *Brucella pseudintermedia* (SRCHD05), as well as *Ochrobactrum* sp. (SRCHD06), remove hexamine [24]. The capacity of bacteria to produce extracellular enzymes is an essential criterion for their identification. These profiles help identify the functional characteristics of bacteria, including their ability to utilize or degrade specific substrates. The extracellular enzyme-producing ability of the isolates (MCC0008, SRCHD03, SRCHD05, SRCHD06, SRCOD5, and SRCHD08) were checked using standard protocols and reported (Gogoi et al., 2024; extracellular protease, amylase, catalase, oxidase, DNase, and lipase as per Nandy et al., 2007; cellulase according to Kasana et al., 2008; pectinase according to Yao et al., 2017; and gelatinase following Cruz et al., 2012) [25–29].

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2.3. Microbial Growth on Different Concentrations of Media Solution

Media solutions were made in different concentrations: 25% Ramie gum (0.25 gm in 100 mL distilled water), 100% LB [1 gm LB {1% Tryptone, 0.5% Yeast Extract and 0.5% sodium chloride (NaCl)} mix in 100 mL distilled water at pH 7.0], and Ramie gum: LB (25%:75%, 25%:50%, 25%:25%) were prepared and sterilized at 121 °C for 15 min. Each concentration of media (200 microliters) was separately inoculated with 1% (*v*/*v*) of the culture {which could be *Bacillus* sp. (MCC0008), *Brevundimonas diminuta* (SRCHD3), *Brucella pseudintermedia* (SRCHD05), *Ochrobactrum* sp. (SRCHD06), *Pseudomonas* sp. (SRCOD5), and *Micrococcus luteus* (SRCHD08)} in biological replicates of three within 96-well plates. Then the plate was placed inside the microplate reader (BioTek, EPOCH2TS) for 28 h at 37 °C, and the growth in terms of optical density (OD) was measured during the entire growth period at 600 nm.

2.4. Biochemical Assay

The chemical oxygen demand (COD) and total carbohydrate content of various media, including 25% Ramie gum in water, 100% LB, 75% LB, and 25% Ramie gum with 75% LB, were quantified to investigate the mechanism by which the gum influences microbial growth. Raw Ramie gum is not totally soluble in water; however, 25% Ramie gum was completely soluble in LB broth. The total carbohydrate content was determined using the phenol-sulphuric acid method [30]. COD was assessed using the dichromate method [25]. All estimations were done in triplicate, followed by statistical validation of the findings.

2.5. Biofilm-Forming Ability

In a sterile 24-well tissue culture plate (Tarsons, Cat. No. 980030), 2 mL sterile media (100% LB and 25% gum with 75% LB) were added, and 1% of actively growing cultures of isolates MCC0008, SRCOD5, and SRCHD08 were inoculated individually. Uninoculated media were considered as negative controls for the experiment. The plate was incubated for 24 h at 37 °C under static conditions. After 24 h, biofilm-forming ability was quantified using Martin's method [31], which involves crystal violet staining. All estimations were done in biological triplicate with statistical validation of the data.

2.6. Bioremedial Study

Bioremediation ability under immobilized conditions was tested for the isolates MCC0008, SRCOD5, and SRCHD08. Actively grown cultures were inoculated in 50 mL Falcon tubes containing a sterile Raschig ring with 15 mL 100% LB and 25% gum in 75% LB broth, respectively. The tubes were incubated at 37 °C for 24 h to establish a biofilm on the surface of the Raschig ring. After incubation, the cultures were decanted from the respective 50 mL Falcon tubes. Tubes with MCC0008 and SRCOD5 biofilm were recharged with 25% Ramie gum in 75% LB broth. On the other hand, the tube with the SRCHD08 biofilm was recharged with 25% Ramie gum in 75% LB broth with 100 mg/L hexamine. Nitrate (after 5 h), ammonia (after 10 h), and hexamine (after 24 h) were quantified using the Salicylic acid method [32], Nessler's reagent method [33], and the Hantzsch reaction method [34], respectively. All estimations were done in biological triplicate with statistical validation of the data.

2.7. In Silico Analysis

The contigs of the MCC0008 genome sequence were submitted to the Rapid Annotation using Subsystem Technology (RAST) server. The number of contigs was 331. The system (SEED viewer) was searched for the presence of genes involved in biofilm formation.

2.8. Statistical Analysis

Each experiment reported above was carried out in triplicate, with three biological replicates in each case. Hence, the data presented have a sample size of 9 ($n = 9$). The statistical analysis included an F-test followed by a one-tailed t-test. The F-test was performed to determine whether equal or unequal variances should be used for the one-tailed t-test. Microsoft Excel 365 was used to conduct the analyses. The statistical analysis was performed at a 95% confidence level.

3. Results

3.1. Ramie Gum Extraction

Ramie fiber grown at Tripura University contains 30–35% Ramie gum, of which only 2.52 ± 0.78% Ramie gum can be easily extracted.

3.2. Enzyme Assay of the Isolates

The enzyme-producing abilities of the six isolates are shown in Table 1. The enzyme production of isolate MCC0008 is reported in Banerjee 2018 [35], while that of isolates SRCHD03, SRCHD05, SRCHD06, and SRCHD08 is reported in Samal et al., 2024 [24].

Table 1: Extracellular enzyme-producing ability of the bacterial isolates.

