Maejo International Journal of
Energy and Environmental Communication
Journal home page: https://ph02.tci-thaijo.org/index.php/MIJEEC

RESEARCH ARTICLE

Novel process integrating the first- and second-generation bioethanol fuel production from arrowroot waste

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ARTICLEINFO

Article history:

Received: 21 November 2025
Revised:27 December 2025
Accepted: 12 January 2026
Published: 13 January 2026

Keywords:

Arrowroot waste
Integrated bioethanol
1G–2G biorefinery
Sulfomethylation
Circular economy

Abstract

Arrowroot waste (ARW) is a starch-processing residue that contains a rare combination of residual starch, cellulose-rich fiber, and low native lignin, making it attractive for integrated first-generation (1G) and second-generation (2G) ethanol production. This study combines compositional analysis, replicated hydrolysis and fermentation trials, pretreatment validation, mass–carbon closure, and a preliminary sustainability assessment. We dried, milled, and sieved ARW into crude starch (CS) and fiber residue (FR), then sulfomethylated FR to obtain cellulose-enriched sulfomethylated treated fiber (STFR). Compositional analysis followed National Renewable Energy Laboratory protocols, and hydrolysis and fermentation were evaluated in triplicate (n = 3) using separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and integrated simultaneous saccharification and co-utilization of starch- and cellulose-derived glucose (SSCF). CS hydrolysis reached 145.7 ± 2.1 g L^{-1} reducing sugars (97.6 ± 1.3% yield), while STFR hydrolysis reached 108.6 ± 2.3 g L^{-1} (88.9 ± 1.7%). The integrated SSCF configuration achieved the highest ethanol titer (82.4 ± 1.2 g L^{-1}) and an overall theoretical yield of 76.9 ± 1.0% after 60 h. Carbon closure reached 95.6% and solids closure 98.0% on a 100 kg dry-ARW basis. A preliminary techno-economic and environmental assessment indicated a net ethanol production cost of approximately USD 0.55 L^{-1}, a greenhouse-gas intensity of 47.8 g CO_2-eq MJ^{-1}, and a net energy ratio of 3.8. Taken together, the results position ARW as a promising single-waste feedstock for integrated 1G–2G ethanol production in starch-processing regions.

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1. Introduction

The transition to low-carbon transport fuels has renewed attention on residue-based ethanol systems that maximize carbon utilization while avoiding direct competition with food. Conventional first-generation (1G) ethanol processes efficiently convert starch or sugar, whereas second-generation (2G) systems unlock cellulose and hemicellulose but often require more severe pretreatment, higher enzyme inputs, and greater capital intensity [4,6,11,14–16,23,24]. Integrating both routes can improve plant-wide economics by sharing utilities, distillation, and fermentation infrastructure [4,9]. Process simulations of sugarcane biorefineries, for example, show that integrated 1G–2G plants achieve lower ethanol production costs than stand-alone 2G plants [25].

Arrowroot (*Maranta arundinacea* L.) is an underutilized tropical starch crop whose industrial processing generates a fibrous waste stream after starch extraction [22]. Because arrowroot starch is extracted

mainly for food and industrial uses, recovery is never complete, and the residual fiber retains both unextracted starch and cellulose [2,22]. Unlike many lignocellulosic residues, arrowroot waste contains both recoverable starch and a comparatively low-lignin fiber fraction, creating a favorable platform for coupled 1G–2G conversion [2]. In Thailand and neighboring Southeast Asian regions, starch industries already operate near dense agricultural and food-processing networks, which makes residue-based integrated biorefineries regionally relevant [18,20,21].

Most previous integrated 1G–2G ethanol studies have focused on combinations such as molasses with bagasse, or they have used separate food-grade and lignocellulosic feedstocks. In contrast, ARW provides both carbohydrate pools within a single industrial residue. That distinction is important: the starch-derived sugars can rapidly initiate fermentation, while the fiber-derived sugars sustain ethanol formation over longer residence times. At the same time, the low-lignin nature of ARW suggests that pretreatment intensity can be moderated relative to more recalcitrant feedstocks.

Accordingly, this study evaluated ARW as a single-residue feedstock for integrated 1G–2G ethanol production. The specific objectives were to (i) characterize the ARW fractions using standard analytical methods, (ii) assess sulfomethylation as a pretreatment for the fiber fraction, (iii) compare SHF, SSF, and integrated SSCF configurations using replicated experiments and statistical testing, (iv) quantify mass and carbon closure, and (v) estimate preliminary techno-economic and environmental performance. Together, these elements form an integrated framework that links fractionation, pretreatment, hydrolysis, fermentation, material balances, and sustainability metrics.

