Characterisation and Optimisation of Succinoglycan from Garden Grass using *Agrobacterium radiobacter* NCIM 2443

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Abstract. Succinoglycan is an exopolysaccharide that is produced mainly by soil-dwelling microorganisms. It has certain unique structural properties, making it a super viscosifying agent and a thermally stable gel. These properties are contributed by its acidic and water-soluble nature. This heteropolymer is made up of one galactose residue for every seven glucose residues and also includes certain non-carbohydrate residues, namely succinate, acetate and pyruvate. The polymer finds various applications as a thickener and emulsifier for the food industry, fluid loss agent for the cement industry, as a cosmetic additive, etc. Currently, commercial production of succinoglycan is mainly through conventional media. This has a direct effect on the production costs and thus limits the market potential of the biopolymer. This calls for researching low-cost carbon sources. One such carbon source is garden grass, which is being produced and wasted in tons every year. The present study aims to hydrolyze the waste garden grass using chemical methods to recover fermentable sugars and to use these sugars along with nutrient supplementation for succinoglycan production. Initially, we have characterized the garden grass, which had 19.13% extractives. Further, the cellulose content was estimated to be 31.11±2.70 %, and lignin content was estimated to be 16.05 %. The maximum reducing sugar of 0.54 g per g of grass was obtained using 2% H_2SO_4, which was further used for the production of succinoglycan using microbial fermentation with the *Agrobacterium radiobacter* NCIM 2443 strain. The carbon source, glucose concentration, and C:N ratio were optimized for maximum succinoglycan production. The produced polymer was then characterized using NMR and FT-IR analysis.

1 Introduction

Biopolymers are basically polymers that are produced by living things like microorganism. It can also be phrased as polymeric biomolecules. The carbohydrate-based polysaccharides are polymeric carbohydrate structures that have linear or branched polymers of sugars within them [1, 2]. Since these polymers are produced by living organisms, they are also biodegradable and their long chain lengths leave a very low carbon footprint [3]. Compared to synthetically produced polymers that have relatively simpler structure, biopolymers tend to have complex structural features and thus adopt unique 3D spatial geometries. It is these unique structures that give rise to biological activity for most of these biopolymers. Microorganisms are known to naturally produce many industrially important polysaccharides, polyesters, and polyamides. These biopolymers have a varying range of applications, from being used as viscosity modifiers to plastics based on their properties. These properties include viscosity-modifying effect, inertness, composition and molecular weights. Some microorganisms are of increasing interest based on their production of multifunctional polysaccharides including intracellular, structural, or exopolysaccharides [4].

Exopolysaccharides are secreted out into the surrounding environment by the microorganisms. There is a multitude of applications of biopolymers that extend to exopolysaccharides in the fields of pharmaceuticals and food industries [5]. Most microbial exopolysaccharides are identical to gums that are in current use [6]. Succinoglycan is one such commercially important polymer, which is acidic, water insoluble made up of β-1,2-glucan monomeric units. Succinoglycan with excellent thermal stability and remarkable viscosity enhancing properties was first isolated from *Alcaligenes faecalis* var. *myxogenes* in 1965 [7]. The polymer is primarily produced by soil dwelling bacteria. The applications of this biopolymer mainly extend to food industry and construction industry owing to its unique rheological and stability characteristics.

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Agrobacterium radiobacter is a facultatively aerobic, heterotrophic bacterium that metabolizes organic compounds for energy and growth. During oxidative respiration, the bacterium stores energy in the form of polyglucose molecules while oxygen acts as the terminal electron acceptor. This is not seen in the case of anaerobic respiration. It can produce succinoglycan-like EPS when grown on carbon sources like ethanol or glucose under diverse growth conditions. Furthermore, its metabolic flexibility and resilience suggest that *A. radiobacter* possesses the physiological robustness advantageous for biotechnological applications [8].

Green waste has increased due to rapid expansion and urbanisation, generating about tons of waste every year [9]. This biomass is rich in lignin, cellulose and hemicellulose, which can be potentially used for bioconversions based on the concept of biorefinery [10]. The major green waste includes the garden grass, whose composition ranges from cellulose, hemicellulose and lignin in the range of 25-40%, 25-50% and 10-30%, respectively. Thus, this is a potential resource for fermentable sugars, which in turn could be used for succinoglycan production.

Hence, this work focuses on the production of succinoglycan fermentation using *A. radiobacter* strain. Parameters like carbon source, carbon concentration, C/N ratio, and precipitation ratio for the maximum production of succinoglycan were optimised. The acid hydrolysate of waste garden grass was used as a carbon source for the succinoglycan fermentation.

