

# Advanced Bio-Material Management: Leveraging Agro-Industrial Waste for Sustainable Dextran Production through Optimized *Leuconostoc mesenteroides* Bioprocessing

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**Abstract**—Dextran, a versatile biopolymer primarily composed of  $\alpha$ -(1 $\rightarrow$ 6) linked D-glucose units, is a cornerstone of modern biotechnology with critical applications in the medical, pharmaceutical, and food industries. This study focuses on the isolation, identification, and optimization of *Leuconostoc* spp., specifically *L. mesenteroides*, from sucrose-rich surfaces at the Manjara Sugarcane Industry. Purified isolates, identified as Gram-positive, catalase-negative cocci, exhibited a characteristic mucoid morphology on sucrose-supplemented MRS agar, indicating robust extracellular polysaccharide production. Optimization of culture parameters using turbidometric analysis (OD 600) revealed that maximum growth and dextran yield were achieved at a temperature of 26°C, a near-neutral pH of 6.5 to 7.0, and a 10% sucrose concentration. Furthermore, a comparative analysis of agro-industrial substrates demonstrated that molasses is a superior, cost-effective carbon source compared to refined sucrose, likely due to its rich mineral and nutrient profile. Laboratory-scale production confirmed that dextran synthesized under these conditions possesses high water solubility and viscosity, validating its potential for sustainable, high-yield industrial applications in drug delivery, food stabilization, and green nanotechnology.

**Index Terms**—Dextran, *Leuconostoc mesenteroides*, Agro-industrial waste, Molasses, Bioprocess optimization, Fermentation, Sustainability

## I. INTRODUCTION

Dextran is a versatile biopolymer primarily synthesized from sucrose by bacteria like *Leuconostoc mesenteroides* through the enzymatic action of dextransucrase. Primarily composed of  $\alpha$ -(1 $\rightarrow$ 6) linked D-glucose units, this polysaccharide is celebrated for its biocompatibility and high solubility, making it indispensable in the medical field as a plasma volume expander and in labs as a staple for gel filtration. As a structural powerhouse with sensitivity to its environment, dextran remains at the forefront of biotechnological research, bridging the gap between microbial fermentation and essential industrial applications.

Dextran stands as a cornerstone of modern biotechnology, bridging the gap between life-saving medical applications, like plasma expansion, and essential industrial uses in food stabilization and molecular purification. By pivoting from refined sugars to sustainable agro-industrial byproducts like molasses and sugarcane juice, production becomes both eco-friendly and cost-effective. This shift, paired with the precise optimization of *Leuconostoc* fermentation, unlocks new potential for the high-yield synthesis of biodegradable polymers. Ultimately, refining these microbial processes paves the way for cutting-edge advancements in green nanotechnology and targeted drug delivery.

Table 1: Applications of Dextran Across Industries

Industry	Primary Functions	Specific Applications
Medical & Pharmaceutical	Plasma expander, drug carrier, anticoagulant	Treatment of shock and hypovolemia (Dextran 40/70), antithrombotic therapy, targeted drug delivery, and tissue engineering scaffolds.
Food Industry	Stabilizer, thickener, humectant	Improving texture in bakery and dairy products, preventing sugar crystallization, moisture retention, and enhancing mouthfeel in sauces.
Biotechnology	Separation matrix, immobilization agent	Gel filtration chromatography (Sephadex) for purifying proteins and nucleic acids, enzyme immobilization, and biosensor development.
Cosmetics	Moisturizer, film-former	Skin hydration in creams and serums, improving product texture, and serving as a biodegradable ingredient in organic skincare.
Nanotechnology	Nanoparticle synthesis, imaging	Development of nanoparticles for targeted therapy, diagnostic imaging agents, and biodegradable hydrogels.
Industrial & Environmental	Flocculant, stabilizer	Wastewater treatment processes and the production of biodegradable films and materials.

*Leuconostoc* species, particularly *L. mesenteroides*, are specialized lactic acid bacteria that thrive in sugar-rich environments, utilizing the phosphoketolase pathway to drive their unique metabolism. As "Generally Recognized as Safe" (GRAS) organisms, they serve as biological factories, employing the extracellular enzyme dextransucrase to transform sucrose into the high-value biopolymer dextran. While their ability to create thick, mucoid solutions can be a challenge in sugar refineries, it is precisely this trait that makes them indispensable for producing industrial stabilizers and medical polymers. By maintaining optimized conditions—typically a near-neutral pH and temperatures between 26°C and 30°C—these bacteria become powerful tools for

sustainable biotechnology and food science.