Table 1 Positioning of the present ARW platform relative to representative 1G, 2G, and integrated ethanol routes.

Platform	Feedstock basis	Strength	Typical limitation	Reported ethanol titer (g L^{-1})	Key differentiator
Conventional starch ethanol (1G)	Single starch stream	High sugar accessibility; high fermentability	Does not recover cellulose-rich residue	40–65	Starch only
Residue-only cellulosic ethanol (2G)	Lignocellulosic residue only	Waste valorization; high carbon benefit	High pretreatment severity and enzyme demand	20–55	Cellulose only
Integrated 1G/2G sugar platforms	Separate sugar and fiber feeds	Shared utilities and distillation	Usually requires two feedstocks and complex integration	60–80	Cross-feedstock integration
This study: ARW integrated 1G–2G biorefinery	Single arrowroot waste feedstock, internally fractionated	Residual starch recovery + low-lignin fiber upgrading + one-pot SSCF	Still requires chemical recovery management	82.4	Single-waste integrated starch/cellulose conversion

2. Materials and methods

2.1. Feedstock preparation and fractionation

Industrial ARW was collected from a starch-processing facility in Nakhon Ratchasima, Thailand. The material was screened to remove stones and extraneous debris, dried at 80 °C for 48 h, milled, and sieved through a 200-mesh screen. The fine fraction was designated crude starch (CS), while the retained coarse fraction was designated fiber residue (FR). Fraction yields were recorded on a dry basis. Food-grade α-amylase, glucoamylase, cellulase, and β-glucosidase were used throughout the study, and *Saccharomyces cerevisiae* served as the ethanologenic microorganism [17].

2.2. Biomass characterization and analytical methods

Compositional analysis was performed following NREL laboratory analytical procedures for structural carbohydrates and lignin [1]. Moisture and total solids were determined gravimetrically. Structural carbohydrates and lignin were measured after two-step sulfuric-acid hydrolysis (72% H_2SO_4 at 30 °C for 60 min, followed by dilution to 4% acid and autoclaving at 121 °C for 60 min). Sugars released in the hydrolysate were quantified by HPLC using an Aminex HPX-87H column with 5 mM H_2SO_4 as the mobile phase (0.6 mL min^{-1}) and refractive-index detection. Acid-insoluble lignin and acid-soluble lignin were determined according to the same protocol. Cellulose retention, lignin removal, and apparent carbohydrate preservation after sulfomethylation were also determined.

2.3. Sulfomethylation pretreatment and structural validation

FR was subjected to sulfomethylation using Na_2SO_3 and formaldehyde solution at 160 °C for 6 h to improve cellulose accessibility. In sulfite-based pretreatment, sulfonation increases the hydrophilicity and solubility of lignin and reduces non-productive binding of cellulases to lignin [3,26]. Briefly, 45 g dry FR was mixed with 255 mL reagent solution (0.5 M Na_2SO_3 + 0.5 M CH_2O). After reaction, the solid fraction was washed to near-neutral pH and dried at 80 °C for 24 h to obtain STFR. Solid recovery

and lignin removal were calculated from gravimetric and compositional measurements.

2.4. Enzyme optimization and hydrolysis conditions

Hydrolysis conditions were optimized through preliminary factorial screening of solids loading and enzyme dosage, after which the best-performing conditions were retained for the comparative experiments. CS hydrolysis was carried out using a 20% (w/v) slurry. Liquefaction employed α-amylase at 1.5 mg g^{-1} starch at 90 °C for 60 min, followed by glucoamylase at 60 °C for 120 min. STFR hydrolysis was conducted at 12.5% (w/v) solids in citrate buffer (pH 5.5) at 50 °C for 48 h using cellulase (1.5 mg g^{-1} substrate) and β-glucosidase (0.2 mg g^{-1} substrate). A preliminary enzyme-loading screen was used to justify the selected glucoamylase dosage and to show the economic trade-off between yield and enzyme cost.

2.5. Fermentation configurations

Fermentation experiments were performed in 250 mL flasks with 100 mL working volume using three configurations: SHF, SSF, and integrated SSCF. In SHF, hydrolysis was completed prior to yeast inoculation. In SSF, enzymes and yeast were present concurrently, which relieves end-product inhibition of the cellulases by glucose [27]. In SSCF, partially liquefied CS hydrolysate and STFR were combined in a single vessel together with yeast and the enzyme cocktail. In this study, the term co-fermentation refers to co-utilization of starch-derived and cellulose-derived glucose streams in one integrated operation; the yeast strain was not engineered for pentose fermentation. Fermentations were run at 35 °C and 200 rpm for up to 60 h. Ethanol was quantified by GC-FID and reducing sugars by DNS assay.

