

Bioethanol from Pearl Millet Stalk, Maize Stalk and *Spirulina Platensis*: A Comparative Review of Pretreatment, Enzymatic Hydrolysis and Fermentation

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Article type: Review Literature

Highlights

- Pretreatment, hydrolysis and fermentation data for three non-food feedstocks are compared.
- Maize and pearl millet stalks hold 71–75% carbohydrate but 17.5–19.4% lignin. While *Spirulina* has no lignin, forms no inhibitors and yields mostly hexose sugars.
- No study has compared the three feedstocks within one experimental framework.

Abstract

Non-food biomass can supply fermentable sugar for bioethanol without competing with food production, which makes it relevant to the energy security of India and other countries of the Global South. This review compares two agricultural residues, maize (*Zea mays*) stalk and pearl millet (*Pennisetum glaucum*) stalk, with the cyanobacterium *Spirulina platensis* (*Arthrospira platensis*) across pretreatment, enzymatic hydrolysis and fermentation. The two stalks contain 71.03–75.34% structural carbohydrate but also 17.5–19.35% lignin, so they need acid pretreatment at 121 °C, and much of their sugar is pentose that wild *Saccharomyces cerevisiae* cannot ferment. Pearl millet stalk gave 44.24 g/L ethanol by simultaneous saccharification and fermentation (SSF) with glucose supplementation, while maize stalk gave up to 25.88 g/L by separate hydrolysis and fermentation (SHF). *Spirulina* contains 46.34% carbohydrate as a lignin-free α -glucan; after freeze–thaw disruption and amylolytic hydrolysis it released 70.45 g/L of reducing sugar within 10 h, and SHF with *S. cerevisiae* gave 15.7 g/L ethanol at 83% efficiency without detoxification or added nutrients, while its residues yielded biomethane. Theoretical ethanol potentials calculated from composition are about 432, 407 and 263 g/kg for maize stalk, pearl millet stalk and *Spirulina* respectively, but 44–47% of the stalk potential lies in pentoses. No feedstock is superior on every criterion. A comparison under a common framework, optimisation of enzyme dose, kinetic modelling and techno-economic analysis are still needed to establish cost-effective bioethanol routes from these feedstocks.

Keywords: bioethanol; SSF; SHF; *Saccharomyces cerevisiae*; pearl millet stalk; maize stalk; *Spirulina platensis*; cost-effective; energy security; India; Global South

1. Introduction

First-generation bioethanol is produced from sugar and starch crops such as maize grain and sugarcane, which means it competes with food supply (Wong and Fikri, 2014; Rempel et al., 2019). Second-generation bioethanol uses lignocellulosic residues instead. It has energetic, economic and environmental advantages over bioethanol from starch or sugar, but the structure of lignocellulose makes its sugars hard to release (Alvira et al., 2010). Third-generation feedstocks, micro- and macroalgae, grow five to ten times faster than common biomass, need no arable land and can use agro-industrial effluents as nutrients (Rempel et al., 2019). In India, the national roadmap set a target of 20% ethanol blending in petrol by 2025 (NITI Aayog, 2021). This has raised interest in feedstocks that do not draw on food grain.

Maize and pearl millet are both widely grown cereals, and their stalks remain after grain harvest. *Spirulina* is a cyanobacterium already cultivated commercially. The three feedstocks therefore represent two contrasting routes to non-food ethanol: lignified crop residues and a lignin-free microbial biomass. Studies on each feedstock have been published separately, with different pretreatments, enzymes, organisms and analytical methods. No review has placed them side by side.

This review compares the three feedstocks across the full conversion chain. Sections 2–4 set out the general principles of pretreatment, enzymatic hydrolysis and fermentation, and show how each has been applied to these feedstocks. Sections 5–7 examine maize stalk, pearl millet stalk and *Spirulina* in turn. Section 8 brings the reported data together in a comparative synthesis, and Section 9 identifies research gaps. The review draws on primary studies that report composition, pretreatment, hydrolysis and fermentation data for these feedstocks, supported by established reviews for general theory. Where a value was calculated in this review from reported data rather than taken directly from a source, the text says so. Fig. 1 outlines the process routes reported in the principal studies.

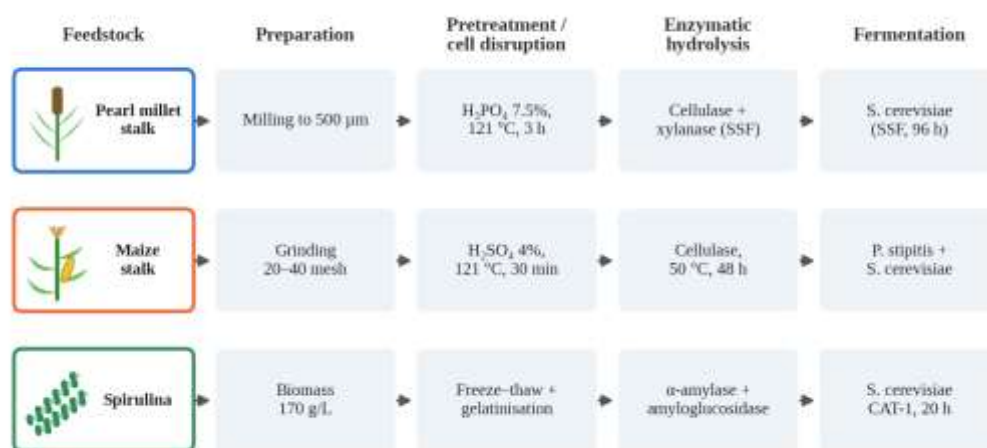


Fig. 1. Process routes reported for the three feedstocks, pearl millet stalk, maize stalk and *Spirulina*. Compiled from Sriramajayam et al. (2022), Wang et al. (2025) and Rempel et al. (2019).

2. Pretreatment Technologies and Strategies for Overcoming Recalcitrance

Lignocellulosic biomass consists of cellulose, hemicellulose and lignin.

- **Cellulose** is a linear chain of glucose units joined by β-1,4 bonds. The chains pack into partly crystalline microfibrils (Sun and Cheng, 2002).
- **Hemicellulose** is a branched polymer of pentose and hexose sugars that is coated over the cellulose (Sun and Cheng, 2002).
- **Lignin** is an aromatic polymer that binds the two carbohydrate fractions together and restricts enzyme access to cellulose (Alvira et al., 2010).

The resulting resistance to breakdown into their components is called recalcitrance. The goal of any pretreatment is to alter these structural and compositional barriers, so that enzymatic hydrolysis becomes faster and the yield of fermentable sugars rises (Mosier et al., 2005). According to Mosier et al. (2005), an effective pretreatment should:

- preserve the pentose (hemicellulose) fraction;
- limit the formation of degradation products that inhibit fermenting organisms;
- keep energy demand and cost low.

2.1. Types of Biomass Pretreatments

Physical and mechanical disruption. Chipping, grinding and milling reduce particle size and cellulose crystallinity. Typical final sizes are 10–30 mm after chipping and 0.2–2 mm after milling (Sun and Cheng, 2002). The energy required depends on the material and on the target particle size. Corn stover milled to 1.6 mm in a knife mill needs 14 kWh/t,

whereas hardwood milled to the same size needs 130 kWh/t (Sun and Cheng, 2002). All the lignocellulosic studies reviewed here began with size reduction:

- milling of pearl millet stalk to 500 μm (Sriramajayam et al., 2022);
- grinding of maize stover to 20–40 mesh (Wang et al., 2025);
- chopping of corn stalk to 10–30 mm and grinding to 0.2–2 mm (Wong and Fikri, 2014).

In the case of *Spirulina*, however, size reduction is not an issue.

Chemical pretreatments (acid, alkali, ozonolysis).

- **Dilute acid**, usually sulfuric acid, hydrolyses much of the hemicellulose and makes the cellulose more accessible. It is run either above 160 °C in continuous flow at 5–10% solids, or below 160 °C in batch at 10–40% solids, and a neutralisation step is needed afterwards (Sun and Cheng, 2002). At high severity it also forms sugar-degradation products that inhibit fermentation (Mosier et al., 2005).
- **Alkali** (sodium hydroxide, lime, ammonia) works by saponifying the ester bonds that cross-link xylan hemicellulose to other components. The fibre swells, its internal surface area rises and its crystallinity falls. The effect depends on the lignin content of the biomass (Sun and Cheng, 2002).
- **Ozonolysis** uses ozone to degrade lignin selectively at room temperature and pressure without leaving toxic residues, but the large amount of ozone needed makes it expensive (Sun and Cheng, 2002).

Physico-chemical methods (steam explosion, AFEX).

- **Steam explosion** exposes biomass to saturated steam at 160–260 °C (0.69–4.83 MPa) for seconds to a few minutes and then releases the pressure suddenly. The combination of heat and explosive decompression hydrolyses hemicellulose and opens the fibre structure (Sun and Cheng, 2002).
- **Ammonia fibre explosion (AFEX)** treats biomass with 1–2 kg of liquid ammonia per kg of dry biomass at 90 °C for 30 min and then releases the pressure rapidly (Sun and Cheng, 2002).

None of the primary maize, pearl millet or *Spirulina* studies reviewed here used either method.