*Leuconostoc mesenteroides* NRRL B-512F remains the gold standard for industrial dextran production, favored for its consistent yield of highly uniform  $\alpha$ -(1→6) linked polymers. Modern research has elevated this process by integrating sustainable agro-industrial wastes—like potato peels and brewer’s spent grain—with advanced techniques such as fed-batch fermentation to prevent substrate inhibition. By meticulously fine-tuning environmental variables and selecting specific microbial strains, scientists can now tailor dextran’s molecular weight and branching to meet the precise demands of the pharmaceutical and food industries.

Table 2: Commercial Strains and Brands of Dextran

Brand Name	Type of Dextran	Molecular Weight	Application
Macrodex	Dextran 70	High Molecular Weight	Plasma volume expander for shock and blood loss.
Rheomacrodex	Dextran 40	Low Molecular Weight	Improves blood flow and microcirculation.
Gentran	Dextran 40/70	Variable	Clinical use for fluid replacement and hypovolemia.
Sephadex	Cross-linked Dextran	Variable	Size-exclusion chromatography for biomolecule purification.
Dextran Sulfate	Modified Dextran	Variable	Anticoagulant and biochemical research tool.

Table 3: Rationale of the Present Study

Key Factor	Challenge or Opportunity	Proposed Solution / Objective
Microbial Source	Need for efficient, high-yielding strains adapted to high sugar environments.	Isolation and characterization of native <i>Leuconostoc</i> spp. from sugarcane industry surfaces.
Production Cost	High expense of refined sucrose and synthetic media in large-scale synthesis.	Utilization of agro-industrial substrates like molasses, sugarcane juice, and beetroot extract.
Sustainability	Waste management and environmental impact of industrial processes.	Promoting waste valorization and eco-friendly bioprocessing through natural resource recycling.
Process Efficiency	Low yields and inconsistent quality due to unoptimized environmental variables.	Systematic optimization of pH, temperature, substrate concentration, and incubation time.
Industrial Applicability	Limited use due to high costs and structural variability of the biopolymer.	Enhancing productivity and tailoring dextran properties for pharmaceutical and food sectors.

## II. MATERIALS AND METHOD

### 2.1 Collection of sample

Samples were aseptically harvested from sucrose-rich surfaces at the Manjara Sugarcane Industry to target natural *Leuconostoc* growth. These specimens were chilled and swiftly transported to the lab to ensure maximum microbial viability for dextran isolation.

### 2.2. Isolation of *Leuconostoc* spp. by Enrichment and Sub-culturing

To isolate *Leuconostoc* spp., begin by aseptically

inoculating samples into a sucrose-rich enrichment broth and incubating at 25–30°C; the appearance of turbidity and viscosity serves as a telltale sign of growth and dextran production. Next, perform serial dilutions in sterile saline before spreading the culture onto MRS agar supplemented with sucrose. After a 24–48 hour incubation, target the characteristically slimy or mucoid colonies for purification via repeated streaking. Finally, through consistent sub-culturing to ensure a single uniform colony, the purified isolates are maintained on agar slants at 4°C, ready for further study.

Table 4: MRS Agar Composition

Sr. No.	Ingredients	Composition (per liter)	Function
1.	Peptone	10.0 g	Nitrogen source; provides amino acids
2.	Beef extract	8.0 g	Source of vitamins, minerals, and growth factors
3.	Yeast extract	4.0 g	Source of vitamins, especially B-complex
4.	Glucose (Dextrose)	20.0 g	Fermentable carbohydrate; energy source
5.	Dipotassium hydrogen phosphate (K <sub>2</sub> HPO <sub>4</sub> )	2.0 g	Buffering agent; maintains pH stability
6.	Sodium acetate	5.0 g	Selectivity; inhibits many Gram-negative bacteria and molds
7.	Triammonium citrate	2.0 g	Selectivity; enhances growth of lactic acid bacteria
8.	Magnesium sulfate (MgSO <sub>4</sub> · 7H <sub>2</sub> O)	0.2 g	Essential cofactor for enzyme activity
9.	Manganese sulfate (MnSO <sub>4</sub> · H <sub>2</sub> O)	0.05 g	Trace element; important for growth
10.	Tween 80	1.0 mL	Supplies fatty acids and growth factors
11.	Agar	15.0 g	Solidifying agent