2.6. Statistical analysis and reproducibility

All hydrolysis and fermentation experiments were performed in triplicate (n = 3). Results are reported as mean ± standard deviation. Differences among final ethanol titers were evaluated by one-way ANOVA followed by Tukey's HSD test, with significance accepted at $p < 0.05$. Error bars in Figs. 2–4 represent standard deviation.

3. Results and discussion

3.1. Fractionation behavior, novelty, and feedstock composition

Fractionation generated two chemically distinct streams from a single waste feedstock. The CS fraction was enriched in starch, whereas FR retained most of the cellulose and lignin. After sulfomethylation, the cellulose fraction increased to 84.8 ± 1.5%, while lignin decreased from 21.4 ± 0.9% in FR to 2.8 ± 0.2% in STFR. This large apparent reduction in lignin is consistent with the strong hydrolysis improvement observed later in the process.

Table 1 positions the present study relative to conventional 1G, stand-alone 2G, and integrated 1G/2G platforms. The central novelty is that both carbohydrate pools originated from one industrial residue rather than from separate sugar and fiber feedstocks. This single-waste integration reduces feedstock complexity while still enabling a high final ethanol titer.

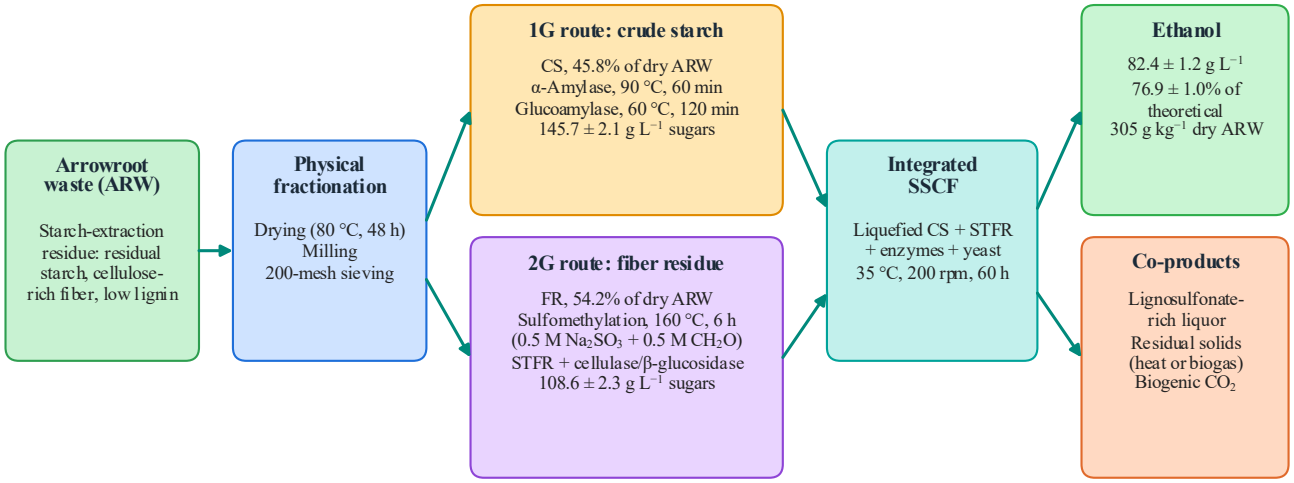

Fig. 1 Integrated workflow for converting arrowroot waste into ethanol via coupled starch and fiber valorization.

Table 2 Composition of whole arrowroot waste and major fractions before and after sulfomethylation.

Feedstock/Fraction	Fraction yield (% dry ARW)	Starch (% dry wt)	Cellulose (% dry wt)	Hemicellulose (% dry wt)	Lignin (% dry wt)	Ash/others (% dry wt)
Whole ARW	100.0	31.6 ± 0.8	40.8 ± 1.1	12.4 ± 0.6	3.4 ± 0.2	11.8
Crude starch (CS)	45.8 ± 1.1	68.9 ± 1.4	9.8 ± 0.6	5.2 ± 0.4	0.8 ± 0.1	15.3
Fiber residue (FR)	54.2 ± 1.1	6.3 ± 0.4	63.7 ± 1.3	11.6 ± 0.7	21.4 ± 0.9	-
Sulfomethylated fiber (STFR)	38.4 ± 0.9	1.2 ± 0.1	84.8 ± 1.5	7.1 ± 0.5	2.8 ± 0.2	4.1

3.2. Enzymatic hydrolysis performance

Under optimized conditions, CS hydrolysis produced 145.7 ± 2.1 g L^{-1} reducing sugars within 3 h, corresponding to 97.6 ± 1.3% of the theoretical starch-derived glucose. In contrast, STFR hydrolysis reached 108.6 ± 2.3 g L^{-1} after 48 h, or 88.9 ± 1.7% conversion relative to cellulose. The slower STFR profile is expected because lignocellulosic deconstruction remains diffusion- and enzyme-access limited even after pretreatment.