Biological pretreatments. Brown-, white- and soft-rot fungi degrade lignin and hemicellulose, and white-rot fungi are the most effective. Biological pretreatment needs little energy and runs under mild conditions, but it is very slow. For example, *Pleurotus ostreatus* converted 35% of straw to reducing sugars in five weeks (Sun and Cheng, 2002).

A further route, not listed in the classical groupings, is genetic: breeding plants that make less lignin. Brown midrib (*bm*) mutants carry mutations in lignin biosynthesis genes and are known in maize, sorghum and pearl millet (Sattler et al., 2010). Wang et al. (2025) showed that the maize *bm2* mutant released more glucose than its wild type under the same acid pretreatment (Section 5).

Table 1. Summary of the main pretreatment types.

Type	Examples	Main action	Main limitation	Source
Physical	Milling, grinding	Increases surface area, lowers crystallinity	High energy demand	Sun and Cheng (2002)
Chemical: acid	Dilute H_2SO_4 , H_3PO_4	Hydrolyses hemicellulose	Inhibitors, corrosion, neutralisation	Sun and Cheng (2002); Mosier et al. (2005)
Chemical: alkali	NaOH , lime, ammonia	Breaks lignin–hemicellulose ester bonds	Depends on lignin content	Sun and Cheng (2002)
Chemical: ozonolysis	Ozone	Degrades lignin selectively	Cost of ozone	Sun and Cheng (2002)
Physico-chemical	Steam explosion, AFEX	Heat and sudden decompression	Pressure equipment	Sun and Cheng (2002)

Type	Examples	Main action	Main limitation	Source
Biological	Rot fungi	Enzymatic degradation	lignin Very slow	Sun and Cheng (2002)
Genetic	<i>bm2</i> maize mutant	Lower lignin in the plant	Needs a special variety	Wang et al. (2025); Sattler et al. (2010)

2.2. Methodologies and Processing Mechanisms

The primary studies show how these general methods are applied in practice.

Pearl millet stalk. Sriramajayam et al. (2022) dried pearl millet stalk at 45 °C, milled it to 500 µm and treated it with ortho-phosphoric acid in an autoclave. They varied four factors:

- solid loading (7.5%, 10%, 12.5%);
- acid concentration (5%, 7.5%, 10%);
- temperature (100 °C, 121 °C);
- time (1, 2, 3 h).

The best combination (12.5% solids, 7.5% acid, 121 °C, 3 h) released 38.96 g/L of reducing sugar and lowered lignin from 17.5% to 9%. The solid residue was neutralised with sodium hydroxide before hydrolysis.

Maize stover. Wang et al. (2025) hydrolysed maize stover with 4% (v/v) sulfuric acid at a solid-to-liquid ratio of 1:5 and 121 °C for 30 min. They checked the liquid for phenolics and furfural, then detoxified it with calcium hydroxide and decolourised it with a macroporous adsorption resin. Hamdy et al. (2021) instead combined autoclaving with dilute oxalic acid, a milder and less corrosive organic acid, to release glucose from corn stover.

Corn stalk. Wong and Fikri (2014) did not use acid. They suspended ground corn stalk in acetate buffer (0.05 M, pH 4.8) at 5% (w/v), autoclaved it at 121 °C for 15 min and then added enzymes directly.

Spirulina. Rempel et al. (2019) needed no delignification, because the biomass contains no lignin. They broke the cells by freezing at –20 °C for 24 h and thawing at 4 °C for 24 h, then heated the suspension at 100 °C for 10 min to gelatinise the storage carbohydrate before hydrolysis.

These examples show the general rule: the pretreatment is chosen to overcome the barrier. For the stalks the barrier is lignin and hemicellulose. For *Spirulina* the barrier is the cell envelope, so physical cell disruption is enough.

3. Enzymatic Hydrolysis

3.1. The Cellulase Enzyme Complex

Cellulose is hydrolysed by three groups of enzymes acting together (Sun and Cheng, 2002):

- **Endoglucanases** attack regions of low crystallinity in the cellulose chain and create free chain ends.
- **Exoglucanases** (cellobiohydrolases) work from these ends and remove cellobiose units.
- **β-Glucosidases** hydrolyse cellobiose to glucose.

Fig. 2 shows this sequence. Among the fungi that produce cellulases, *Trichoderma* has been the most extensively studied (Sun and Cheng, 2002). Hemicellulose needs further enzymes, mainly xylanases, to release xylose; the pearl millet study reviewed below used a xylanase for this purpose (Sriramajayam et al., 2022).

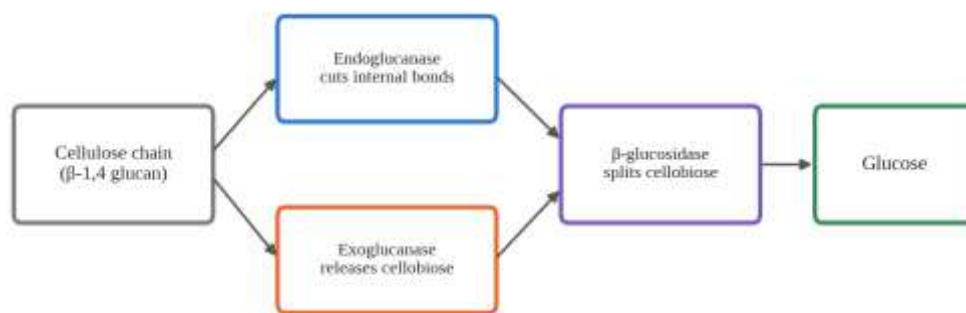


Fig. 2. Synergistic action of the three cellulase enzyme groups on cellulose, based on the description in Sun and Cheng (2002).

The primary studies used different enzyme systems:

- **Pearl millet stalk:** cellulase at 40 FPU/g and xylanase at 25 U/mL. Both activities were checked beforehand, by the filter-paper assay and on xylan respectively, at 50 °C and pH 4.8 (Sriramajayam et al., 2022).
- **Maize stover:** the acid-treated cellulose residue was neutralised with 1% sodium bicarbonate, suspended at 20% (w/v) in citrate buffer (pH 4.8) and hydrolysed with cellulase (400 U/mg) at 30 mg/g of dry substrate, at 50 °C and 250 rpm for 48 h (Wang et al., 2025).
- **Corn stalk:** 0.3% (v/v) of a commercial cellulolytic enzyme mixture (Viscozyme® L) with 2% Tween 20 as surfactant, for 48 h (Wong and Fikri, 2014).
- **Spirulina:** amylolytic enzymes instead of cellulases, because its storage carbohydrate is an α -glucan and not cellulose (Section 7).

3.2. Substrate-Enzyme Interactions

Enzymatic hydrolysis happens at a solid–liquid interface, so its rate depends on how accessible the cellulose is to the enzymes. Increasing this accessibility is the main goal of pretreatment (Alvira et al., 2010). Lignin and hemicellulose covering the cellulose limit access, which is why removing lignin raises sugar yield.

Two findings illustrate this:

- In the *bm2* maize mutant, 8.01% less acid-insoluble lignin coincided with 25.17% more glucose after pretreatment, with no change in cellulose content (Wang et al., 2025).
- In pearl millet stalk, the pretreatment that removed the most lignin (to 9%) also released the most sugar (Sriramajayam et al., 2022).

Temperature matters as well. Wong and Fikri (2014) hydrolysed corn stalk at 30, 40, 50 and 60 °C with 10, 20 and 30 g loadings (Fig. 3).

- At 50 °C, ethanol rose with loading to a maximum of 48.90% at 30 g, compared with 30.33% at 30 °C.
- At 40 °C, ethanol fell as loading increased, from 22.77% (10 g) to 12.57% (20 g) and none at 30 g. The authors attributed this to contamination, probably by *Acetobacter* sp., converting ethanol to acetic acid.
- At 60 °C, no ethanol was formed, which they attributed to the temperature exceeding the enzyme's optimum.

Their ethanol values are percentages derived from an HPLC calibration, not concentrations in g/L, so they can only be compared within that study.

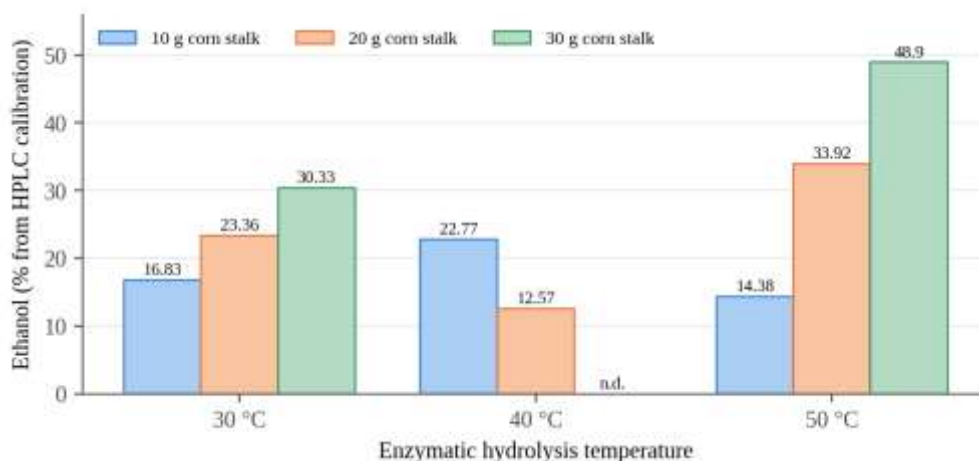


Fig. 3. Effect of enzymatic hydrolysis temperature and corn stalk loading on ethanol output (n.d. = not detected). Values are percentages derived from HPLC calibration. Data from Wong and Fikri (2014).