Enzymes	MCC0008 [35]	SRCHD03 [24]	SRCOD5 (Unpublished Data)	SRCHD06 [24]	SRCHD08 [24]	SRCHD05 [24]
Catalase	+	-	+	+	+	+
Oxidase	+	+	+	+	-	+
Protease	+	-	-	-	+	-
Amylase	+	-	+	-	-	-
Lipase	+	-	-	-	-	-
DNase	+	-	-	-	-	-
Cellulase	+	-	+	-	-	-
Pectinase	+	-	+	-	+	-
Gelatinase		+	-	+	+	+

3.3. Microbial Growth Enhancement by Ramie Gum

Based on the previous report by Banerjee et al. (2018) [35], the initial experiments were conducted using the following media compositions: 100% LB and a combination of 25% gum with 75% LB (Figure 1). The result showed a significant growth enhancement (Table 2) in 25% Ramie gum with 75% LB broth as compared to 100% LB broth for *Bacillus* sp. (MCC0008), *Micrococcus luteus* (SRCHD08), and Pseudomonas sp. (SRCOD5), as shown in Figure 1 and Table 3. However, the *Brucella pseudintermedia* (SRCHD05), *Brevundimonas diminuta* (SRCHD03), and *Ochrobactrum* sp. (SRCHD06) showed no significant growth difference (Table 2) among 100% LB and gum to LB ratio of 25%:75%.

Table 2: Statistical analysis of the growth difference of bacterial isolates in the presence of 25% gum with 75% LB and 100% LB alone.

Isolates	LB 100% vs. 25% Gum:75% LB	*p* Value
MCC0008	Growth enhanced	7×10^{-3}
SRCHD03	No growth enhancement	2.13×10^{-1}
SRCHD05	No growth enhancement	5.7×10^{-2}
SRCHD06	No growth enhancement	4.7×10^{-1}
SRCOD5	Growth enhanced	6×10^{-6}
SRCHD08	Growth enhanced	8.82×10^{-7}

Table 3: Analysis of fold change in bacterial growth enhancement in 25% gum with 75% LB compared to 100% LB alone.

Isolates	25% Gum:75% LB	Fold Increase
MCC0008	Enhanced log phase	1.44
SRCOD5	Enhanced log phase	2.16
SRCHD08	Enhanced log phase	1.88

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Based on the above data, three isolates (MCC0008, SRCOD5, and SRCHD08), which showed visible growth differences, were selected for the growth curve assessment in the varying concentrations of the media (Figure 2): (1) only 25% Ramie gum, (2) Ramie gum: LB (25%:50%), (3) Ramie gum: LB (25%:25%), (4) Ramie gum: LB (25%:75%), (5) only 100% LB. At 8 h, SRCOD5 showed an OD of 0.411 in 100% LB and 0.874 in gum: LB (25:75 %) media at 600 nm. For SRCHD08, the OD was 0.437 in 100% LB and 0.889 in 25% gum:75% LB media. For MCC0008, growth in 100% LB and 25% gum:75% LB media was 0.732 and 1.057, respectively, at 12 hours of incubation. *Bacillus* sp., *Pseudomonas* sp., and *Micrococcus luteus* exhibited higher growth in 25% gum and 75% LB broth compared to other concentrations. For *Micrococcus luteus*, the highest growth was observed in 25% gum and 25% LB, though it was not significantly different (p-value 0.241) from the growth in 25% gum and 75% LB. Hence, the latter was considered for subsequent studies. The lowest growth of *Bacillus* sp. and *Pseudomonas* sp. was observed in 25% gum while that for *Micrococcus luteus* was observed in 25% gum, while that of *Micrococcus luteus* was observed in 100% LB.

Further investigation was carried out to determine the mechanism of growth enhancement in the isolates using biochemical methods. The total carbohydrate estimation revealed that the addition of 25% gum in 75% LB media enhanced the carbohydrate content by 3.34-fold compared to 100% LB (Figure 3).