The enzyme-loading screen showed why glucoamylase was fixed at 0.20 mg g^{-1} starch in the integrated process. Below that loading, starch conversion and ethanol yield dropped noticeably. Above that loading, the yield gain became marginal whereas enzyme cost continued to increase almost linearly. The chosen point therefore balances conversion performance and economic discipline.

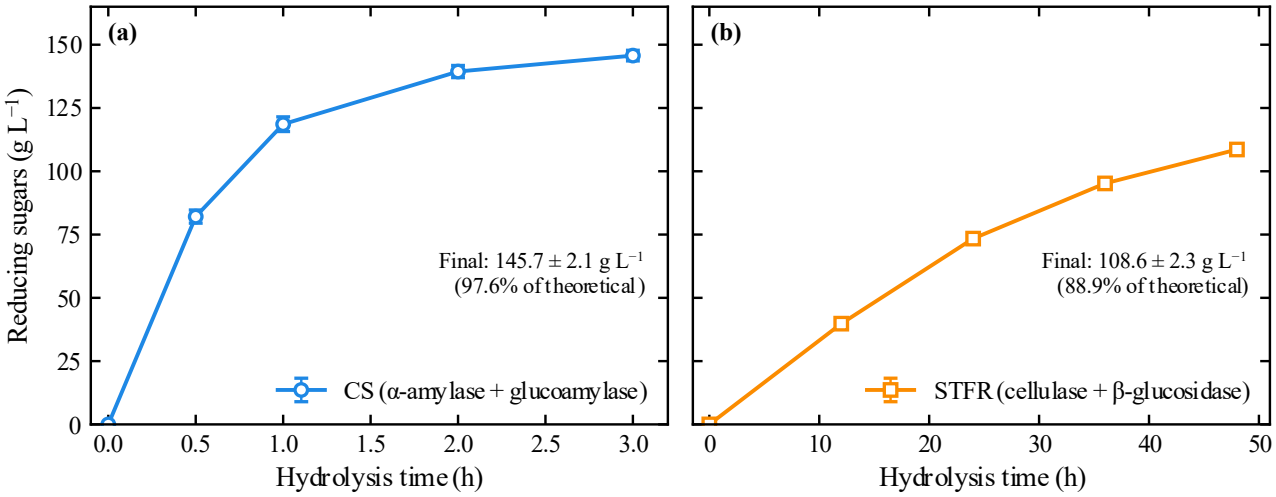

Fig. 2 Hydrolysis profiles of crude starch (CS) and sulfomethylated treated fiber (STFR); points represent mean ± SD (n = 3).

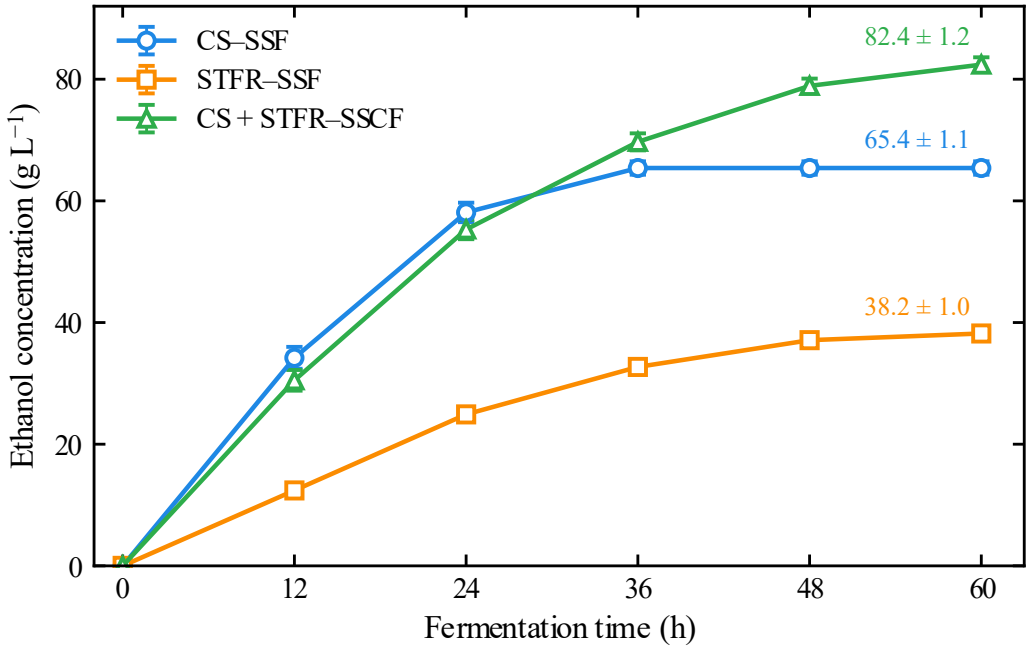

Fig. 3 Ethanol production profiles for CS–SSF, STFR–SSF, and integrated CS + STFR–SSCF; points represent mean ± SD (n = 3).