3.3. Limitations and Bottlenecks (Enzyme Inhibition, Feedback Mechanisms, and Cost)

Product inhibition. Cellulase activity is inhibited by cellobiose and, to a lesser extent, by glucose, so hydrolysis slows as sugars build up. A high substrate concentration can also cause substrate inhibition (Sun and Cheng, 2002). This feedback is one of the main reasons for running hydrolysis and fermentation together in one vessel, because the yeast then removes sugar as it forms (Section 4.3).

Inhibitors from pretreatment. Acid pretreatment can form sugar-degradation products that inhibit the fermenting organism (Mosier et al., 2005). The primary studies dealt with this differently:

- Wang et al. (2025) tested their maize hydrolysate for phenolics and furfural and removed them before fermentation.
- Sriramajayam et al. (2022) did not report inhibitor levels for pearl millet hydrolysate.
- For *Spirulina*, Rempel et al. (2019) noted that the absence of cellulose and lignin means that no fermentation inhibitors are generated, an advantage over lignocellulosic biomass.

Enzyme dose. All the primary studies used fixed enzyme doses rather than optimising them (Sriramajayam et al., 2022; Wang et al., 2025; Rempel et al., 2019). Whether lower doses would give comparable sugar yields on these feedstocks therefore remains a research gap.

4. Fermentation Systems: Yeast Metabolism and Ethanol Production

4.1. Yeast Metabolic Pathways for Bioethanol Synthesis

Saccharomyces cerevisiae is the organism most widely used for industrial ethanol production because it tolerates ethanol well and ferments hexose sugars efficiently under anaerobic conditions (Zabed et al., 2017). Wild strains cannot ferment pentose sugars such as xylose (Zabed et al., 2017). For this reason, two of the primary studies also used the xylose-fermenting yeast *Pichia stipitis* (Sriramajayam et al., 2022; Wang et al., 2025).

The organisms used in the primary studies were:

- *S. cerevisiae* NCIM 3204, compared with *P. stipitis* NCIM 3298, for pearl millet (Sriramajayam et al., 2022);
- *P. stipitis* CICC1960 followed by *S. cerevisiae* P1 for maize (Wang et al., 2025);
- *S. cerevisiae* CAT-1 for *Spirulina* (Rempel et al., 2019);
- commercial baker's yeast (Mauripan) as the source of *S. cerevisiae* for corn stalk (Wong and Fikri, 2014).

4.2. Hexose (Glucose) Fermentation Mechanics

Glucose is converted to ethanol in two stages (Fig. 4). In glycolysis, one glucose molecule is split into two pyruvate molecules, with a net gain of two ATP and two NADH. Under anaerobic conditions, pyruvate decarboxylase then removes carbon dioxide from each pyruvate to form acetaldehyde, and alcohol dehydrogenase reduces acetaldehyde to ethanol, regenerating NAD^+ so that glycolysis can continue. The overall reaction is:



By mass, one gram of glucose can give at most 0.511 g of ethanol. On this basis, fermentation efficiency (η) is calculated as $\eta (\%) = [\Delta E / (0.511 \times (S_0 - S))] \times 100$, where ΔE is the ethanol produced and S_0 and S are the initial and final reducing sugar concentrations (Hang et al., 1981, as applied by Rempel et al., 2019).

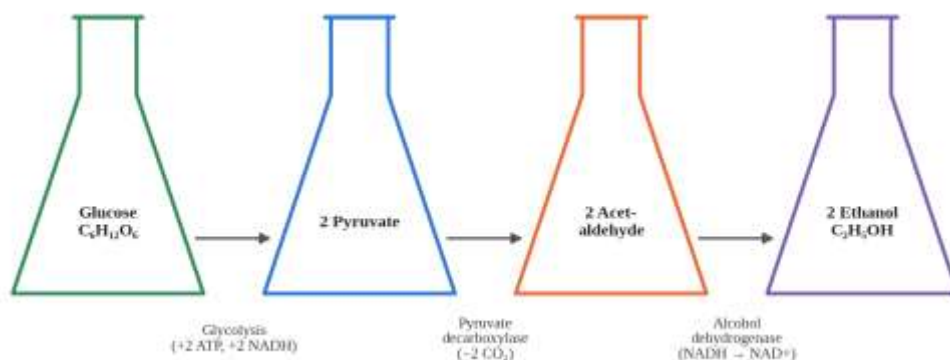


Fig. 4. Anaerobic conversion of glucose to ethanol by yeast.

4.3. Simultaneous Saccharification and Fermentation (SSF)

Hydrolysis and fermentation can be carried out in two ways. In separate hydrolysis and fermentation (SHF), the two steps run in sequence in separate vessels, whereas in simultaneous saccharification and fermentation (SSF) they run together in a single vessel.

- **SSF** has several advantages: less product inhibition, a lower enzyme requirement, higher yields, a reduced need for sterile conditions, a shorter process time and a single reactor (Sun and Cheng, 2002). Its main drawback is that both steps must run at a compromise temperature of about 38 °C, between the optimum for hydrolysis (45–50 °C) and that for fermentation (30 °C). Ethanol also inhibits cellulase: its activity fell by 9%, 36% and 64% at 9, 35 and 60 g/L of ethanol (Wu and Lee, 1997, as cited in Sun and Cheng, 2002).
- **SHF** lets each step run at its own best temperature (Sun and Cheng, 2002).

SSF in the pearl millet study. Sriramajayam et al. (2022) used SSF for pearl millet stalk: 100 mL working volume in 250 mL flasks, cellulase and xylanase added after autoclaving, 10% (v/v) yeast inoculum, anaerobic incubation and sampling at 24, 48, 72 and 96 h. They tested three hydrolysate conditions:

- hydrolysate alone;
- hydrolysate with glucose added to reach 60 g/L total sugar;
- hydrolysate with 10% (w/v) yeast extract added.

The glucose-supplemented condition with *S. cerevisiae* gave the most ethanol, 32.13 g/L at 96 h, with 58.48 g/L of sugar consumed. Optimising temperature (25, 30, 35 °C) and agitation (75, 100, 125 rpm) for this condition raised ethanol to 44.24 g/L at 30 °C and 100 rpm. This concentration lies in the range where Sun and Cheng (2002) report a substantial loss of cellulase activity, so ethanol may have limited hydrolysis late in the run. The study did not test this.

SHF in the other studies. Wang et al. (2025) and Rempel et al. (2019) both ran hydrolysis at 50 °C and fermentation at 30 °C in separate steps.

4.4. Fermentation Kinetics and Modelling

Monod (1949) divided the growth of a batch culture into six phases:

- lag (growth rate zero);
- acceleration (growth rate increasing);
- exponential (growth rate constant);
- retardation (growth rate decreasing);
- stationary (growth rate zero);
- decline (growth rate negative).

Monod (1949) also described the growth rate R as a hyperbolic function of the concentration C of the limiting nutrient:

$$R = R_K \cdot C / (C_1 + C)$$

where R_K is the maximum rate and C_1 is the concentration at which the rate is half of R_K . In modern notation this is written $\mu = \mu_{\max} \cdot S / (K_s + S)$. Although Monod worked with bacteria, the model is commonly applied to yeast fermentation. He also defined the growth yield as the cell mass formed per unit of limiting nutrient consumed, and found it independent of nutrient concentration (Monod, 1949).

This yield concept underlies the coefficients now used to summarise fermentation performance (Schmidell et al., 2011, as cited in Rempel et al., 2019):

- $Y_{X/S}$: grams of cells formed per gram of substrate consumed;
- $Y_{P/S}$: grams of ethanol formed per gram of substrate consumed;
- $Y_{P/X}$: grams of ethanol formed per gram of cells;
- **productivity**: ethanol formed per litre per hour.

Rempel et al. (2019) reported this kind of kinetic data for *Spirulina* hydrolysate:

- a lag phase of up to 4 h;
- a fall in reducing sugar between 4 and 16 h, with a matching rise in yeast and ethanol;
- ethanol productivity peaking at 16 h, at 1.57 g/L/h in the best condition.

Their full yield coefficients are reviewed in Section 7.4. None of the primary studies fitted a Monod-type model to its data. All reported yields at fixed sampling times instead (Sriramajayam et al., 2022; Wang et al., 2025; Rempel et al., 2019).

5. Maize Stalks and Stems

5.1. Compositional Analysis of Maize Stems (Cellulose, Hemicellulose, Lignin, and Ash Fractions)

Wong and Fikri (2014) reported corn stalk to contain 40.28% cellulose, 35.06% pentosans (hemicellulose sugars) and 19.35% lignin (Fig. 5). They described the pentosan fraction as easily hydrolysable. They did not report ash content. The remaining 5.31% shown in Fig. 5 is therefore calculated by difference and includes ash and other minor components.