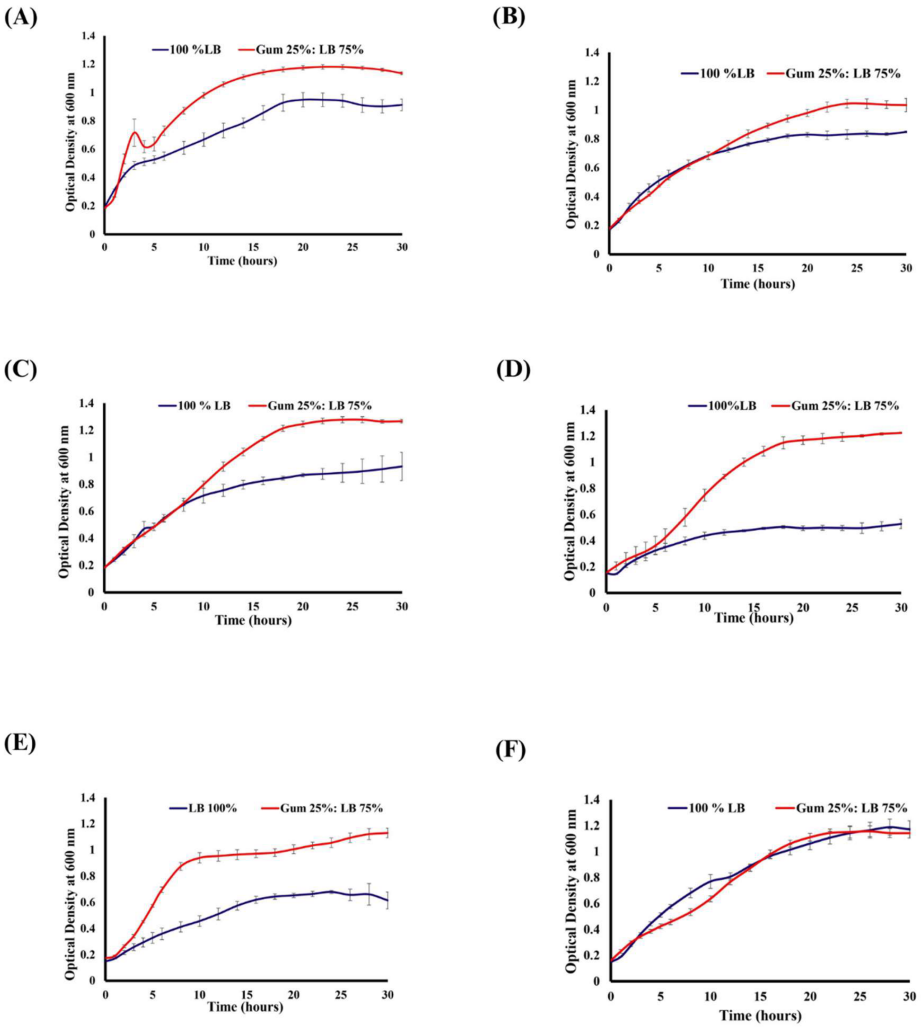

Figure 1: Growth curves of the isolates were done in biological triplicates using a 96-well microplate reader (BioTek, EPOCH2TS) for 28 h at 37 °C. In this study, 100% LB broth and 25% gum:75% LB were used. (**A**) Growth curve for isolate MCC0008, (**B**) Growth curve for isolate SRCHD03, (**C**) Growth curve for isolate SRCHD06, (**D**) Growth curve for isolate SRCHD08, (**E**) Growth curve for isolate SRCOD5, (**F**) Growth curve for isolate SRCHD05.

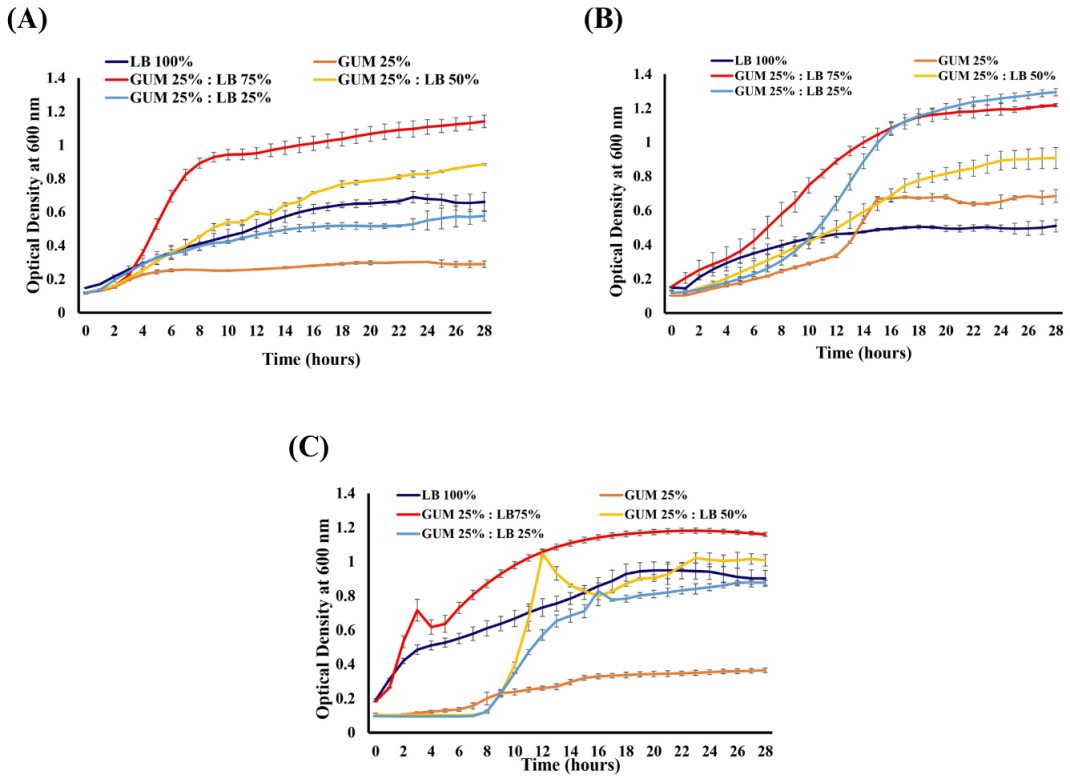

Figure 2: Growth curves of the isolates were assessed in biological triplicates using a 96-well microplate reader (BioTek, EPOCH2TS) for 28 h at 37 °C. In this study, 100% Luria Bertani broth and 25% gum:75% LB, 25% gum:25% LB, 25% gum:50% LB, and 25% gum were used. (**A**) Growth curve for isolate SRCOD5, (**B**) Growth curve for isolate SRCHD08, (**C**) Growth curve for isolate MCC0008.