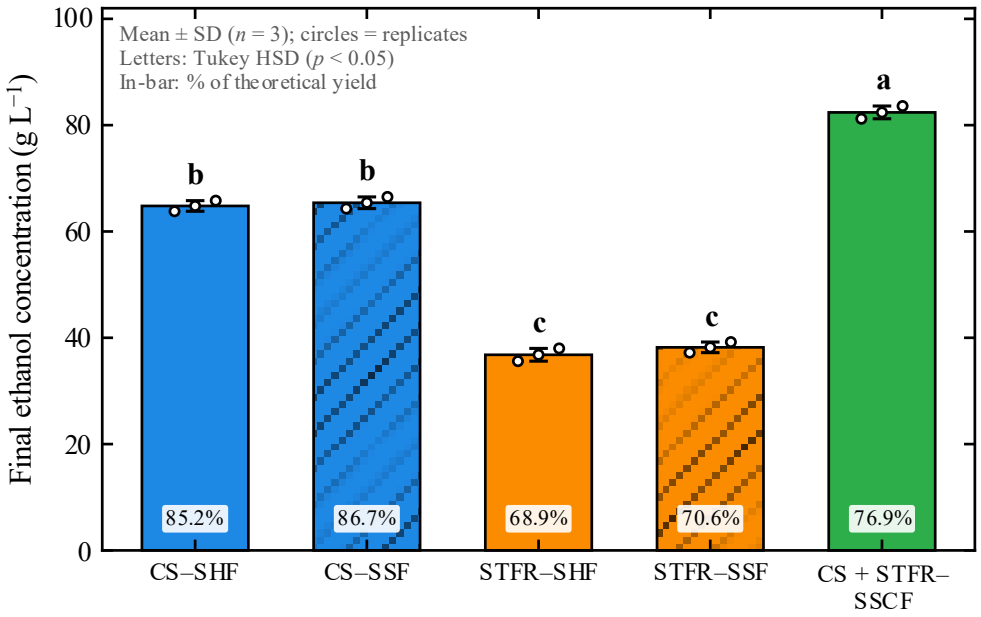

Fig. 4 Final ethanol concentration across process configurations. Error bars show SD (n = 3); letters denote Tukey groups at $p < 0.05$.

Table 3 Summary of hydrolysis and fermentation performance across SHF, SSF, and SSCF configurations.

Process	Hydrolysis yield (%)	Final ethanol (g L^{-1})	Ethanol yield (% theoretical)	Volumetric productivity (g L^{-1} h^{-1})	Residual sugar at end (g L^{-1})
CS–SHF	97.6 ± 1.3	64.8 ± 1.0	85.2 ± 1.4	2.31	<1.0
CS–SSF	97.6 ± 1.3	65.4 ± 1.1	86.7 ± 1.2	2.34	<0.8
STFR–SHF	88.9 ± 1.7	36.8 ± 1.2	68.9 ± 1.6	0.61	1.6 ± 0.2
STFR–SSF	88.9 ± 1.7	38.2 ± 1.0	70.6 ± 1.4	0.64	1.1 ± 0.1
CS + STFR–SSCF	93.4 ± 1.6	82.4 ± 1.2	76.9 ± 1.0	1.37	<0.5

3.3. Fermentation configuration and ethanol performance

The fermentation comparison confirmed that process integration improved final titer without sacrificing stability. CS–SSF slightly outperformed CS–SHF, and STFR–SSF slightly outperformed STFR–SHF, indicating that overlapping hydrolysis and fermentation helped relieve product inhibition. The integrated CS + STFR–SSCF configuration delivered the highest ethanol concentration, 82.4 ± 1.2 g L^{-1}, which was significantly higher than all other configurations ($p < 0.05$).

From a process viewpoint, SSCF benefits from complementary sugar-release kinetics: rapidly available starch-derived glucose allows early yeast growth and ethanol formation, whereas the slower cellulose-derived glucose from STFR extends substrate availability and sustains production over time. That temporal complementarity explains why the integrated configuration produced a higher titer even though the final theoretical yield remained below the starch-only route.