Wang et al. (2025) measured lignin (acid-insoluble and acid-soluble), cellulose (glucan) and hemicellulose (xylan) by the NREL standard procedures, using five biological replicates each pooled from three plants. They reported that the *bm2* mutant had 8.01% less acid-insoluble lignin than the wild type, with no significant change in cellulose or hemicellulose.

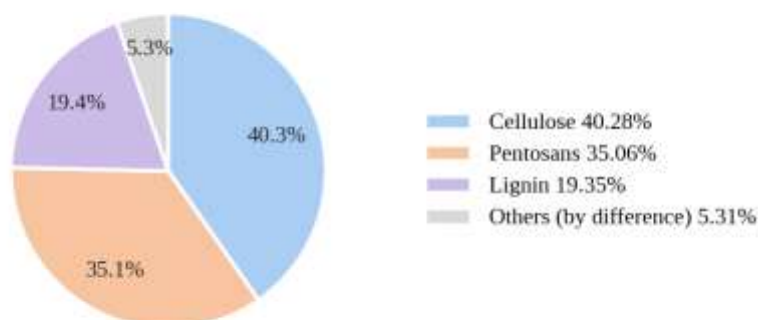


Fig. 5. Composition of maize (corn) stalk. "Others" is calculated by difference. Data from Wong and Fikri (2014).

5.2. Comparative Biomass Response and Recalcitrance of Maize Stems

Maize stalk is one of the more lignified cereal residues reviewed here (19.35% lignin; Wong and Fikri, 2014). Wang et al. (2025) accordingly used a relatively strong acid pretreatment (4% sulfuric acid at 121 °C) followed by detoxification.

Hamdy et al. (2021) approached the same barrier through milder pretreatment chemistry: autoclaving with oxalic acid instead of sulfuric acid, which reduces corrosion and neutralisation needs. Genetic and chemical routes can thus complement each other. A low-lignin variety would need a less severe chemical treatment, although no study reviewed here tested the two together.

5.3. Bioethanol Yield Benchmarking and Empirical Results

Wang et al. (2025) fermented two streams from each stover sample:

- Stream 1.** The acid hydrolysate, containing glucose and xylose, was adjusted to 30 g/L reducing sugar and fermented for 72 h by *P. stipitis* CICC1960. The *bm2* mutant gave 3.05 g/L ethanol, 59.07% more than the wild type.
- Stream 2.** The cellulose residue was hydrolysed enzymatically, concentrated to 50 g/L glucose and fermented for 72 h by *S. cerevisiae* P1. The *bm2* mutant gave 25.88 g/L ethanol, 38.58% more than the wild type.

Both fermentations were shaken at 120 rpm for 12 h and 90 rpm for the next 12 h, then left sealed and still. Ethanol was measured by HPLC. Fig. 6 shows these results. The wild-type ethanol values in the right-hand panel were calculated in this review from the reported percentage increases.

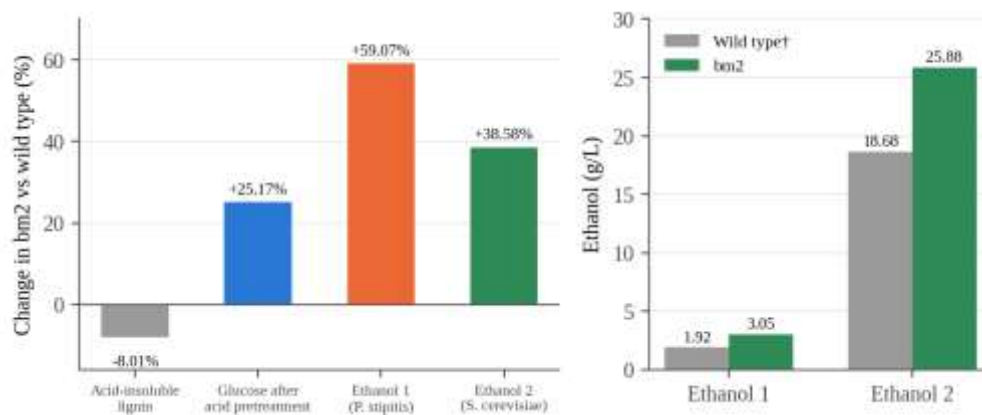


Fig. 6. Maize *bm2* mutant compared with the wild type: percentage changes (left) and ethanol concentrations (right). †Wild-type values calculated from the reported percentage increases. Data from Wang et al. (2025).

Wong and Fikri (2014) obtained their highest ethanol output from corn stalk at 50 °C hydrolysis with the largest loading (30 g) (Fig. 3). They concluded that production was successful but the yield was still small, and that further optimisation was needed.

6. Pearl Millet Stems

6.1. Structural Properties of Pearl Millet Stems

Pearl millet (*Pennisetum glaucum*) is a drought-tolerant cereal. Sriramajayam et al. (2022) characterised its stalk using ASTM methods for moisture and ash and the Goering and Van Soest method for fibre, and reported:

- cellulose 39.98%, hemicellulose 31.05% and lignin 17.5% (Fig. 7);
- moisture 4.13% and ash 2.73%;
- water extractives 5.83% and ethanol extractives 6.61%;
- bulk density 100.01 kg/m³.

The cellulose and hemicellulose fractions together make up 71.03% of the stalk.

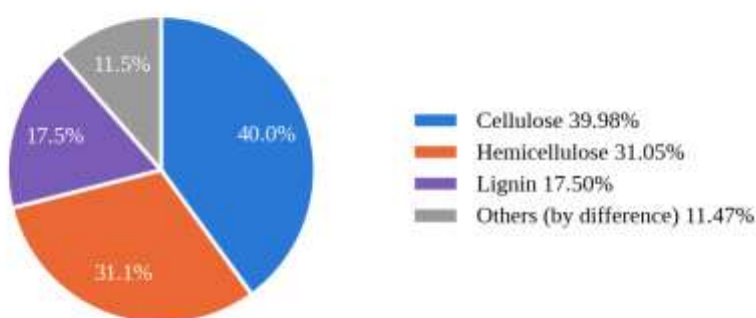


Fig. 7. Composition of pearl millet stalk. “Others” is calculated by difference and includes ash and extractives. Data from Sriramajayam et al. (2022).

6.2. Compositional Comparison: Maize Stems vs. Pearl Millet Stems

Fig. 8 and Table 2 compare the two stalks. Their cellulose contents are almost the same (39.98% and 40.28%). Maize stalk has more hemicellulose or pentosans (35.06% against 31.05%) and more lignin (19.35% against 17.5%). The higher lignin content of maize fits its use of a stronger acid (sulfuric) in the study reviewed, compared with the ortho-phosphoric acid found sufficient for pearl millet. The two stalk studies used different analytical methods (Van Soest for pearl millet; unspecified for Wong and Fikri, 2014), so small differences should be read with caution.

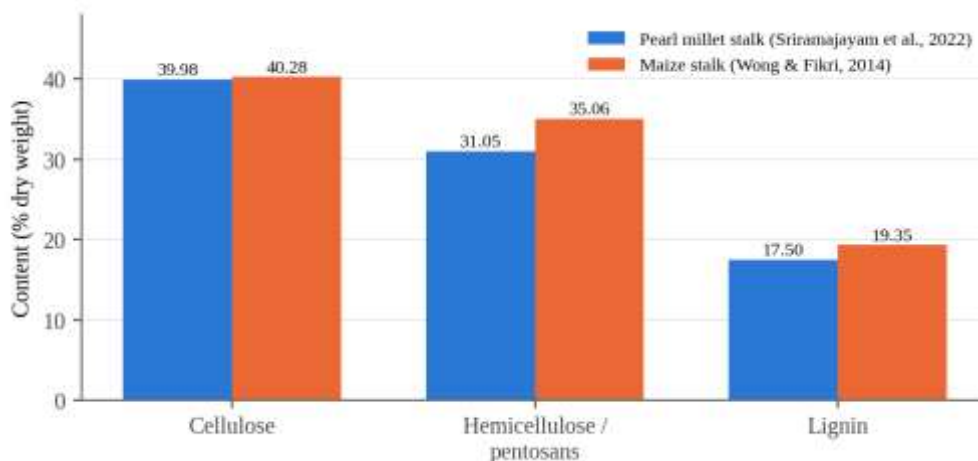


Fig. 8. Structural composition of pearl millet stalk and maize stalk. Data from Sriramajayam et al. (2022) and Wong and Fikri (2014).

Table 2. Composition of maize stalk and pearl millet stalk.

Component (% dry weight)	Maize stalk	Pearl millet stalk
Cellulose	40.28	39.98
Hemicellulose / pentosans	35.06	31.05
Lignin	19.35	17.50
Ash	Not reported	2.73
Moisture	Not reported	4.13
Source	Wong and Fikri (2014)	Sriramajayam et al. (2022)

6.3. Predicted and Empirical Bioethanol Yields for Pearl Millet

Theoretical yield. A theoretical ethanol yield can be estimated from composition. This review assumed complete hydrolysis (cellulose to glucose with a hydration factor of 1.111; hemicellulose to xylose with a factor of 1.136) and complete conversion at 0.511 g ethanol per gram of sugar. On that basis, one kilogram of dry pearl millet stalk could give about 227 g of ethanol from cellulose and 180 g from hemicellulose, 407 g in total (Fig. 9). The xylose share is only available with a pentose-fermenting organism.