2 Materials and methods

2.1 Procurement of chemicals

Sucrose, peptone, $MgSO_4$, Agar Type 1, $NH_4H_2PO_4$, sodium hydroxide and hydrochloric acid were purchased from Himedia Laboratories Pvt. Ltd. Yeast extract, galactose, $ZnCl_2$, acetone and isopropanol were purchased from Sisco Research Laboratories Pvt. Ltd. Glucose, KH_2PO_4, $FeSO_4.7H_2O$, $MnSO_4.H_2O$ and $CoCl_2.6H_2O$ were purchased from Merck Specialities Private Limited. Sulphuric acid was procured from Thermo Fischer Scientific India Pvt. Ltd. Nitric acid was purchased from Paxmy Speciality Chemicals.

2.2 Biomass collection and characterisation

2.2.1 Estimation of moisture and extractives

The biomass garden grass was collected from the premises of SASTRA Deemed to be University, Thanjavur. The sample was sieved with sieves of particle size (0.405 mm), and the experiments for each category were carried out. All characterisation protocols were followed as per NREL protocols.

For the moisture content, in a crucible, 1 g of grass was taken and kept at 105ºC for 4 h on a hot plate. The moisture content was calculated as follows:

$$\text{Moisture (\%)} = ((a - b)/a) \times 100 \quad \text{(1)}$$

Where, *a = initial weight of biomass before drying and b = weight of biomass after drying.*

For the extractives, about 20 g of grass was taken, and Soxhlet extraction was carried out with acetone as solvent. The sample was dried until a constant weight was reached. The weight loss was considered to be the extractives that include oils and terpenoids. The extractives (%) were given by:

$$\text{Extractives (\%)} = ((a-b)/a) \times 100 \quad \text{(2)}$$

Where, *a = initial weight of the sample as received and b = weight of the dried sample after extraction.*

2.2.2 Estimation of cellulose and lignin

For the cellulose estimation, about 0.5 g of extractive-free grass was taken and added with 3 mL of reagent containing 80% acetic acid and nitric acid in the ratio 10:1. The mixture was heated to 100ºC for 30 minutes. The sample was filtered using vacuum filtration. The residue containing the cellulose was washed until the colour changed to white and weighed to estimate the percentage of cellulose in the sample.

For the lignin estimation, about 0.5 g of the extract-free sample was taken and added with 3 mL of 72% H_2SO_4. The mixture was then incubated at 30ºC with constant stirring of every 15 minutes for a period of 1 hour. The sample was filtered and diluted with distilled water to reach a final concentration of 4% H_2SO_4. The sample was autoclaved at 121°C and filtered with filter paper after adjusting the pH. For the estimation, about 2 mL of the filtrate was taken, along with 2 mL of 3% H_2SO_4. The optical density of the sample was measured at 208 nm using a UV-Visible spectrophotometer. The percentage of acid-soluble lignin (ASL) was estimated using the eqn. 3.

$$\%\text{ASL} = \frac{A}{\varepsilon}\frac{V}{W_{od}} \times DF \times 100 \quad \text{(3)}$$

Where, A= optical density of the sample at 208 nm, ε = extinction coefficient of lignin at 208 nm (110 $L \cdot g^{-1} \cdot cm^{-1}$, NREL standard), DF = dilution factor, V = total volume of filtrate (L), and W_{od} = oven-dried weight of extractives free sample (g).

The solid residue obtained after filtering the sample was taken and dried at 108ºC until constant weight was reached. The amount of acid-insoluble lignin (AIL) present was estimated using the following formula:

$$\%\ AIL = \frac{W_{ad}}{W_{od}} \text{x} 100 \quad \text{(4)}$$

Where, w_{ad} = weight of the sample after drying, and w_{od} = oven-dried weight of extractives-free sample.

2.2.3 Estimation of total reducing sugar

10 mL of the filtered sample was taken, added with 10 mL of H_2SO_4 and kept for 1 h. After which, 1 mL of the

solution was taken and diluted in the ratio 1:100 with distilled water for further analysis. The sugar content in the sample was estimated using the DNS reagent. A DNS standard was also run simultaneously with known aliquots of glucose as a standard.

2.3 Microbial culture conditions and growth curve analysis

Strain of *Agrobacterium radiobacter* NCIM 2443 was obtained from National Centre for Cell Sciences, Pune. The culture was maintained in glycerol stocks at -20°C. *A. radiobacter* NCIM 2443 was grown on YEB medium containing 0.5 g/L of meat extract, 0.1 g/L of yeast extract, 0.5 g/L of peptone, 0.5 g/L of sucrose and 30 mg/L of $MgSO_4$ in 100 mL of distilled water. The cells were grown at 28°C for 48 h, at 150 rpm, and were used as seed culture for further experimentation.