Sr. No.	Ingredients	Composition (per liter)	Function
12.	Distilled water	1000 mL	Solvent
13.	Final pH	6.2 ± 0.2	

### 2.3. Screening of Dextran-Producing Isolates

Primary Screening: Potential dextran producers are identified by streaking isolates onto sucrose-supplemented MRS agar and selecting colonies that exhibit a distinctively mucoid or slimy morphology.

Secondary Screening: Selected isolates are confirmed through sucrose-broth inoculation, where the highest-performing strain is chosen based on significant increases in viscosity, turbidity, and visible string formation.

### 2.4. Biochemical and Carbohydrate Fermentation Tests

To ensure accurate identification, purified isolates underwent standard biochemical characterization alongside carbohydrate fermentation profiling across various sugars. These combined tests confirmed the metabolic signature of *Leuconostoc* spp., providing a robust profile to validate their specific identity.

### 2.5. Optimisation of Culture Parameters for Dextran Production

To optimize culture conditions, the isolates are inoculated into MRS broth where a single parameter

such as substrate concentration, pH, or temperature is varied while all others remain constant. Growth is monitored through turbidometric analysis at OD (600) at regular intervals, with the maximum absorbance value identifying the ideal environment for peak microbial growth and dextran production potential.

### 2.6. Lab-Scale Production of Dextran Using MSE and MRS Broth

For lab-scale production, an active *Leuconostoc* inoculum is prepared in vancomycin-supplemented MRS broth at 26°C before being aseptically transferred sterile MRS or MSE media at a 5–10% concentration. The fermentation proceeds at 25–30°C for up to 72 hours under static or mild shaking conditions, with extracellular dextran production signaled by rising viscosity and turbidity. To recover the product, the broth is heated to 60°C to release the dextran and inactivate cells, followed by centrifugation to isolate the supernatant. Finally, the crude dextran is precipitated with chilled ethanol overnight, washed for purity, and dried or lyophilized into a fine powder.

Table 5. MSE Broth Composition

(Mayeux–Sandine–Elliker Medium)

MSE broth is a sucrose-rich medium used for dextran production by *Leuconostoc* spp. (Lactic acid bacteria).

Sr. No.	Ingredients	Composition (per liter)	Function
1.	Sucrose	100 g	Carbon source; high sucrose concentration promotes dextran production
2.	Peptone	10 g	Nitrogen source; provides essential amino acids
3.	Yeast extract	5 g	Source of vitamins, growth factors and B-complex
4.	Dipotassium phosphate (K <sub>2</sub> HPO <sub>4</sub> )	2 g	Buffering agent; maintains pH and provides phosphate
5.	Magnesium sulfate (MgSO <sub>4</sub> ·7H <sub>2</sub> O)	0.2 g	Essential cofactor for enzyme activity
6.	Manganese sulfate (MnSO <sub>4</sub> ·H <sub>2</sub> O)	0.05 g	Trace element; important for enzyme activation
7.	Sodium chloride	1 g	Maintains osmotic balance
8.	Distilled water	1000 mL	Solvent
	Final pH	6.8 – 7.0	

2.7. Comparative Study of Agro-Industrial Substrates

To evaluate the efficiency of various carbon sources for dextran production by *Leuconostoc* spp., a comparative study was conducted using agro-industrial substrates like molasses, sugarcane juice, and both diluted and concentrated beetroot juice. Each substrate was sterilized and inoculated with a standardized culture before being incubated under optimized conditions to determine which alternative source yielded the best results.