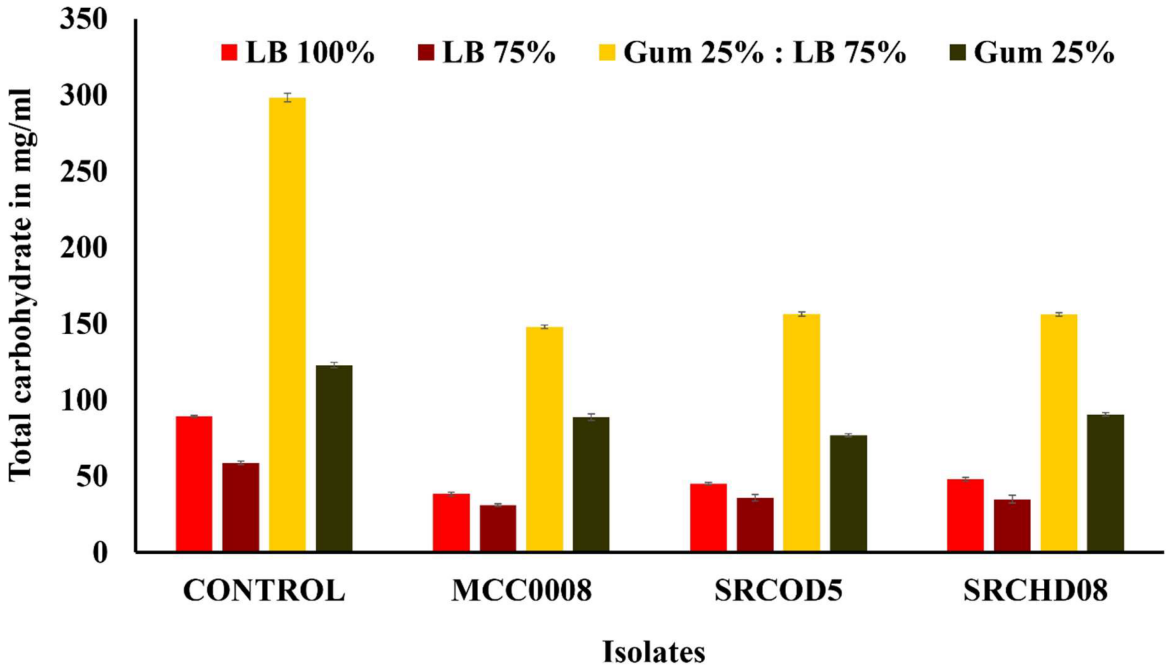

Figure 3: Total carbohydrate estimation in 100% LB (89.2 mg/L), 75% LB (58.8 mg/L), 25% gum with 75% LB (298.4 mg/L) and 25% gum (122.8 mg/L) before (control) and after inoculum with the isolates {MCC0008 (38.3, 31, 147.8, 88.6 mg/L), SRCOD5 (45, 35.8, 156.4, 76.8 mg/L), and SRCHD08 (47.9, 34.9, 156, 90.5 mg/L). All estimations were done in biological triplicate with statistical validation of the data.

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Before implementing this modified medium (25% gum with 75%) to enhance the growth of the isolates for pilot/industrial-scale application, it was necessary to understand its impact on the environment. Therefore, COD of the medium before and after growth of the isolates was assessed. There was 22.28% lower COD in 25% gum with 75% LB as compared to 100% LB (Figure 4). So, it was an enhanced carbohydrate with reduced COD, which would stimulate bacterial growth with reduced impact on the environment due to lower COD of the discharged media. Based on their COD-reduction abilities, MCC0008, SRCOD5, and SRCHD08 were selected for further experimental validation. The biofilm-forming ability of the isolates MCC0008, SRCOD5and SRCHD08 showed 1.46 (p-value 1.3×10^{-10}), 1.97 (p-value 1.9×10^{-17}), and 1.13 (p-value 6×10^{-5}) fold higher enhancement in biofilm-forming ability (Figure 5).

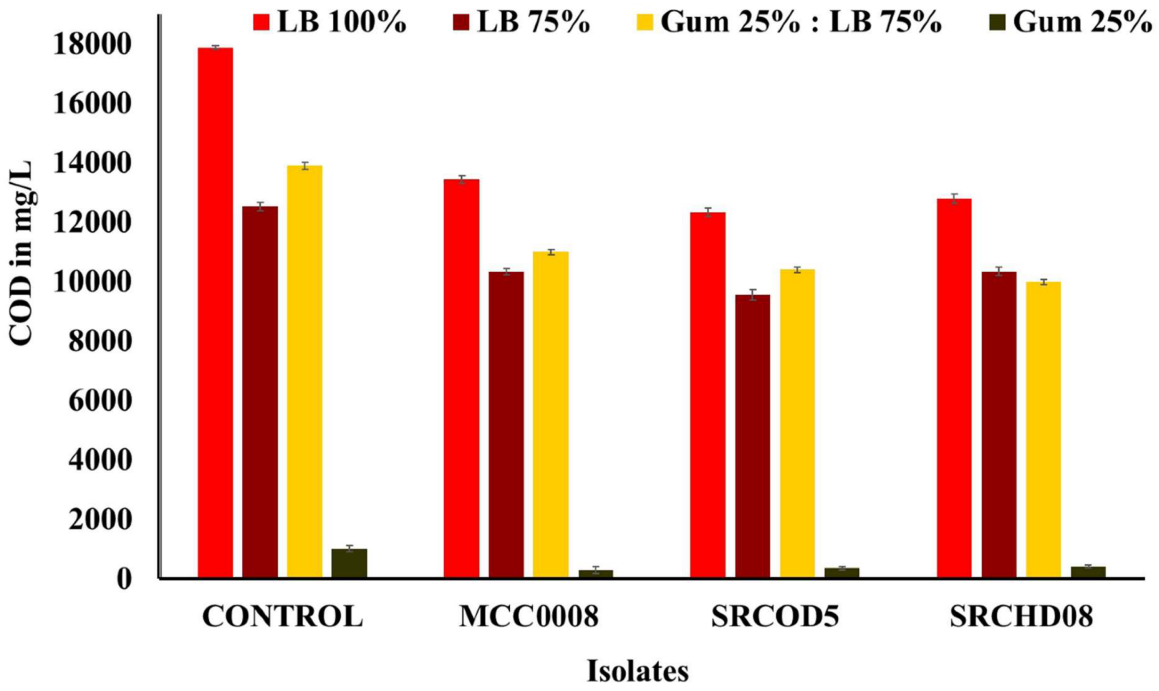

Figure 4: COD estimation in 100% LB (17,875.8 mg/L), 75% LB (12,519.4 mg/L), 25% gum with 75% LB (13,891.3 mg/L) and 25% gum (1012.05 mg/L) before (control) and after inoculum with the isolates {MCC0008 (13,437.7, 10,330.4, 10,997.6, 296.1 mg/L), SRCOD5 (12,328.3, 9562, 10,405.4, 359.8 mg/L), SRCHD08 (12,789.3, 10,341.7, 9981.8, 412.3 mg/L). All estimations were done in biological triplicate with statistical validation of the data.