For the growth curve, 1 % of the *A. radiobacter* cells were inoculated in YEB medium and kept at 150 rpm. The cell growth was measured at regular intervals of 2 h by measuring the optical density of the sample at 600 nm for a period of 5 days.

2.4 Sudan black screening test for succinoglycan

The grown cells of *A. radiobacter* were quadrant streaked on YEB agar plates and incubated at 28°C for 48 hours. Colonies were observed and added with 5 mL of 0.2 % Sudan Black reagent, and rinsed with 10 mL of absolute ethanol after 10 minutes.

2.5 Production media for succinoglycan

The production media contained 25 g/L sucrose, 1 g/L KH_2PO_4, 0.25 g/L $MgSO_4$, 1 g/L $NH_4H_2PO_4$ and 1 mL of trace elements solution (0.5 g $FeSO_4.7H_2O$, 0.2 g $MnSO_4.H_2O$, 0.1 g $CaCl_2$ and 0.1 g of $ZnCl_2$ in 100 mL of 0.1N HCl) in 100 mL distilled water [11]. The pH of the media was adjusted to 7. Seed culture of 10 mL was inoculated in the media, and a temperature of 28°C with 150 rpm was maintained.

2.6 Effect of carbon source

Three different sugars are taken as carbon sources, namely, glucose, sucrose and galactose, were formulated with other media components. The media contains 25 g/L carbon source (Glucose/ Sucrose/ Galactose), 1 g/L KH_2PO_4, 0.25 g/L $MgSO_4$, 1 g/L $NH_4H_2PO_4$ and 1 mL of trace elements solution (0.5 g $FeSO_4.7H_2O$, 0.2 g $MnSO_4.H_2O$, 0.1 g $CaCl_2$ and 0.1 g of $ZnCl_2$ in 100 mL of 0.1 N HCl) in 100 mL distilled water. The pH of the media was adjusted to 7. 10 mL of the seed culture was inoculated in the media, and a temperature of 28°C and an agitation of 150 rpm were maintained. After choosing the best carbon source, the concentration of the carbon source was varied (25 g/L, 50 g/L, 75 g/L and 100 g/L) to study the influence of the concentration of the carbon source.

2.7 Effect of carbon-nitrogen (C:N) ratio

The basic media contained 25 g/L glucose, 1 g/L KH_2PO_4, 0.25 g/L $MgSO_4$, $NH_4H_2PO_4$ (1.4 g/L, 0.547 g/L and 2 g/L) and 1 mL of solution containing trace elements (0.5 g $FeSO_4.7H_2O$, 0.2 g $MnSO_4.H_2O$, 0.1 g $CaCl_2$ and 0.1 g of $ZnCl_2$ in 100 mL of 0.1 N HCl) in 100 mL distilled water. Fermentation was carried out at pH 7, a temperature of 28°C, with an agitation speed of 150 rpm.

2.8 Recovery of succinoglycan

In the optimised media, the cells were inoculated, and the samples were withdrawn every 24 hours from day 0 to day 5. 10 mL of the sample was taken and centrifuged at 4°C at 8000 rpm for 15 minutes. The supernatant and the pellet were recovered and preserved separately. The pellet was dried in the hot air oven at 40°C, and the dry cell weight was calculated. 10 mL of the supernatant was taken and added to 10 mL of antisolvent. Acetone and isopropanol were chosen as antisolvents and added in ratios of 1:1, 2:1, 3:1, 4:1, and 5:1 to the supernatant. The precipitated polymer was dried at 40°C until it reached constant weight.

2.9 Production media using garden grass hydrolysate

The grass sample after acid hydrolysis was taken and filtered. The sample pH was adjusted to 7 and filtered again. 50 mL of the sample was taken and made up to 100 mL using distilled water and other production media components. Fermentation was carried out at pH 7, at temperature 28°C, with an agitation speed of 150 rpm.

2.10 Characterisation of succinoglycan

The produced succinoglycan sample was analysed using NMR and FT-IR to confirm its structural and functional groups.

3 Results and discussion

3.1 Biomass collection and characterisation

The moisture content of the garden grass was found to be 0.225 ± 0.014%. The extractives were found to be 19.14 ± 0.34%, and the total lignin content of the extractive-free sample was 19.85 ± 1.21%. In the present study, effectiveness of H_2SO_4 pretreatments for hydrolyzing grass biomass was assessed based on the yield of reducing sugars using the DNS assay. Acid pretreatment using 2% H_2SO_4 resulted in the highest sugar yield, 54.2 ± 0.82 g/100 g of grass biomass (Figure 1). This yield is consistent with established literature, wherein dilute acid hydrolysis effectively breaks down hemicellulose and partially hydrolyzes cellulose, liberating monomeric sugars [12, 13]. The mechanism involves hydrothermal degradation, where the concentrated acid facilitates cleavage of glycosidic linkages in hemicellulose and opens the lignocellulosic matrix, thereby improving cellulose accessibility.