III. RESULTS AND DISCUSSION

3.1 Isolation and Identification of *Leuconostoc* spp.

1. Sample Source: Potential dextran-producing bacteria were successfully isolated from sugar-rich surfaces at the Manjara Sugarcane Industry, specifically from crushing units and juice collection areas.
2. Cultural Characteristics: Isolates grown on MRS agar supplemented with sucrose displayed a characteristic mucoid and slimy appearance, which serves as a primary indicator of extracellular polysaccharide (dextran) production. The purified isolates were identified as Gram-positive, non-spore-forming, cocci-shaped bacteria arranged in pairs or short chains.

Table 1: Morphological Characteristics of *Leuconostoc* spp.

Microscopic observations confirm the cellular structure of the isolate.

Characteristic	Observation
Gram Reaction	Gram-positive (Purple)
Cell Shape	Cocci
Arrangement	Pairs / Short chains
Spore Formation	Non-spore forming

Table 4: Screening Phases for Dextran-Producing Isolates

Screening Phase	Medium Type	Key Selection Criteria	Physical Indicators of Success
Primary Screening	Sucrose-rich Solid Media	Colony Morphology	Formation of highly mucoid (slimy) colonies
Secondary Screening	Liquid MRS Broth	Physical Transformation	Increased turbidity and viscosity
Confirmation Test	Liquid MRS Broth	Stability / Polymerization	Notable "string" formation when handled

3) Biochemical traits: All selected isolates were catalase-negative and exhibited obligate heterofermentative metabolism, confirming their classification within the lactic acid bacteria group.

Table 2: Biochemical Test Results

Standard biochemical assays used to identify the metabolic profile of the genus.

Biochemical Test	Observation
Indole Test	Negative (-ve)
Methyl Red (MR)	Negative (-ve)
Voges-Proskauer (VP)	Positive (+ve)
Citrate Utilization	Negative (-ve)
Catalase Test	Negative (-ve)

Table 3: Carbohydrate Fermentation Profile

The ability of the isolate to ferment various sugars with the production of acid and gas.

Carbohydrate Source	Observation (Acid & Gas)
Fructose	Positive (+ve)
Glucose	Positive (+ve)
Lactose	Positive (+ve)
Mannitol	Positive (+ve)

3.2 Screening for Dextran Production

- Primary Screening: Selection was based on the formation of highly mucoid colonies on sucrose-rich media.
- Secondary Screening: In liquid MRS broth, production was confirmed through increased viscosity, turbidity, and "string" formation. One specific isolate showing maximum growth and viscosity was prioritized for the optimization phase.

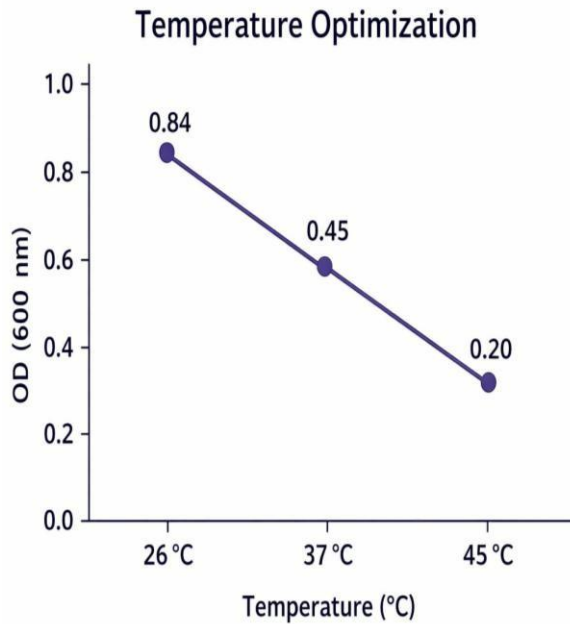
### 3.3 Optimization of Production Parameters

The study utilized turbidometric analysis (OD at 600 nm) to determine the ideal conditions for microbial growth and correlated dextran production:

Temperature: Maximum production was achieved when incubated at 25–30°C.