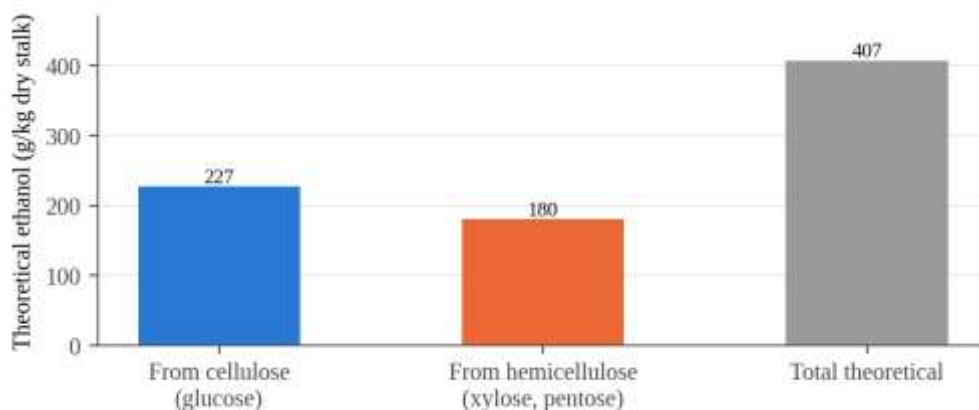


Fig. 9. Theoretical ethanol from pearl millet stalk, calculated in this review from the composition reported by Sriramajayam et al. (2022).

Empirical yield. Sriramajayam et al. (2022) reported the following:

- **Pretreatment:** 38.96 g/L of sugar.
- **SSF with glucose supplementation:** 32.13 g/L ethanol at 96 h with *S. cerevisiae*, which outperformed *P. stipitis*.
- **After temperature and agitation optimisation:** 44.24 g/L at 30 °C and 100 rpm after 96 h.

Because glucose was added to reach 60 g/L of sugar, the final figure is not a yield from the stalk alone. In addition, *S. cerevisiae* does not ferment xylose, so most of the hemicellulose contribution in Fig. 9 was probably not captured.

7. *Spirulina* Microalgae (Non-Terrestrial Biomass)

7.1. Advantages of Microalgal Biomass over Terrestrial Feedstocks

Rempel et al. (2019) summarised the case for third-generation feedstocks as follows:

- **First generation** feedstocks raise the food-versus-fuel debate.
- **Second generation** requires energy-intensive pretreatment and gives low yields.
- **Micro- and macroalgae** grow five to ten times faster than common biomass, need no arable land and can use agro-industrial effluents as a nutrient source, which reduces eutrophication and the need for synthetic nutrients.

They also note that, because microalgae lack cellulose and lignin, their processing does not generate fermentation inhibitors, and that their hydrolysate consists mostly of hexoses rather than pentoses (citing Pancha et al., 2016, and Lee et al., 2015). These two properties remove the two main difficulties of the lignocellulosic route: recalcitrance and pentose fermentation.

7.2. Compositional and Structural Analysis of *Spirulina*

Spirulina (*Arthrospira platensis*) is a filamentous cyanobacterium grown in alkaline, bicarbonate-rich media. Its cell envelope lacks cellulose or lignin (Rempel et al., 2019).

Rempel et al. (2019) cultivated it in 20% Zarrouk's medium under non-sterile conditions and harvested it at the stationary phase. The dried biomass contained, on a dry basis (Fig. 10):

- carbohydrate $46.34 \pm 0.2\%$;
- protein $40.30 \pm 1.02\%$;
- lipid $3.5 \pm 0.3\%$;
- ash $10.8 \pm 1.2\%$.

The authors note that *Spirulina* grown in nutrient-rich media usually contains 50–70% protein. They attribute the higher carbohydrate content to harvesting in the decline phase, when microalgae accumulate storage reserves.

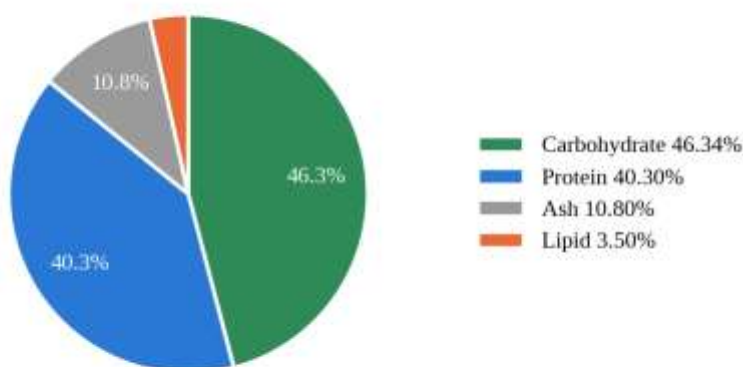


Fig. 10. Composition of *Spirulina platensis* biomass harvested at the stationary phase (dry basis). Data from Rempel et al. (2019).

7.3. Carbohydrate Profile Comparison Matrix (Tabulated Breakdown)

Table 3. Carbohydrate profile of the three feedstocks.

Property	Maize stalk	Pearl millet stalk	<i>Spirulina</i>
Main carbohydrate	Cellulose + pentosans	Cellulose + hemicellulose	+ Storage α -glucan
Carbohydrate content	75.34% (40.28 + 35.06)	71.03% (39.98 + 31.05)	46.34%
Main linkage	β -1,4 (cellulose)	β -1,4 (cellulose)	α -1,4 and α -1,6
Sugars released	Glucose + xylose	Glucose + xylose	Mostly hexoses
Lignin barrier	19.35%	17.5%	None
Enzymes needed	Cellulase (+ hemicellulases)	Cellulase + xylanase	α -Amylase + amyloglucosidase
Organism needed for all sugars	Glucose- and xylose-fermenting	Glucose- and xylose-fermenting	<i>S. cerevisiae</i> alone
Source	Wong and Fikri (2014); Wang et al. (2025)	Sriramajayam et al. (2022)	et Rempel et al. (2019)

The stalks hold more total carbohydrate than *Spirulina*, but it is locked in a lignified structure and part of it is pentose. *Spirulina* holds less carbohydrate, but it is a readily hydrolysed α -glucan that gives mostly hexose sugars.

7.4. Fermentation Dynamics and Processing of *Spirulina* Biomass

Cell disruption and hydrolysis. Rempel et al. (2019) suspended the biomass at 170 g/L in 0.2 mol/L sodium phosphate buffer (pH 5.5) and processed it as follows:

- freezing at -20°C for 24 h and thawing at 4°C for 24 h;
- gelatinisation at 100°C for 10 min;
- hydrolysis with α -amylase (Liquozyme® Supra 2.2X, from *Bacillus licheniformis*, 300 KNU/g) and amyloglucosidase (AMG® 300 L, from *Aspergillus niger*, 300 AGU/mL), each added together at 1% (v/v), at 50°C and 100 rpm in a 1.5 L reactor.

About 50% hydrolysis was reached in 2 h. Sugar release stabilised after 10 h at 70.45 g/L of reducing sugar, an 82% conversion of the storage carbohydrate. α -Amylase breaks the α -1,4 bonds into dextrans and oligosaccharides, and amyloglucosidase then cleaves both α -1,4 and α -1,6 bonds to release glucose.

Other hydrolysis routes. Other *Spirulina* studies released less sugar (Fig. 11):

- **Werlang et al. (2020):** 27 g/L of monosaccharides, a 67.5% saccharification yield. Three enzymes were applied one after another: α -amylase at 90 °C for 2 h, glucoamylase at 60 °C for 2 h and cellulase at 50 °C for 40 h.
- **Simon et al. (2025):** 19.06 g/L of glucose from bioflocculated *Spirulina*, at about 70% hydrolysis efficiency, with amylase and amyloglucosidase at pH 4.5 and 60 °C.
- **Hossain et al. (2015):** 15.2 g/L of reducing sugar by sulfuric acid hydrolysis.
- **Ma'mun et al. (2024):** at most 1.19 g/L of glucose, by microwave-assisted hydrolysis with 0.2 M sulfuric acid at 100 °C for 90 min.

For cyanobacteria more generally, repeated freeze–thaw destroyed more than 80% of cells in one to five cycles (Greenstein et al., 2021). Simon et al. (2025) found that combining ultrasound with autoclaving gave better hydrolysis than either alone.

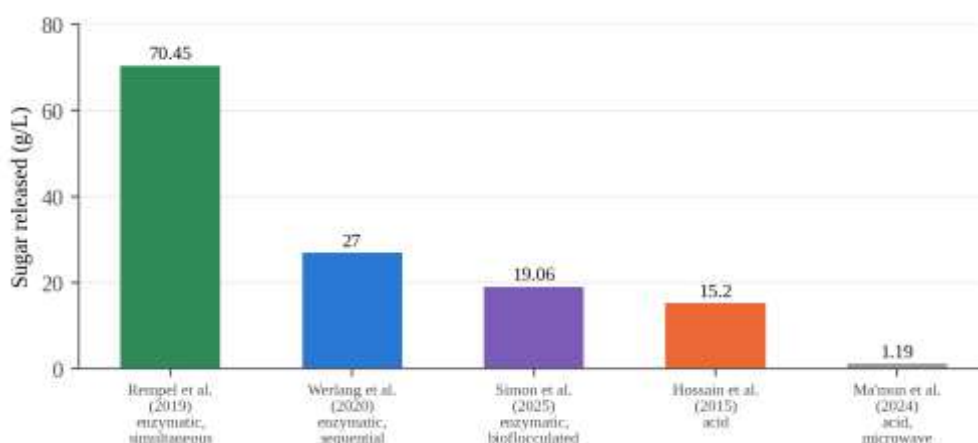


Fig. 11. Sugar released from *Spirulina* biomass by different hydrolysis routes. Data from the studies named on the axis.