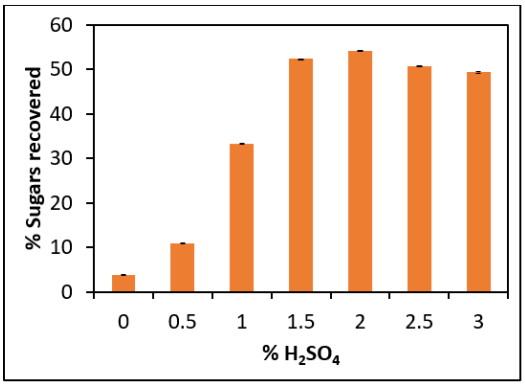

Fig. 1. Sugar recovered using different concentrations of sulphuric acid.

3.2 Sudan black test

The exopolysaccharide succinoglycan produced by certain rhizobial and soil bacteria, plays a significant role in biofilm formation, symbiotic interactions, and osmotic stress tolerance. In the current experiment, the Sudan Black B staining method was employed to assess the production of succinoglycan by *A. radiobacter*. Sudan Black B is a lipophilic azo dye commonly used to stain lipid-rich substances, including polyhydroxybutyrate (PHB) and other neutral lipids. However, in polysaccharide detection assays, it has a secondary application: it can bind non-specifically to hydrophobic components but does not interact with highly hydrated, gel-forming exopolysaccharides like succinoglycan. As reported by Liu et al. (1998), colonies that do not produce succinoglycan or other similar EPS typically absorb Sudan Black dye and exhibit black or dark blue coloration [12]. In contrast, succinoglycan-producing colonies retain a white or creamy appearance due to the exclusion of the dye by the gelatinous EPS layer, which forms a hydrated barrier around the cells and prevents dye penetration.

As shown in Figure 2, the bacterial streaks remain white and unstained following treatment with Sudan Black B. This indicates that the bacteria are actively producing a thick, hydrated layer of succinoglycan, which prevents the lipophilic dye from binding to the underlying cell wall or membrane structures. The uniformity and intensity of the unstained zone across multiple streaks further suggest robust and consistent EPS production across the bacterial population. This visual outcome serves as a qualitative confirmation of succinoglycan production. The gelatinous nature of the EPS is typical of succinoglycan, which is composed predominantly of glucose and galactose residues with pyruvyl, succinyl, and acetyl modifications that confer its anionic and viscous properties [14].

Fig. 2. Sudan black test for the production of succinoglycan by the *A. radiobacter*.

3.3 Growth curve analysis and succinoglycan production

The growth profile of the bacterial isolate was evaluated by measuring optical density at 600 nm over a 48-hour incubation period, as shown in Figure 3. The organism exhibited a distinct three-phase growth pattern typical of bacterial cultures. The initial lag phase, spanning 0–6 hours, likely reflected a period of physiological adaptation where the bacteria adjusted to the new growth environment and initiated metabolic reprogramming. This was followed by a pronounced exponential (log) phase from approximately 6 to 36 hours, which rapid cellular proliferation was observed, indicating active nutrient uptake and biomass accumulation. After 36 hours, the growth curve plateaued, signifying entry into the stationary phase, where nutrient depletion and accumulation of waste products began to limit further population expansion. This phase is often associated with the biosynthesis of secondary metabolites and extracellular biopolymers, including exopolysaccharides (EPS) such as succinoglycan.

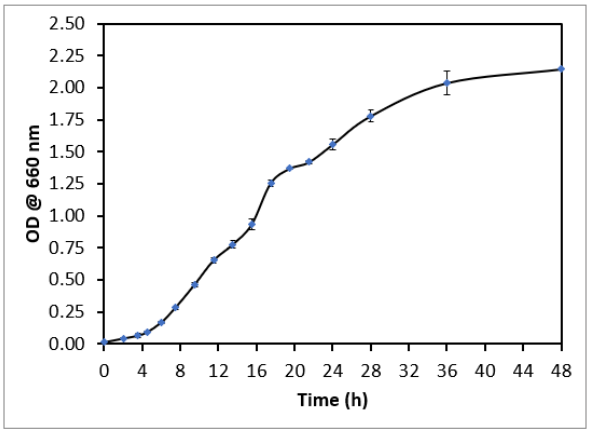

Fig. 3. Microbial growth curve representing the growth of the organism *A. radiobacter*.