Table 5: Optimization of Temperature for Growth and Production

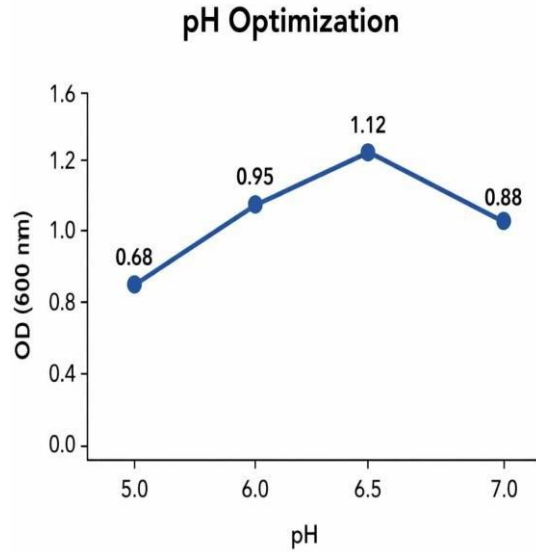
Temperature (°C)	OD (600nm)
26°C	0.84
37°C	0.45
45°C	0.20



pH Levels: The optimal pH for enhancing yield was identified as pH 6.5 to 7.0.

Table 6: Optimization of pH for Growth and Production

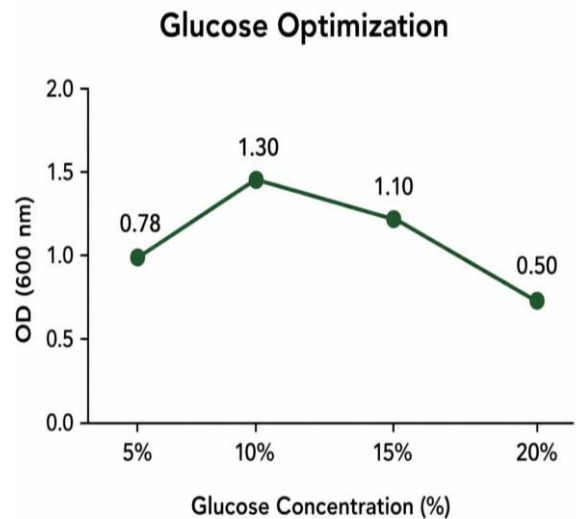
Medium pH	Optical Density (OD at 600 nm)
5.0	0.68
6.0	0.95
6.5 (High Growth)	1.12
7.0	0.88



Substrate Concentration: A 10% sucrose concentration was found to be the most efficient for maximizing dextran yield.

Table 7: Optimization of Substrate (Glucose) Concentration

Substrate Concentration (%)	Optical Density (OD at 600 nm)
5%	0.78
10% (Optimum)	1.30
15%	1.10
20%	0.50



Incubating Time: Optimal yields were typically recorded after 20 to 24 hours of incubation.

### 3.4 Evaluation of Agro-Industrial Substrates

- **Substrate Efficiency:** The study successfully utilized low-cost substrates, including molasses and sugarcane juice, as alternative carbon sources.

- **Comparative Yield:** Results indicated that these agro-industrial wastes not only supported substantial growth but also improved the cost-effectiveness of large-scale production compared to refined sucrose media.

Table 8: Comparative Analysis of Dextran Production by Substrate

Substrate	Visual Turbidity	Consistency / Viscosity	Relative Dextran Yield
Beetroot Juice (Control)	Low	Thin / Low Viscosity	Minimum
Molasses	High	Thick / High Viscosity	Maximum
Sugarcane Juice	Low	Thin / Low Viscosity	Minimum

### 3.5 Lab-Scale Production and Characterization

- **Production Medium:** Successful dextran synthesis was demonstrated in both MSE and MRS broth.
- **Physicochemical Properties:** The extracted dextran exhibited key industrial properties, including high water solubility, viscosity, and biocompatibility, making it suitable for pharmaceutical and food applications.

## IV. CONCLUSION

The study highlights the successful isolation of *Leuconostoc* spp. from sucrose-rich sugarcane industry surfaces, showcasing their natural adaptation for high-yield dextran production. Results indicate that MSE broth, with its higher sucrose concentration, significantly outperforms MRS broth by directly fueling the enzymatic activity of dextranase. While optimal physiological conditions were found at a pH of 7.0–7.5 and a temperature of 26°C, the choice of substrate proved equally critical; molasses emerged as the superior low-cost nutrient source, likely due to its enriched mineral and nutrient profile. These findings confirm that by leveraging microbial optimization and agro-industrial waste, particularly molasses, industries can achieve sustainable, economical, and high-quality dextran production for food and pharmaceutical applications.

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