The isolates MCC0008, SRCOD5, and SRCHD08 were tested for their biofilm-based bioremediation ability to remove organic pollutants (nitrate, ammonia, and hexamine), where it was found that the developed biofilm bioreactor in 25% Ramie gum in 75% LB showed a significant increase of 12.1% (p value 6.4×10^{-6}) of ammonia and 4.8% (p value 8.9×10^{-6}) of hexamine removal compared to the biofilm system in 100% LB. On the other hand, MCC0008 could completely remove nitrate from an initial concentration of 4.3 mg/L in both systems.

3.4. In Silico Analysis

RAST data of the draft genome sequence of *Bacillus* sp. MCC0008 [23] revealed the presence of genes for Spo0A (matrix-producing and sporulation gene), SinI, SinR, Eps C and Eps D (for exopolysaccharide biosynthesis), as well as Tas A protein (matrix protein component), pointing towards the possible pathway of biofilm induction. From the RAST analysis, the closest neighbors of strain MCC0008 were *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*. All the species were reported to contain the Spo0A gene for biofilm stimulation. In *Bacillus thuringiensis*, Spo0A is very important for biofilm formation [36]. Another study reported that the Spo0A mutant of *Bacillus thuringiensis is incapable of forming biofilms at an air-liquid interface, and that* Spo0A-P directly induces biofilm formation. Spo0A controls expression of sinI, which represses the biofilm repressor SinR [37]. Spo0A, SinI, and SinR are reported to be

present in *Bacillus cereus* and involved in biofilm formation [38]. It was also reported that *Bacillus anthracis* contains the Spo0A gene [39].

The literature reports biofilm formation by both Pseudomonas sp. and *Micrococcus luteus*. *P. aeruginosa* develops biofilms in various environments. The biofilm structure consists of exopolysaccharides, alginate, Pel, and Psl. Alginate is an essential polymer for biofilm protection and stability, while Psl is crucial for biofilm formation and stability. Pel, a glucose-rich polysaccharide, is also present in the biofilm. Surface proteins like the PpgL protein and the PA4204 gene play crucial roles in biofilm formation [40]. Genes involved in biofilm formation in the case of *Micrococcus luteus* were phnA, phnB, cyaB, vfr, vps, glgC, wecB, wecC, and cysE [41].

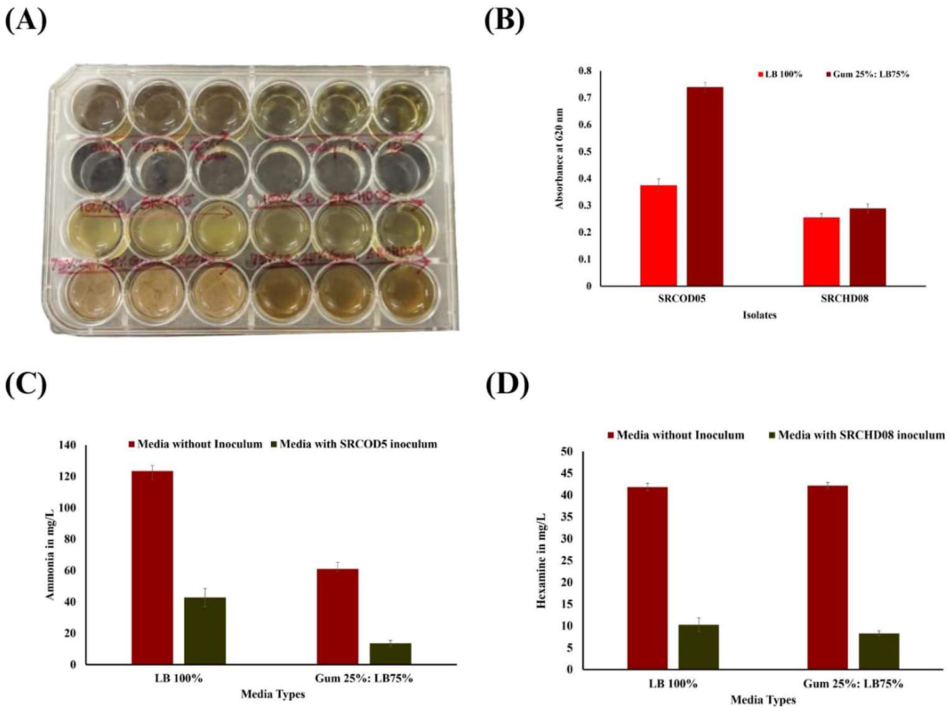

Figure 5: (**A**) Biofilm formation ability in a 24-well tissue culture plate; (**B**) Biofilm quantification using Martin's method, in which the absorbance of isolate MCC0008 at 620 nm in 100% LB and Gum 25%: LB75% were 0.551 and 0.802, respectively, for isolate SRCOD5 was 0.37 and 0.74, respectively. For isolated SRCHD08, it was 0.25 and 0.29, respectively. (**C**) SRCOD5 showed 65.42% ammonia removal in 100% LB (initial and final concentration was 123.5 and 42.77 mg/L) and 77.7% ammonia removal in Gum 25%: LB 75% (initial and final concentrations were 61.1 and 13.67 mg/L); (**D**) SRCHD08 showed 75.3% hexamine removal in 100% LB (initial and final concentration was 41.8 and 10.3 mg/L) and 80.28% hexamine removal in Gum 25%: LB 75% (initial and final concentrations were 42.1 and 8.3 mg/L). All estimations were done in biological triplicate with statistical validation of the data.