Succinoglycan production was targeted specifically during the stationary phase, given that stress conditions such as nutrient limitation often upregulate EPS biosynthetic pathways. After fermentation, the culture broth was centrifuged to separate bacterial cells from the extracellular matrix. The supernatant was treated with an anti-solvent isopropanol to precipitate succinoglycan. A white, floating precipitate was clearly visible, as

illustrated in Figure 4. The precipitate was recovered from the solution, consistent with characteristics described for succinoglycan-like EPS in previous studies. Isopropanol was chosen due to its polarity difference from water and its ability to precipitate high-molecular-weight polysaccharides without degradation. The extraction process was repeated from day 2 to day 5, confirming consistent and reproducible production. The white precipitate's appearance and timing strongly suggest successful recovery of succinoglycan, a high-viscosity biopolymer primarily composed of glucose and galactose, with succinyl, acetyl, and pyruvyl side groups contributing to its physical and chemical properties.

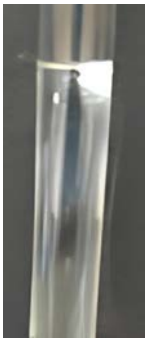

Fig. 4. Shows the white precipitate as a result of acetone precipitation

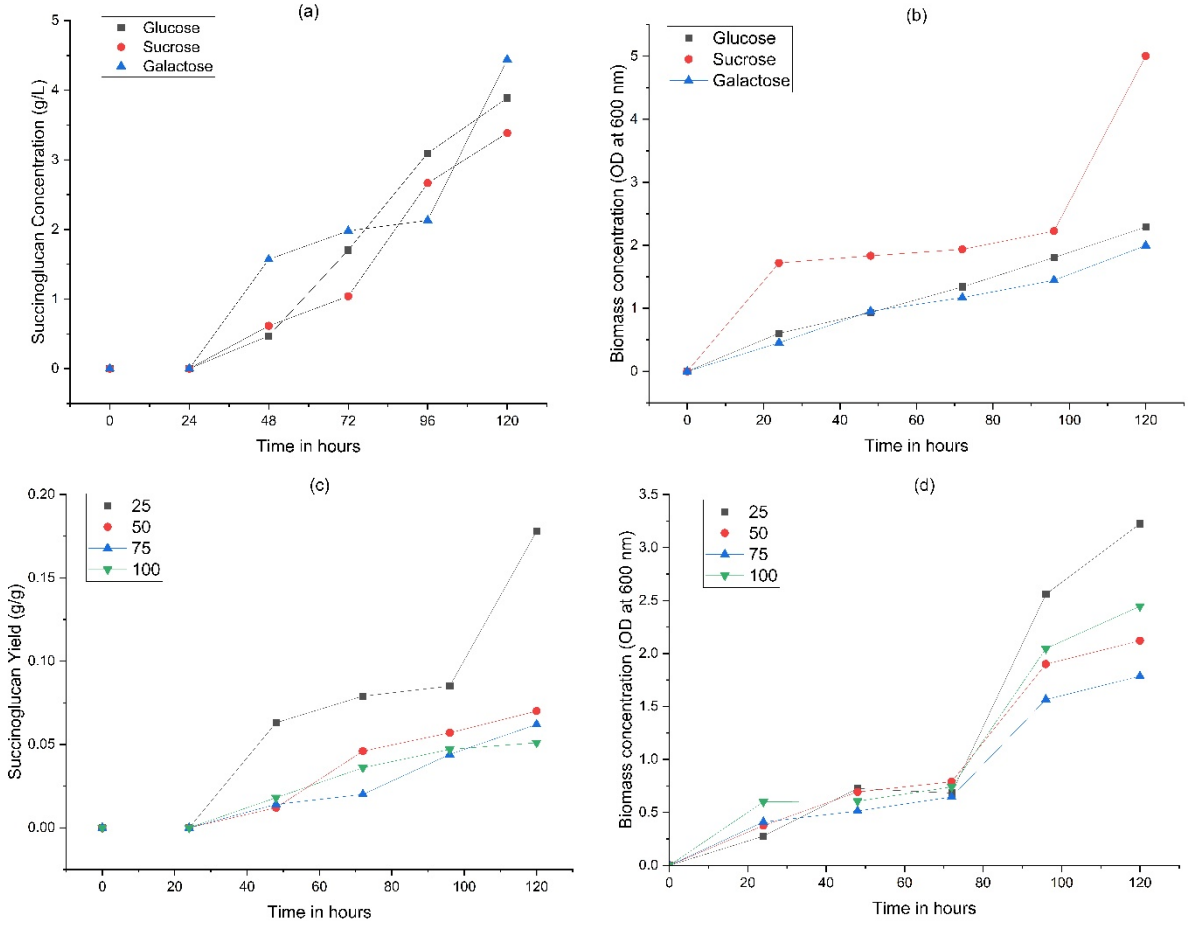

Fig. 5. Optimisation of Succinoglycan production: (a) succinoglycan concentration with different carbon sources, (b) biomass yield with different carbon sources, (c) succinoglycan yield at various glucose concentrations, and (d) biomass yield at various glucose concentrations.