3.4. Pretreatment validation, inhibitor control, and mechanistic interpretation

Pretreatment performance was validated through compositional, by-product, and inhibitor analyses. Compositional evidence showed approximately 90.8% lignin removal from FR and strong cellulose retention.

Potential fermentation inhibitors were low after washing of the pretreated fiber. This is important because furan aldehydes, weak acids, and phenolic compounds formed during pretreatment are the main inhibitors of yeast in lignocellulosic hydrolysates [28,29]. Furfural and 5-HMF were below detection, while acetic acid remained at 0.58 ± 0.05 g L^{-1} in the SSCF broth. Glycerol was the dominant by-product at 4.6 ± 0.3 g L^{-1}, consistent with redox balancing and osmotic adaptation by yeast. These observations suggest that the sulfomethylation route, when followed by sufficient washing and neutralization, can suppress inhibitor carryover to a level compatible with yeast fermentation.

Table 4 Qualitative benchmark of sulfomethylation against commonly discussed pretreatment strategies for residue ethanol systems.

Pretreatment	Expected effect on substrate	Key drawback	Relevance to ARW
Dilute acid	Moderate–high hemicellulose solubilization; moderate lignin removal	Possible furfural/HMF formation; corrosion	Widely used benchmark; higher inhibitor risk for residue ethanol
AFEX	Good fiber swelling; limited sugar degradation	Ammonia recovery required; capital-intensive	Strong option for herbaceous residues but less selective for lignin extraction
DES	High delignification selectivity and tunable chemistry	Solvent recovery still developing; cost varies with formulation	Promising green benchmark for future ARW upgrading
Sulfomethylation (this study)	Very high apparent lignin removal (≈91%) and strong hydrolysis response	Requires sulfite/formaldehyde handling and wastewater control	Well suited to low-lignin, starch-bearing residue where mild-to-moderate pretreatment is sufficient

3.5. Mass and carbon balance closure

To verify closure of the mass and carbon balances, the material-flow assessment was normalized to 100 kg dry ARW. After sieving, 45.8 kg entered the starch route and 54.2 kg entered the fiber route. Pretreatment recovered 38.4 kg STFR and produced a lignosulfonate-rich dissolved-solids stream. The integrated fermentation stage yielded 30.5 kg ethanol, 29.2 kg biogenic CO_2, and 25.5 kg residual solids/biomass. Solids closure reached 98.0% and carbon closure 95.6%, which are acceptable values for bench-scale material accounting.

These balances also help explain the circular-economy potential of the process. The pretreatment liquor is not merely a waste stream; it may contain lignosulfonate-rich material that can be valorized, while the post-fermentation solids remain suitable for energy recovery, including anaerobic digestion to biogas, or soil-oriented applications after appropriate treatment [13,19,20].