Fermentation. The hydrolysate was adjusted to pH 4.5–5.0 with acetic acid, sterilised at 121 °C for 20 min and inoculated with 10% (v/v) *S. cerevisiae* CAT-1. Fermentation ran in a 1 L flask (450 mL working volume) at 30 °C under anaerobic conditions for 20 h (Rempel et al., 2019). Three inoculum preparations were compared:

- 3% hydrolysate in the inoculum (FS3%);
- 15% hydrolysate (FS15%);
- 15% hydrolysate plus synthetic nutrients (FNS15%).

The results (Fig. 12) were:

- **Efficiency:** 83.36% for FS3% and 83.56% for FNS15%, not significantly different; FS15% was lower at 74.11%.
- **Ethanol yield ($Y_{P/S}$):** 0.41–0.46 g/g, not significantly different between conditions.
- **Cell yield ($Y_{X/S}$):** highest in FS15% (0.08 g/g) and lowest in FNS15% (0.05 g/g).

Adding nutrients therefore did not increase yeast growth. The biomass itself supplied enough minerals. By comparison, Pancha et al. (2016) needed peptone and yeast extract to reach 78% efficiency with *Scenedesmus* hydrolysate (as cited in Rempel et al., 2019). The final ethanol concentration was 15.7 g/L.

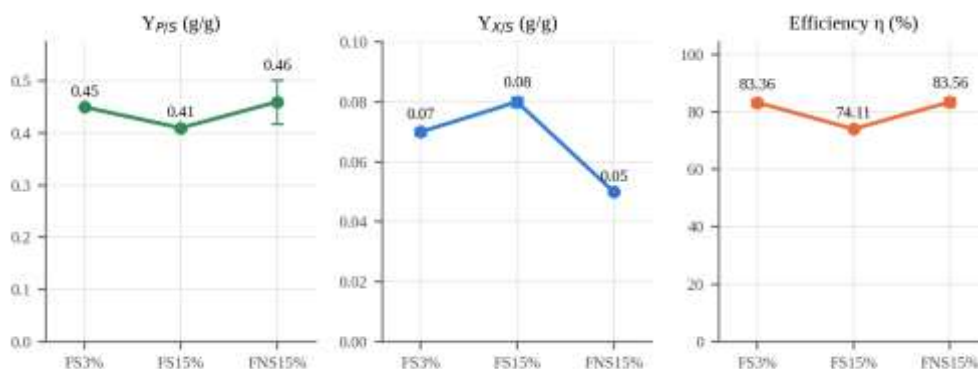


Fig. 12. Kinetic parameters of *Spirulina* hydrolysate fermentation under three inoculum preparations (mean $\pm$ SD). Data from Rempel et al. (2019).

Comparison across studies. Table 4 compares the *Spirulina* studies. They reported ethanol in different units, so the values are given as published.

- Werlang et al. (2020) reached 92% conversion of the glucose in the hydrolysate in 9 h with the engineered bacterium *Escherichia coli* MS04. This gave 12.7 g/L ethanol, a productivity of 1.4 g/L/h and 0.13 g of ethanol per gram of biomass.
- Simon et al. (2025) obtained 7.29 g/L ethanol (about 79% conversion efficiency) after 12 h with *S. cerevisiae* CAT-1, the same strain used by Rempel et al. (2019).
- Hossain et al. (2015) reported ethanol contents of 0.85–1.0% after fermentation with *S. cerevisiae*, and described the yield as not economical.
- Ma'mun et al. (2024) reported a bioethanol yield of 2.89% after 96 h of fermentation and distillation.

Table 4. Hydrolysis and fermentation outcomes reported for *Spirulina* biomass.

Study	Hydrolysis	Sugar released	Organism	Ethanol	Efficiency; time
Rempel et al. (2019)	Freeze–thaw, gelatinisation, α -amylase + amyloglucosidase	70.45 g/L	<i>S. cerevisiae</i> CAT-1	15.7 g/L	83%; 20 h
Werlang et al. (2020)	α -Amylase, glucoamylase, cellulase in sequence	27 g/L	<i>E. coli</i> MS04	12.7 g/L	92%; 9 h
Simon et al. (2025)	Amylase + amyloglucosidase, bioflocculated biomass	19.06 g/L glucose	<i>S. cerevisiae</i> CAT-1	7.29 g/L	$\approx$ 79%; 12 h
Hossain et al. (2015)	H ₂ SO ₄	15.2 g/L	<i>S. cerevisiae</i>	0.85–1.0%	Not reported
Ma'mun et al. (2024)	Microwave-assisted H ₂ SO ₄	1.19 g/L glucose	<i>S. cerevisiae</i>	2.89% yield	Not reported; 96 h

7.5. Projected *Spirulina* Bioethanol Yields: Theoretical vs. Practical Outputs

The practical yield can be traced step by step from the data of Rempel et al. (2019), per kilogram of dry biomass (Fig. 13, left):

1. **Carbohydrate:** 463.4 g (46.34%).
2. **Reducing sugar:** at 82% hydrolysis, about 380 g.
3. **Theoretical ethanol:** at 0.511 g/g, this sugar could give about 194 g.
4. **Practical ethanol:** at 83% fermentation efficiency, about 161 g.

This step-by-step calculation was made in this review. It agrees with the bioethanol energy potential that Rempel et al. (2019) themselves reported, 4,664 kJ/kg of biomass. The result, about 0.16 g of ethanol per gram of biomass, is also

close to the 0.13 g/g reported by Werlang et al. (2020) by a different route. If the hydration of glucan to glucose (factor 1.111) is included, the full theoretical maximum rises to about 263 g/kg (Section 8).

Rempel et al. (2019) also compared energy routes (Fig. 13, right):

- converting the whole biomass directly to biomethane: 16,770 kJ/kg;
- bioethanol alone: 4,664 kJ/kg;
- bioethanol followed by biomethane from the saccharification and fermentation residues: 13,945 kJ/kg.

Producing both bioethanol and biomethane from the same *Spirulina* biomass could therefore contribute to energy security.

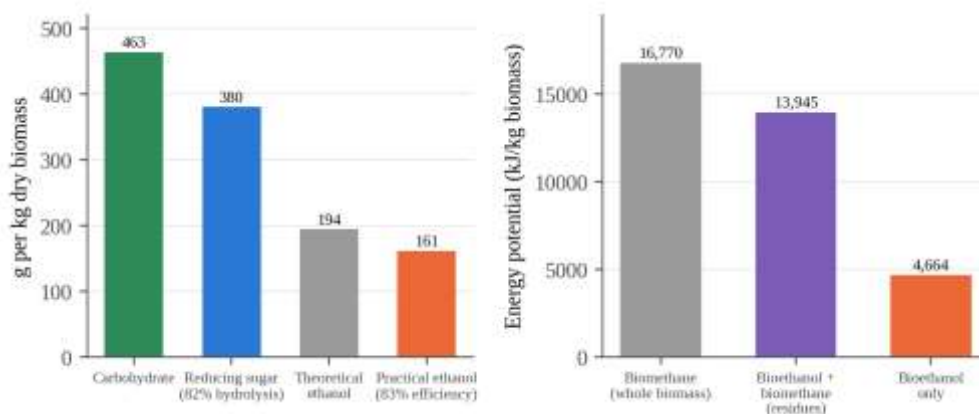


Fig. 13. Left: theoretical and practical ethanol per kilogram of *Spirulina*, calculated in this review from the efficiencies reported by Rempel et al. (2019). Right: energy potential of the three routes reported by Rempel et al. (2019).

7.6. Comparative Advantages of *Spirulina* over Maize Stem and Pearl Millet Stem

The literature reviewed points to the following advantages of *Spirulina* over the two stalks:

1. **No lignin.** Only cell disruption is needed; no acid pretreatment, neutralisation or detoxification (Rempel et al., 2019).
2. **No inhibitors.** No cellulose or lignin means no furan or phenolic inhibitors (Rempel et al., 2019).
3. **Mostly hexose sugars.** A single yeast, *S. cerevisiae*, is sufficient (Rempel et al., 2019).
4. **Fast hydrolysis.** 70.45 g/L of reducing sugar within 10 h with two enzymes (Rempel et al., 2019).
5. **Self-sufficient nutrition.** Fermentation reached 83% efficiency without added nutrients (Rempel et al., 2019).
6. **Useful residues.** The fermentation residue gave 422 ± 15 NL CH₄/kg of volatile solids, more than the whole biomass (326 ± 2) or the saccharification residue (296 ± 10) (Fig. 14) (Rempel et al., 2019).
7. **Cultivation.** No arable land is needed and growth is five to ten times faster than common biomass (Rempel et al., 2019).