Similar behavior has been reported in *A. radiobacter*, as described by [15], where succinoglycan production peaked during late-log and early stationary phases. This temporal association between growth kinetics and EPS production provides a basis for optimizing inoculum timing, culture duration, and recovery protocols in future bioprocess development.

3.4 Effect of carbon source

To investigate the influence of various carbon sources on succinoglycan production, three conventional sugars—glucose, galactose, and sucrose—were evaluated. Glucose and galactose were selected due to their known role as the principal monomeric constituents of succinoglycan [8]. Sucrose was included based on recent studies demonstrating its effectiveness in enhancing EPS production [11]. As shown in Figure 5 (a), succinoglycan production increased progressively with time for all three carbon sources. Among them, sucrose supported the highest final yield, reaching approximately 4.5 g/L by 120

hours. Glucose and galactose also promoted significant production, with final yields of approximately 3.8 g/L and 3.5 g/L, respectively. These trends are consistent with existing literature and support the hypothesis that sucrose acts as a more readily utilizable substrate for EPS biosynthesis. Furthermore, a conversion yield of 0.208 g of succinoglycan per gram of sucrose was recorded, indicating efficient biopolymer synthesis. Despite sucrose yielding the highest biomass and EPS production, glucose was chosen as the primary carbon source for subsequent optimization experiments. This choice was based on the relevance to practical applications involving lignocellulosic feedstocks, particularly grass biomass, which primarily contains cellulose composed of glucose units rather than disaccharides like sucrose.

To study the effect of concentration of glucose for succinoglycan production, fermentation trials were conducted at 25 g/L, 50 g/L, 75 g/L, and 100 g/L of glucose. The highest conversion yield was observed at 25 g/L, with a value of 0.177 ± 0.0018 g of succinoglycan per gram of glucose, aligning well with reports on optimal sucrose levels from previous studies. In contrast, increasing glucose concentration resulted in progressively lower yields: 0.07 g/g at 50 g/L, 0.062 g/g at 75 g/L, and 0.051 g/g at 100 g/L (Figure 5 (c)). This inverse relationship between glucose concentration and succinoglycan yield suggests possible catabolite repression or osmotic inhibition at higher sugar levels, which could impair metabolic flux toward EPS biosynthesis. Similar patterns have been observed in other polysaccharide-producing microbes, where moderate substrate concentrations favor polymer synthesis, while excessive sugar levels suppress secondary metabolite pathways.

3.5 Effect of Carbon/Nitrogen ratio

In this study, glucose was selected as the fixed carbon source based on its relevance to lignocellulosic hydrolysates, while ammonium dihydrogen phosphate was used as the nitrogen source. The carbon concentration was maintained constant, and the C:N ratio was varied by changing nitrogen levels to obtain molar ratios of 25:1, 50:1, and 75:1, respectively. These ratios were chosen based on previous studies suggesting that nitrogen-limiting conditions may favor exopolysaccharide production, particularly succinoglycan, in several microbial systems. As illustrated in Figure 6(a), the highest biomass yield was achieved at a C:N ratio of 25, followed closely by C:N 50. In contrast, the C:N 75 condition resulted in significantly reduced biomass accumulation. These findings indicate that higher nitrogen availability supports more cell growth, likely due to enhanced protein and nucleic acid biosynthesis necessary during exponential and stationary growth phases. Surprisingly, as shown in Figure 6(b), the highest succinoglycan yield was also observed at C:N 25, reaching 0.54 g of succinoglycan per gram of glucose consumed. Yields declined progressively with increasing C:N ratio, with the lowest production observed at C:N 75. This result is not fully consistent with previous literature, which often reports enhanced exopolysaccharide production under nitrogen-limited conditions, presumably as a microbial stress response mechanism that redirects carbon flux toward secondary metabolite pathways. The divergence observed in the present study may be attributed to the enhanced biomass productivity under higher nitrogen conditions, which may in turn support greater overall EPS biosynthesis through increased enzyme expression and metabolic activity. A similar observation was made by Pinedo et al. (2008), where moderate nitrogen availability promoted EPS production in certain rhizobial strains [16]. It is also plausible that the threshold for nitrogen limitation in this strain is higher than expected, and further experimentation with broader C:N ratios or different nitrogen sources may help clarify the optimal window for succinoglycan overproduction.