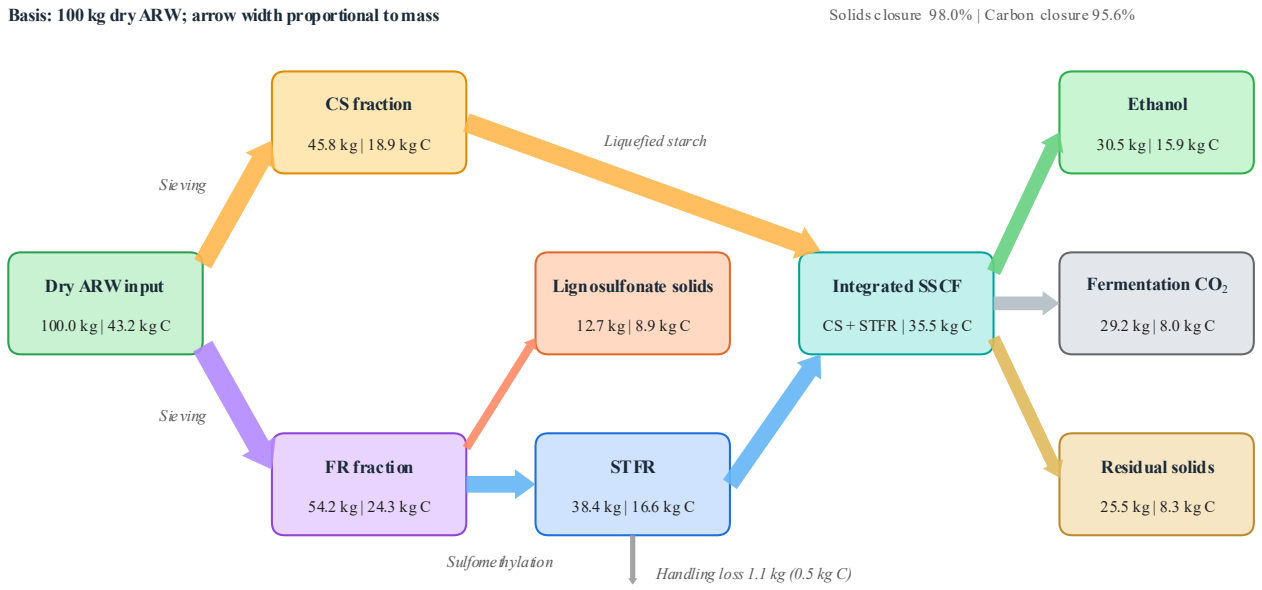

Fig. 5 Simplified mass and carbon flow for the integrated process normalized to 100 kg dry ARW.

3.6. Comparison with alternative pretreatment and integration strategies

ARW is unusually favorable compared with many lignocellulosic residues because it couples a residual starch fraction with a relatively low-lignin fiber fraction. That combination reduces the gap between starch-based and cellulosic ethanol routes. Table 4 benchmarks sulfomethylation against dilute acid, AFEX, and deep eutectic solvent (DES) pretreatments. Sulfomethylation performed strongly in terms of apparent lignin removal and hydrolysis response, although its chemical sustainability depends on efficient reagent recovery and wastewater management.

Mechanistically, the process can be interpreted as an accessibility problem rather than a simple composition

problem. The pretreatment selectively reduced lignin-associated barriers, increasing water uptake and enzyme penetration. Once the starch route initiated fermentation, the SSCF system maintained low free-sugar concentrations, which reduced osmotic stress on yeast and helped preserve hydrolysis efficiency.

Table 5 Mass and carbon balance summary for the integrated ARW-to-ethanol process.

Stream	Mass (kg per 100 kg dry ARW)	Carbon (kg C)	Comment
Dry ARW input	100.0	43.2	Basis of calculation
CS fraction after sieving	45.8	18.9	Starch-rich stream to liquefaction
FR fraction after sieving	54.2	24.3	Fiber-rich stream to pretreatment
STFR recovered after sulfomethylation	38.4	16.6	Solid recovery = 70.9% of FR
Lignosulfonate-rich dissolved solids	12.7	8.9	Potential co-product stream
Unaccounted pretreatment handling loss	1.1	0.5	Wash/fines loss
Ethanol product	30.5	15.9	Equivalent to 305 g kg^{-1} dry ARW
Fermentation CO_2	29.2	8.0	Stoichiometric biogenic CO_2 from fermented hexose
Post-fermentation residual solids/biomass	25.5	8.3	Digestible residue + yeast + ash
Overall solids closure	98.0	95.6	Mass closure 98.0%; carbon closure 95.6%

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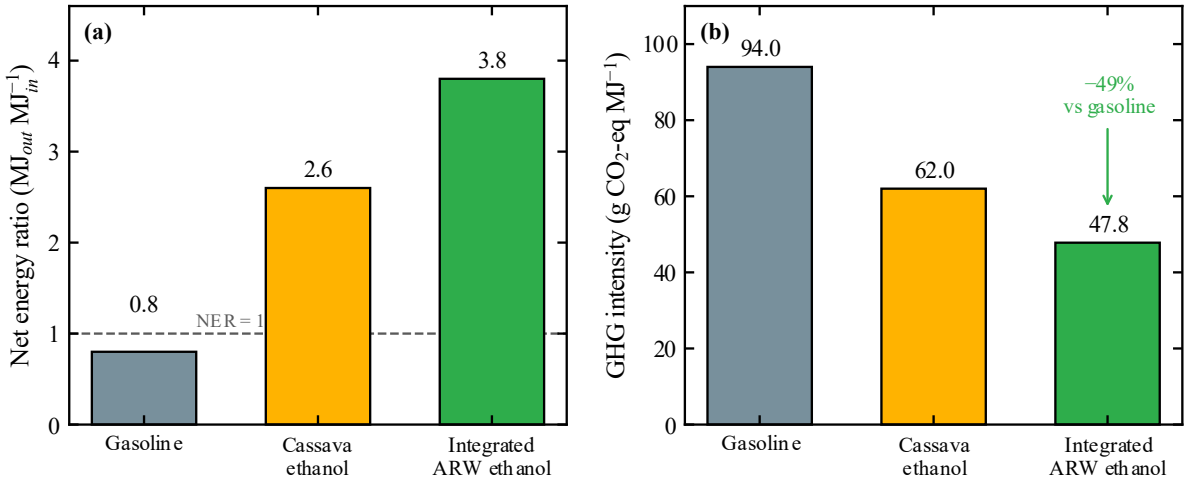

Fig. 6 Comparative energy and greenhouse-gas performance of integrated ARW ethanol relative to representative reference fuels.