Its disadvantages are:

- lower carbohydrate content than the stalks (Table 3);
- a long 48 h freeze–thaw step in the best reported route;
- the need for dedicated cultivation, whereas the stalks are residues of existing crops.

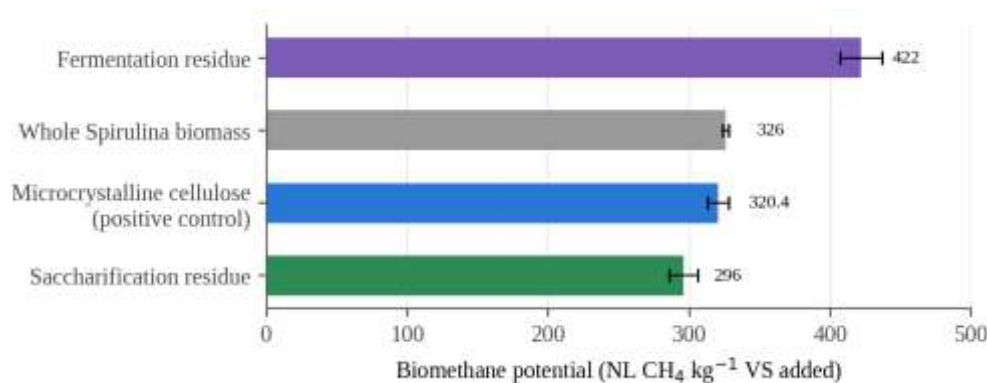


Fig. 14. Biomethane potential of *Spirulina* biomass and of the residues from bioethanol production (mean $\pm$ SD). Data from Rempel et al. (2019).

8. Comparative Synthesis of Feedstock Performance: Maize Stem, Pearl Millet Stem and *Spirulina*

8.1. Consolidated Evaluation Matrix

Table 5 brings together the process conditions and results reported in the primary studies. The figures come from independent studies with different scales, loadings and analytical methods. They show what each study reported and do not come from a head-to-head laboratory comparison.

Table 5. Consolidated evaluation matrix.

Parameter	Maize stalk	Pearl millet stalk	<i>Spirulina</i>
Generation	Second	Second	Third
Carbohydrate (% dry weight)	75.34	71.03	46.34
Lignin (%)	19.35	17.5	0
Pretreatment	4% H ₂ SO ₄ , 121 °C, 30 min + detoxification	7.5% H ₃ PO ₄ , 121 °C, 3 h	Freeze–thaw (48 h) + gelatinisation (100 °C, 10 min)
Enzymes	Cellulase 400 U/mg, 30 mg/g	Cellulase 40 FPU/g + xylanase 25 U/mL	α -Amylase + amyloglucosidase, 1% (v/v) each
Hydrolysis result	+25.17% glucose (<i>bm2</i> vs wild type)	38.96 g/L (pretreatment)	70.45 g/L (82% conversion, 10 h)
Process mode	SHF, two-stage fermentation	SSF	SHF
Organism	<i>P. stipitis</i> + <i>S. cerevisiae</i>	<i>S. cerevisiae</i>	<i>S. cerevisiae</i> CAT-1
Fermentation time	72 h per stage	96 h	20 h
Ethanol	3.05 + 25.88 g/L (<i>bm2</i>)	44.24 g/L (with added glucose)	15.7 g/L (83%)
Theoretical ethanol (g/kg) [†]	$\approx$ 432	$\approx$ 407	$\approx$ 263
Inhibitors	Tested and removed	Not reported	Not generated
Co-products reported	None	None	Biomethane
Source	Wong and Fikri (2014); Wang et al. (2025)	Sriramajayam et al. (2022)	et Rempel et al. (2019)

[†] Calculated in this review from composition, assuming complete hydrolysis and fermentation (factors 1.111 for glucan, 1.136 for xylan, 0.511 g ethanol per g sugar).

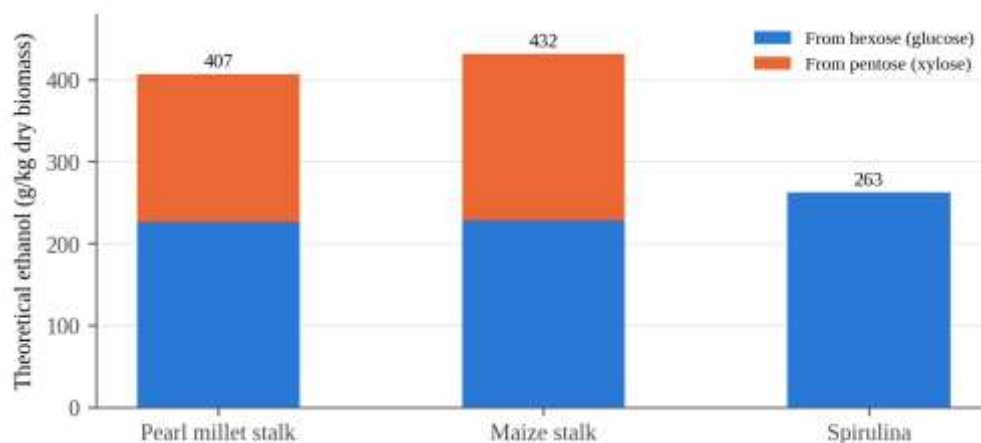


Fig. 15. Theoretical ethanol per kilogram of dry biomass, divided into the hexose and pentose contributions. Calculated in this review from the compositions reported by Wong and Fikri (2014), Sriramajayam et al. (2022) and Rempel et al. (2019).

Fig. 15 shows an important contrast. On paper, the two stalks have a higher theoretical ethanol potential than *Spirulina* (about 432 and 407 g/kg against 263 g/kg). However, 44–47% of the stalk potential lies in pentose sugars, which *S. cerevisiae* cannot use, and the rest is locked behind lignin. All of *Spirulina*'s potential is in hexose, and Rempel et al. (2019) converted 82% of its carbohydrate to sugar within 10 h.

8.2. Techno-Economic and Yield Efficiency Trade-offs

None of the primary studies reported a techno-economic analysis. The trade-offs below are therefore based on the process requirements each study describes, and Table 6 summarises them.

Process time. From the start of pretreatment to the end of fermentation, *Spirulina* took about 78 h, pearl millet about 99 h and maize about 120 h (Fig. 16). Most of the *Spirulina* time is the 48 h freeze–thaw step. The stalks spend most of their time in fermentation.

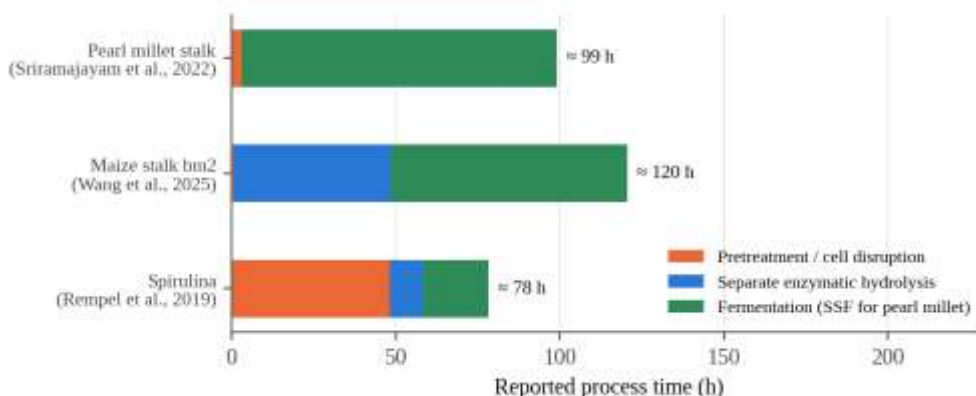


Fig. 16. Reported process time by stage. For maize, the *P. stipitis* fermentation of the acid hydrolysate can run while the cellulose residue is being hydrolysed, so only one 72 h fermentation is shown. Data from Sriramajayam et al. (2022), Wang et al. (2025) and Rempel et al. (2019).

Chemicals and equipment. Both stalks need acid pretreatment at 121 °C, which requires acid-resistant vessels and a neutralisation step. The maize route of Wang et al. (2025) also needs detoxification with calcium hydroxide and an adsorption resin. *Spirulina* needs a freezer and a 100 °C water bath but no acid (Rempel et al., 2019).

Enzymes. The stalks need a multi-enzyme cellulase–xylanase system. *Spirulina* needs two amylolytic enzymes. In every study, enzyme doses were fixed rather than optimised.

Feedstock supply. Pearl millet and maize stalks are residues of existing grain crops and need no extra land. *Spirulina* needs dedicated cultivation, although no arable land is required (Rempel et al., 2019).

Yield and co-products. The stalks offer more carbohydrate per kilogram. *Spirulina* gives faster, inhibitor-free conversion and a biomethane co-product.

Table 6. Techno-economic and yield trade-offs identified from the reviewed studies.