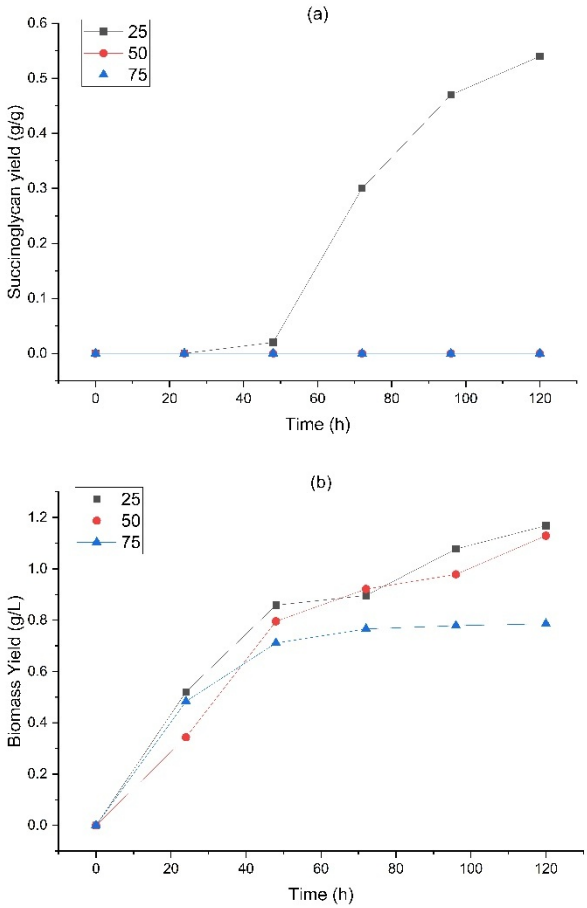

Fig. 6. Effect of carbon to nitrogen ratio (a) product yield and (b) biomass yield.

3.6 Succinoglycan recovery Total reducing sugar content

Downstream processing plays a major role in the efficient recovery and quantification of biopolymers such as succinoglycan and is equally important as upstream optimization. In this study, solvent precipitation was employed as the primary recovery technique, leveraging the poor solubility of succinoglycan in organic solvents such as acetone and isopropanol (IPA), as previously described by [17] To optimize recovery efficiency, different sample-to-solvent volume ratios (1:1 to 1:5) were evaluated for both acetone and IPA. The concentration of succinoglycan recovered under each condition is presented in Figure 7. Among the tested

conditions, isopropanol at a 1:2 (supernatant: solvent) ratio was found to be the most effective, yielding a maximum succinoglycan concentration of 7.12 g/L, while at 1:1 ratio yield was only 4.7 g/L . Upon increasing the IPA ratio beyond 2, succinoglycan yield . In case of acetone, from 1:1 to 1:3 ratio, there was an increase in succinoglycan production from 3.42 g/L to 4.62 g/L, and at 1:4 ratio, the succinoglycan of about 6.2 g/L was obtained. Further increase in acetone ratio reduced polymer yield. This condition clearly outperformed all others, including corresponding acetone ratios, which consistently yielded lower concentrations. Isopropanol proved superior to acetone across all solvent ratios tested.