4. Discussion

It has been reported that tryptone in LB media provides amino acids, yeast extract provides nitrogen sources and a low concentration of organic carbon, and NaCl maintains osmotic balance [42], supporting the growth of many culturable bacteria in the laboratory. On the other hand, Ramie gum contains cellulose (68.6–76.2%), hemicellulose (13.1–16.7%), lignin (0.6–0.7%), and pectin (1.9%), which are rich in carbon sources [16]. In this study, the total carbohydrate content data revealed that the medium containing 25% gum and 75% LB exhibited a 3.34-fold higher carbohydrate content compared to 100% LB (Figure 3). The addition of a natural carbon source, 25% Ramie gum in 75% LB (with protein and limited carbohydrates), enables extracellular enzyme-producing bacterial isolates (cellulase, amylase, and pectinase) to thrive better, reaching higher cell densities and forming structured biofilms (except for SRCH08). The isolates MCC0008, SRCOD5, and SRCHD08 in 25% Ramie gum with 75% LB could utilize 150.6, 140, and 140.2 mg/L of total carbohydrate, respectively, after 10 h of incubation at 37 °C, boosting their cell growth in the log phase. The carbohydrate utilization in 100% LB was 50.94, 44.19, and 41.29 mg/L for isolates MCC0008, SRCOD5, and SRCHD08, respectively.

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On the other hand, isolates that lacked the ability to produce these extracellular enzymes were unable to utilize the additional polysaccharides present in 25% Ramie gum and showed insignificant growth differences between 100% LB and 25% gum with 75% LB. COD data showed that 25% gum with 75% LB had a lower COD load than 100% LB. The total carbohydrate utilization, COD load, and growth enhancement emphasized the potential application of the Ramie gum as a microbiological growth enhancer. These selected isolates (MCC0008, SRCOD5, and SRCHD08) are potential bioremediants; therefore, further investigation was carried out towards their bioremediation ability. The presence of 25% Ramie gum in 75% LB not only enhanced the cell growth but ensured strong biofilm formation except in the case of SRCHD08 (showing structured biofilm formation). Literature reported an increase in the biofilm-forming ability in the presence of plant phytochemicals in many bacterial species. Ghosh et al. (2016) reported that the presence of phytochemicals in the leaf extract of *Nyctanthes arbour-tristis* stimulates the biofilm-forming ability of the epiphytic bacteria significantly, showing 1.4 to 8.38 times enhancement; for the *Azadirachta indica* extracts, it was 1.2 to 3.6 times, and for *Ocimum sanctum* and *Mentha spicata*, it was 1 to 7.5 times and 2 to 6 times higher biofilm stimulation [43]. Complex carbohydrates of plant origin have been shown to support bacterial growth and often reduce the antagonistic effects exhibited by simple carbon sources, such as glucose, during microbial community interactions [19]. *Bacillus subtilis* also uses these plant polysaccharides by converting them to UDP-galactose, which gets incorporated into the Extracellular Polymeric Substances of the matrix. This response of *Bacillus* sp. to plant polysaccharides was observed among different strains of the genus [44].

Biofilm quantification data from this study revealed that the presence of 25% Ramie gum with 75% LB significantly enhanced the biofilm-forming ability of MCC0008, SRCOD5, and SRCHD08 compared with 100% LB. The literature suggests that the extracellular matrix is composed of two major components: EPS and the TasA protein [44]. The expression of enzymes involved in EPS production and the TasA protein is regulated by the Spo0A gene, which serves as the principal regulatory gene for biofilm formation in Bacillus sp. [44]. Activation of Spo0A occurs when it is phosphorylated through the kinases (kinase A to kinase E). The kinases phosphorylate the Spo0A after receiving the environmental signal. Though the low level of Spo0A stimulates the matrix formation, higher levels of Spo0A induce sporulation. The Spo0A-activated cells secrete toxins during food scarcity, which kill their sensitive neighbouring cells and delay sporulation [45]. RAST data for the draft genome sequence of MCC0008 [23] revealed that it contains Spo0A (matrix-producing and sporulation gene), SinI, SinR, Eps C and Eps D (for exopolysaccharide biosynthesis), and the Tas A protein (matrix protein component). SinI is the repressor of SinR (transcriptional repressor of matrix genes). Activated Spo0A accumulation results in SinI synthesis. It was reported that a mutation in the SinI or Spo0A gene leads to inhibition of plant polysaccharides-induced biofilm stimulation [44]. Ramie gum contains polysaccharides such as pectin and xylan, which can act as environmental cues for biofilm stimulation when used at 25% and 75% in LB (Figure 6).

It was reported that cellulose is composed of glucose molecules and can be readily degraded by cellulase [46]. Cellulolytic bacteria use cellulose as a substrate to produce glucose molecules [47]. Hemicellulose can be degraded by many enzymes, yielding hexose sugars such as mannose, glucose, and galactose, and pentose sugars such as arabinose and xylose [46]. Lignin can be degraded by ligninolytic enzymes like oxidases and peroxidases and produce coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol [18,46]. Pectin can be degraded into galacturonic acid, rhamnose, arabinose, glucuronic acid, galactose, xylose, and fucose [48].