Factor	Maize stalk	Pearl millet stalk	<i>Spirulina</i>
Feedstock cost basis	Crop residue	Crop residue	Cultivation required
Pretreatment chemicals	Sulfuric acid, Ca(OH) ₂ , resin	Ortho-phosphoric acid, NaOH	None (freeze–thaw, heat)
Enzyme system	Cellulase	Cellulase + xylanase	α -Amylase + amyloglucosidase
Fermentation organisms	Two	One	One
Pentose utilisation	Yes (<i>P. stipitis</i>)	No	Not needed
Longest step	Fermentation (72 h per stage)	SSF (96 h)	Freeze–thaw (48 h)
Co-product	Not reported	Not reported	Biomethane

No feedstock is best on every measure. Maize stalk offers the most carbohydrate and a proven gain from genetic lignin reduction, but it needs the most processing steps. Pearl millet stalk offers a simple single-vessel SSF route, but its pentose fraction was not used. *Spirulina* offers the fastest and cleanest conversion and a biomethane co-product, but it has the lowest carbohydrate content, needs cultivation, and relies on a 48 h freeze–thaw step.

These findings leave two open questions. First, no study has compared the three feedstocks under one framework. Second, no study has shortened or strengthened *Spirulina* cell disruption by using its alkaline growth chemistry (pH shock).

9. Research Gaps and Future Perspectives

The review identifies the following gaps:

1. **No common framework.** No study has processed maize stalk, pearl millet stalk and *Spirulina* under the same analytical methods and reporting basis. The values in Table 5 therefore cannot be compared directly.
2. **Pentose utilisation in pearl millet.** About 44% of the theoretical ethanol from pearl millet stalk is in its hemicellulose, and the best reported route used a hexose-only yeast.
3. **Low-lignin varieties combined with mild pretreatment.** Brown midrib mutants exist in maize and pearl millet (Sattler et al., 2010), and milder acids have been tested on corn stover (Hamdy et al., 2021). No study has combined the two.
4. **Faster *Spirulina* disruption.** The freeze–thaw step accounts for most of the process time. Shorter or combined disruption methods, including ones that exploit the alkaline growth chemistry of *Spirulina*, have not been evaluated for ethanol production.
5. **Enzyme dose and kinetics.** Enzyme doses were fixed in every study, and no study fitted a Monod-type kinetic model to its fermentation data.
6. **Techno-economics.** No study reported costs or life-cycle impacts for these feedstocks.

10. Conclusions

Maize stalk, pearl millet stalk and *Spirulina* offer different routes to non-food bioethanol.

- **The two stalks** are available as crop residues and hold 71–75% carbohydrate. However, they need acid pretreatment at 121 °C, carry 17.5–19.35% lignin, and have much of their sugar in pentose form. The best reported concentrations were 44.24 g/L for pearl millet (with added glucose) and 25.88 g/L for a lignin-reduced maize mutant.

- *Spirulina* holds less carbohydrate (46.34%), but it is a lignin-free α -glucan. It hydrolysed to 70.45 g/L of reducing sugar in 10 h and fermented at 83% efficiency without detoxification or added nutrients, and its residues can be converted to biomethane.

The choice between the feedstocks depends on whether carbohydrate quantity or ease and speed of conversion matters more. A controlled comparison under a single framework is the most pressing need.

Declarations

CRedit authorship contribution statement.

- Ashish Pushkar: Conceptualization, Investigation (literature survey), Data curation, Visualization, Writing – original draft.
- Devendra Nath Thakur: Supervision, Conceptualization, Writing – review & editing.

Funding. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability. All data discussed are available in the cited publications.

Declaration of generative AI use. During the preparation of this work the authors used Claude (Anthropic) to assist with drafting, structuring and preparing figures. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Acknowledgements. The authors thank the University Department of Chemistry, Patna University, for providing facilities and support.

References

- Alvira, P., Tomás-Pejó, E., Ballesteros, M., & Negro, M.J. (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology*, 101(13), 4851–4861. <https://doi.org/10.1016/j.biortech.2009.11.093>
- Greenstein, K.E., Zamyadi, A., & Wert, E.C. (2021). Comparative assessment of physical and chemical cyanobacteria cell lysis methods for total microcystin-LR analysis. *Toxins*, 13(9), 596. <https://doi.org/10.3390/toxins13090596>
- Hamdy, A., Elhafez, S.E., et al. (2021). The interplay of autoclaving with oxalate as pretreatment technique in the view of bioethanol production based on corn stover. *Polymers*, 13(21), 3762. <https://doi.org/10.3390/polym13213762>
- Hang, Y.D., Lee, C.Y., & Woodams, E.E. (1981). Production of alcohol from apple pomace. *Applied and Environmental Microbiology*, 42(6), 1128–1129.
- Hossain, M.N.B., Basu, J.K., & Mamun, M. (2015). The production of ethanol from micro-algae *Spirulina*. *Procedia Engineering*, 105, 733–738. <https://doi.org/10.1016/j.proeng.2015.05.064>
- Lee, O.K., Oh, Y.K., & Lee, E.Y. (2015). Bioethanol production from carbohydrate-enriched residual biomass obtained after lipid extraction of *Chlorella* sp. KR-1. *Bioresource Technology*, 196, 22–27. (As cited in Rempel et al., 2019.)
- Ma'mun, S., Prasetyo, M.W., & Anugrah, A.R. (2024). Bioethanol production from *Spirulina* (*Arthrospira platensis*) by microwave-assisted acid hydrolysis pretreatment. *Jurnal Bahan Alam Terbarukan*, 13(1), 1–9. <https://doi.org/10.15294/jbat.v13i1.49344>
- Monod, J. (1949). The growth of bacterial cultures. *Annual Review of Microbiology*, 3, 371–394. <https://doi.org/10.1146/annurev.mi.03.100149.002103>

- Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., & Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, 96(6), 673–686. <https://doi.org/10.1016/j.biortech.2004.06.025>
- NITI Aayog. (2021). *Roadmap for ethanol blending in India 2020–25: Report of the expert committee*. Government of India, New Delhi.
- Pancha, I., Chokshi, K., Maurya, R., Bhattacharya, S., Bachani, P., & Mishra, S. (2016). Comparative evaluation of chemical and enzymatic saccharification of mixotrophically grown de-oiled microalgal biomass for reducing sugar production. *Bioresource Technology*, 204, 9–16. (As cited in Rempel et al., 2019.)
- Rempel, A., Sossella, F.S., Margarites, A.C., Astolfi, A.L., Steinmetz, R.L.R., Kunz, A., Treichel, H., & Colla, L.M. (2019). Bioethanol from *Spirulina platensis* biomass and the use of residuals to produce biomethane: An energy efficient approach. *Bioresource Technology*, 288, 121588. <https://doi.org/10.1016/j.biortech.2019.121588>
- Sattler, S.E., Funnell-Harris, D.L., & Pedersen, J.F. (2010). Brown midrib mutations and their importance to the utilization of maize, sorghum, and pearl millet lignocellulosic tissues. *Plant Science*, 178(3), 229–238.
- Schmidell, W., Lima, A.U., Aquarone, E., & Borzani, W. (2011). *Biotechnologia Industrial*. E. Blücher, São Paulo. (As cited in Rempel et al., 2019.)
- Simon, V., Freitag, J.F., da Silva, J.L., & Colla, L.M. (2025). Optimizing the enzymatic hydrolysis of bioflocculated microalgae for bioethanol production. *Processes*, 13(2), 364. <https://doi.org/10.3390/pr13020364>
- Sriramajayam, S., Padhi, T., Ramesh, D., Gitanjali, J., & Palaniselvam, V. (2022). Process optimization of simultaneous saccharification and fermentation system for bioethanol production from pearl millet stalk. *International Journal of Agriculture Sciences*, 14(6), 11381–11385.
- Sun, Y., & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Technology*, 83(1), 1–11. [https://doi.org/10.1016/S0960-8524\(01\)00212-7](https://doi.org/10.1016/S0960-8524(01)00212-7)
- Wang, J., Tuo, H., Cheng, P., Chen, F., Li, Y., Yuan, M., Lin, W., Bao, X., Sun, J., Guo, Y., Wang, Z., Wang, Y., Li, H., Mu, X., Zhang, Q., Yu, Y., & Gou, M. (2025). Boosting lignocellulosic bioethanol production: Fermentation of corn stalk using a lignin-reduced brown midrib 2 maize mutant. *Biotechnology for Biofuels and Bioproducts*, 19, 1. <https://doi.org/10.1186/s13068-025-02722-6>
- Werlang, E.B., Julich, J., Muller, M.V.G., Neves, F.F., Sierra-Ibarra, E., Martinez, A., & Schneider, R.C.S. (2020). Bioethanol from hydrolyzed *Spirulina* (*Arthrospira platensis*) biomass using ethanologenic bacteria. *Bioresources and Bioprocessing*, 7, 27. <https://doi.org/10.1186/s40643-020-00315-9>
- Wong, Y.C., & Fikri, A.M. (2014). Synthesis of bio-ethanol from corn stalk by fermentation process. *Oriental Journal of Chemistry*, 30(2). <https://doi.org/10.13005/ojc/300232>
- Wu, Z., & Lee, Y.Y. (1997). Inhibition of the enzymatic hydrolysis of cellulose by ethanol. *Biotechnology Letters*, 19, 977–979. (As cited in Sun and Cheng, 2002.)
- Zabed, H., Sahu, J.N., Boyce, A.N., & Faruq, G. (2017). Bioethanol production from renewable sources: Current perspectives and technological progress. *Renewable and Sustainable Energy Reviews*, 71, 475–501. <https://doi.org/10.1016/j.rser.2016.12.076>