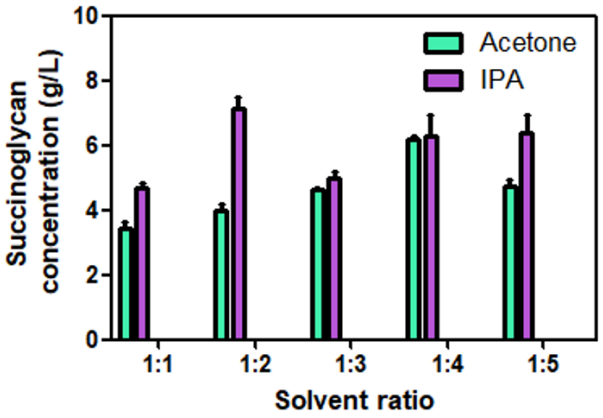

Fig. 7. Succinoglycan precipitation upon two different solvents

3.7 Characterization of succinoglycan

The structural features of the extracted biopolymer were characterized using ¹H Nuclear Magnetic Resonance (¹H-NMR) and Fourier Transform Infrared (FT-IR) Spectroscopy. The ¹H-NMR spectrum, presented in Figure 8, provides key insights into the monomeric composition and substitution pattern of the polymer. The chemical shifts observed at δ = 0.965 and 1.165 ppm represent methyl protons of pyruvyl groups. The acetyl protons are identified at δ = 1.808 ppm. The cluster ranging from δ = 3.052 to 4.022 ppm indicates the presence of β-glycosidic linkages between glucose and galactose units, confirming the polysaccharide backbone structure (Figure 8). The peak chemical shift at 4.075 gives out the solvent peak [8]. The lack of succinate addition to the polymer is evident from the result. It is postulated that the bacterium might be an exoH mutant, i.e. it lacks the gene to add the succinate moiety.

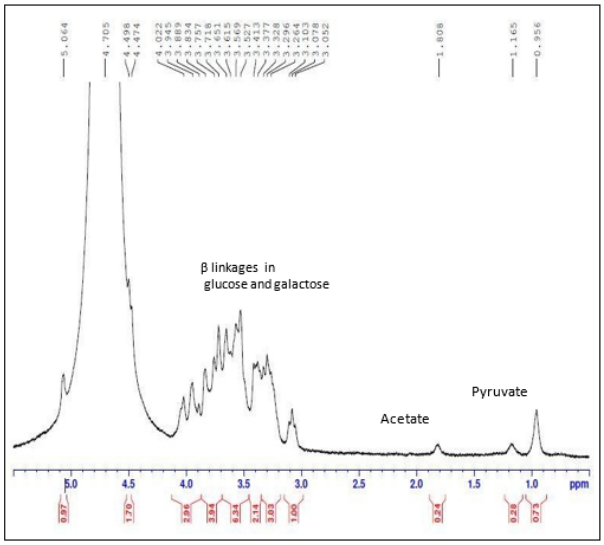

Fig. 8. H-NMR analysis of the biopolymer.

Further confirmation was obtained via FT-IR analysis, shown in Figure 9. A broad absorption band around 3300 cm^{-1} corresponds to -OH stretching vibrations, typical of polysaccharides. The band near 1700 cm^{-1} is assigned to -CH bending, while the signal at 1313 cm^{-1} is attributed to carboxylic acid (COOH) functional groups. A strong band at 1070 cm^{-1} confirms the presence of C–O–C and C–O stretching vibrations, reinforcing the polysaccharide structure [18]. However, subtle deviations in the FT-IR profile, particularly the diminished absorbance in regions typically associated with succinate groups, support the hypothesis that the biopolymer lacks succinylation. This observation aligns with prior reports on exoH-deficient strains, which produce succinoglycan without succinate moieties, leading to minor but consistent spectral shifts [19].

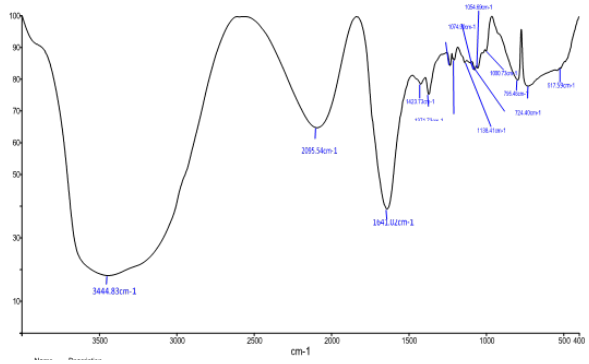

Fig. 9. FT-IR analysis of the biopolymer.

4 Conclusion

The compositional analysis of garden grass revealed its suitability as a lignocellulosic feedstock for biopolymer production. The biomass contained 19.13% extractives, comprising waxes, fatty acids, resin oils, and terpenoids. The cellulose content was determined to be 31.11 ± 2.70 %, while lignin accounted for 16.05 % of the dry weight. The moisture content was relatively low, at 0.18 %, indicating good storage stability and minimal pre-processing requirements. Acid hydrolysis using 2 % sulfuric acid yielded a maximum of 0.5909 g of reducing

sugars per gram of grass, demonstrating the efficiency of the pretreatment method in converting polysaccharides into fermentable sugars. These hydrolysates were subsequently utilized as a substrate for the production of succinoglycan, an industrially significant extracellular biopolymer. Through systematic optimization of the conventional production media, it was found that a glucose concentration of 25 g/L combined with a carbon-to-nitrogen (C:N) ratio of 25 supported maximum succinoglycan synthesis. Given its abundance, low cost, and sustainable nature, garden grass serves as a viable alternative to conventional carbon sources for succinoglycan production, thus enhancing the economic feasibility of biopolymer manufacturing processes.

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