The literature suggests that bacterial growth in LB broth is limited due to a shortage of utilizable carbon sources, and that the addition of glucose can support growth [49] after utilizable carbon is exhausted in LB. Hence, cultivation of six bacterial strains was checked with 75% LB medium along with 25% Ramie gum (as a carbon source). The extracellular enzyme-producing isolates degraded the Ramie gum and led to higher cell growth, feeding on the degraded materials.

Bacillus sp. was reported for its wax-utilizing ability [50]. The degraded wax is used as the carbon source. Rapid Annotation using Subsystem Technology (RAST)-based data analysis of strain MCC0008 has shown that it harbors a gene cluster for glucose, fructose, and mannose utilization. The enzyme assay showed that this strain has cellulase, amylase, and pectinase-producing ability, which can degrade cellulose, hemicellulose, and pectin present in Ramie gum into glucose, fructose, and mannose. It was reported that *Bacillus subtilis* and other Bacillus prefer glucose as a carbon and energy source [51]. The most probable reason for the sudden drop in growth in 25% gum and 75% LB at the 4th hour was due to preferential utilization of glucose first and then switching to other carbon sources. This isolate, MCC0008, also showed higher growth on 25% gum and 75% LB than in other growth conditions. *Brevundimonas* sp., *Ochrobactrum* sp., and *Brucella pseudintermedia* were known to produce oxidase [52–54].

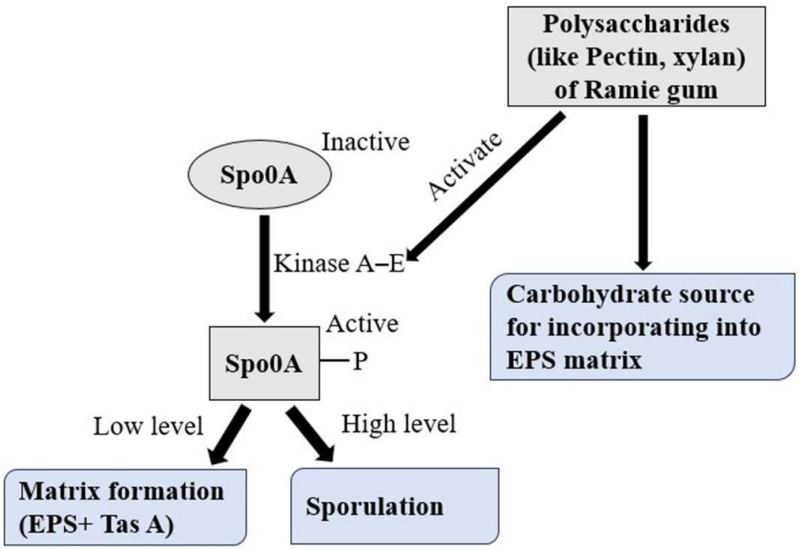

Figure 6: Possible mechanism for biofilm stimulation using 25% Ramie gum and 75% LB. Plant polysaccharides can induce biofilm formation either by acting as an environmental cue to activate kinases, which in turn activate the Spo0A gene for EPS production, or by serving as a carbohydrate source for incorporation into the EPS matrix.

SRCHD03 (*Brevundimonas diminuta*), SRCHD06 (*Ochrobactrum* sp.), and SRCHD05 (*Brucella pseudintermedia*) used in this study have the ability to produce oxidase, as shown in Table 1, and thereby could degrade lignin and feed on it. However, the pathway for lignin metabolism in bacterial systems is highly intricate. Bacteria can degrade lignin to produce small aromatics, which they then consume [55]. According to the literature, Pseudomonas species are known to produce cellulase enzymes [56]. The cellulose present in Ramie gum can be degraded into glucose by cellulase-producing bacteria, which can further sustain their growth. The *Pseudomonas* sp. (SRCOD5) used in this study has the ability to produce cellulase and pectinase, as shown in Table 1. When grown on 25% gum with 75% LB broth, they utilized the additional carbon source from the gum, along with the amino acids and organic compounds present in the 75% LB broth, resulting in much higher growth than in 100% LB media. When 50% LB was combined with 25% gum, bacterial growth decreased compared to 75% LB with 25% gum, but remained higher than in 100% LB broth. However, when 25% gum was provided with 25% LB broth, the growth of the isolate SRCOD5 decreased compared to 100% LB broth, indicating that the additional carbon source was not solely responsible for the enhanced growth and a higher amount of catabolizable amino acids was also required for the growth of the isolate.

The carbon source used by the bacteria from Ramie gum can be cellulose-derived glucose or pectin-derived galacturonic acid/rhamnose/arabinose/glucuronic acid/galactose/xylose/fucose. It was reported that bacteria can utilize carbon sources sequentially (one carbon source after another) or simultaneously (co-utilization) [57]. Co-utilization of a carbon source is required for good bacterial growth. As LB broth contains catabolizable amino acids as a carbon source, the isolate SRCOD5 may co-utilize the LB carbon source along with other carbon sources present in Ramie gum, resulting in higher cell growth than in 100% LB broth. It has been reported that certain bacteria, including *Bacillus subtilis* and *Escherichia coli*, preferentially utilize glucose over other sugars [58]. They use a carbon catabolite repression (CCR) mechanism for inhibiting other secondary carbon sources, as shown in Figure 7 [59]. Bacteria tend to utilize other carbon sources when glucose is exhausted [60]. However, strategies for microbial carbon utilization remain complex. It was also reported that, at low glucose concentrations, co-utilization of glucose and other pentoses can occur [59]. The isolate may utilize glucose as a carbon source or co-utilize glucose and other sugars by reducing the CCR mechanism.

The other isolate, SRCHD08, has pectinase-producing ability and thereby degrades pectin into galacturonic acid, rhamnose, arabinose, glucuronic acid, galactose, xylose, and fucose. The isolate utilized carbon sources from 25% Ramie gum, along with amino acids and other organic compounds from 75% LB broth, to achieve higher cell numbers. Literature suggests that in the absence of glucose, bacteria preferentially utilize arabinose first, followed by xylose [59].

This approach can enhance bacterial growth and promote stable biofilm formation, enabling the development of biofilm systems with improved performance in biore-

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mediation or enzyme production. Additionally, it can reduce the COD of the spent medium, supporting the economical and eco-friendly pilot-scale implementation of bacterial processes.

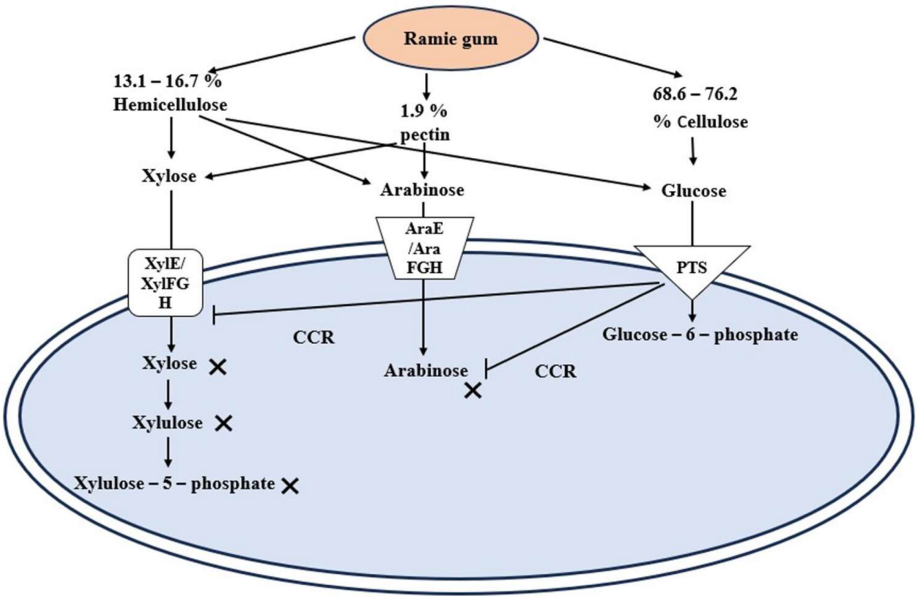

Figure 7: Mechanism of CCR by the phosphotransferase system (PTS) in the presence of glucose and other sugars. When glucose is present, the PTS induces CCR, which inhibits the transportation of xylose and arabinose. CCR (carbon catabolite repression); XylE (xylose-proton symporter); XylFGH (xylose ABC transporters); AraE (arabinose-proton symporter); AraFGH (arabinose ABC transporter).

5. Conclusions

This approach allows waste from the Ramie fiber extraction process to be converted into a value-added substrate that sustains the growth of microorganisms with extracellular enzyme activity. These enzymes can degrade Ramie gum into simple carbohydrates, which can then be utilized for cell growth. Such bacterial species (having polysaccharides-degrading extracellular enzymes producing ability) can utilize the amino acids and organic compounds from LB broth (75%) and additional sugars from Ramie gum for achieving higher growth and biofilm stimulation. This supplementation reduces the requirement for commercial growth medium. On one hand, the cost for microbial cultivation goes down due to less commercial medium requirement; on the other hand, the extent of microbial growth increases, enhancing the microbial activity (be it enzyme production or bioremediation). In addition, environmental concerns associated with the discharge of spent medium are largely mitigated due to the reduction in its COD. Hence, a waste from plant origin is being valorized as a microbial growth medium supplement, supporting environmental protection. After extraction, the Ramie gum was converted into Ramie flakes to enable longer-term storage (Figure 8).

Figure 8: Flakes prepared from Ramie gum.

Hence, sustainable utilisation of Ramie gum is possible by using it as a bacterial feed.

List of Abbreviations

AraE	Arabinose-Proton Symporter
AraFGH	Arabinose ABC Transporter

ATP	Adenosine Triphosphate
CCR	Carbon Catabolite Repression
COD	Chemical Oxygen Demand
DNA	Deoxyribonucleic Acid
LB	Luria Bertani
OD	Optical Density
PTS	Phosphotransferase System
RAST	Rapid Annotation Using Subsystem Technology
RNA	Ribonucleic Acid
XylE	Xylose-Proton Symporter
XylFGH	Xylose ABC Transporters

Author Contributions

Formal analysis, data curation, original draft preparation, validation, visualization/figures, software: E.P. and S.K.W.A.; formal analysis: S.S.; Supervision, Investigation, resources, writing---review and editing, Conceptualization, methodology, funding acquisition, project administration: S.R.C. All authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials

The data supporting the findings of this study are included within the manuscript.

Ethics Committee Approval and Consent to Participate

The Ramie (*Boehmeria nivea*) fiber used in this study was obtained from our experimental field (cultivated source) at Tripura University. No permissions or special approvals were required for the use of this plant material.

Conflicts of Interest

The authors declare no conflicts of interest.

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AI Declaration

The authors confirm that no AI tools were used to generate any content of this manuscript.

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