Table 6 Base-case cost structure for the integrated ARW ethanol process.

Cost component	USD L^{-1} ethanol	Basis
Feedstock collection, dewatering, and milling	0.12	Includes transport within 35 km radius
Pretreatment chemicals (Na_2SO_3 + CH_2O) net of partial recovery	0.09	Recovery efficiency assumed at 70%
Enzymes and nutrients	0.11	Optimized loadings reduce starch-enzyme demand
Utilities (steam, electricity, cooling water)	0.1	Includes liquefaction, pretreatment, SSCF, distillation
Labor and maintenance	0.05	Semi-centralized plant assumption
Wastewater treatment and environmental control	0.03	Includes sulfite-containing stream conditioning
Capital recovery charge	0.09	15-year straight-line depreciation
Subtotal	0.59	Base-case minimum ethanol production cost
Co-product credit (lignosulfonates + residual solids energy)	-0.04	Conservative circular-economy credit
Net production cost	0.55	Indicative integrated ARW ethanol cost

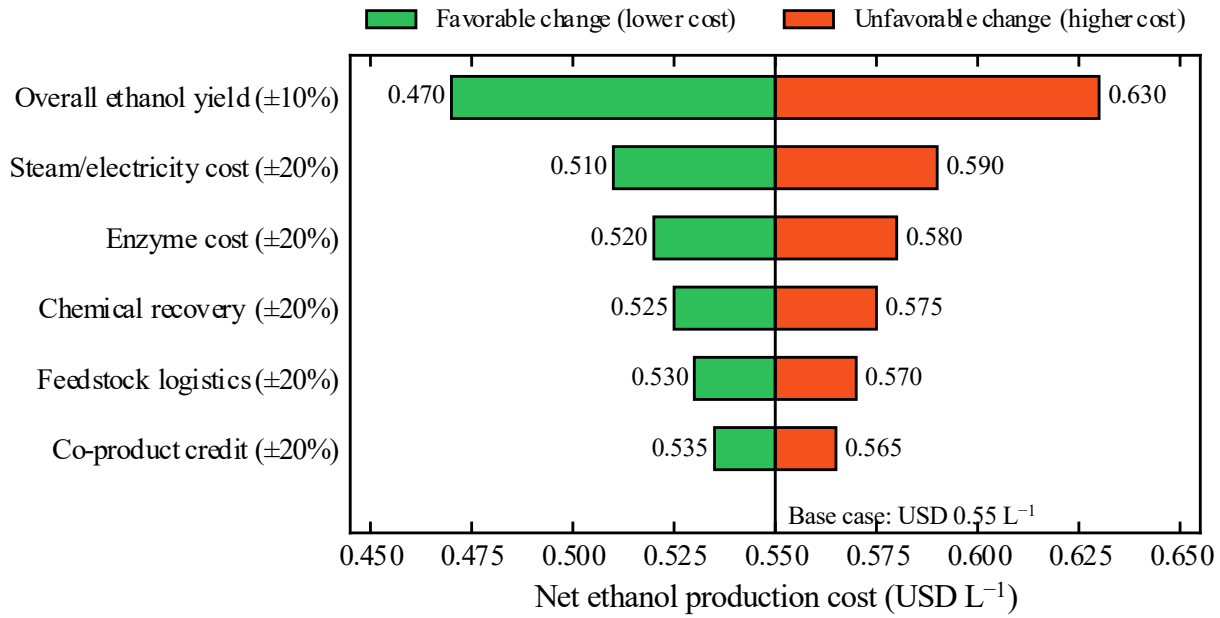

Fig. 7 Sensitivity of net ARW ethanol production cost to key technical and economic variables.

4. Techno-economic, environmental, and scale-up assessment

A preliminary techno-economic analysis, including a sensitivity analysis, was performed to assess the economic feasibility of the process, following the cost-structure approach commonly applied to biochemical ethanol plants [30]. The base case assumed a semi-centralized plant using waste ARW collected within a 35 km radius, partial recovery of sulfite chemistry, and value credits for lignosulfonate-rich streams and residual solids. Under these assumptions, the net ethanol production cost was approximately USD 0.55 L^{-1}, with chemicals, enzymes, and utilities together accounting for the largest share of production cost.

Sensitivity analysis showed that process economics were most responsive to overall ethanol yield, followed by steam/electricity demand, enzyme cost, and chemical recovery efficiency. This means that conversion performance and process integration are more influential than feedstock price alone. From an engineering standpoint, improvements in high-solids mixing, heat integration, and chemical recycling would likely have a larger effect on plant competitiveness than marginal reductions in collection cost.

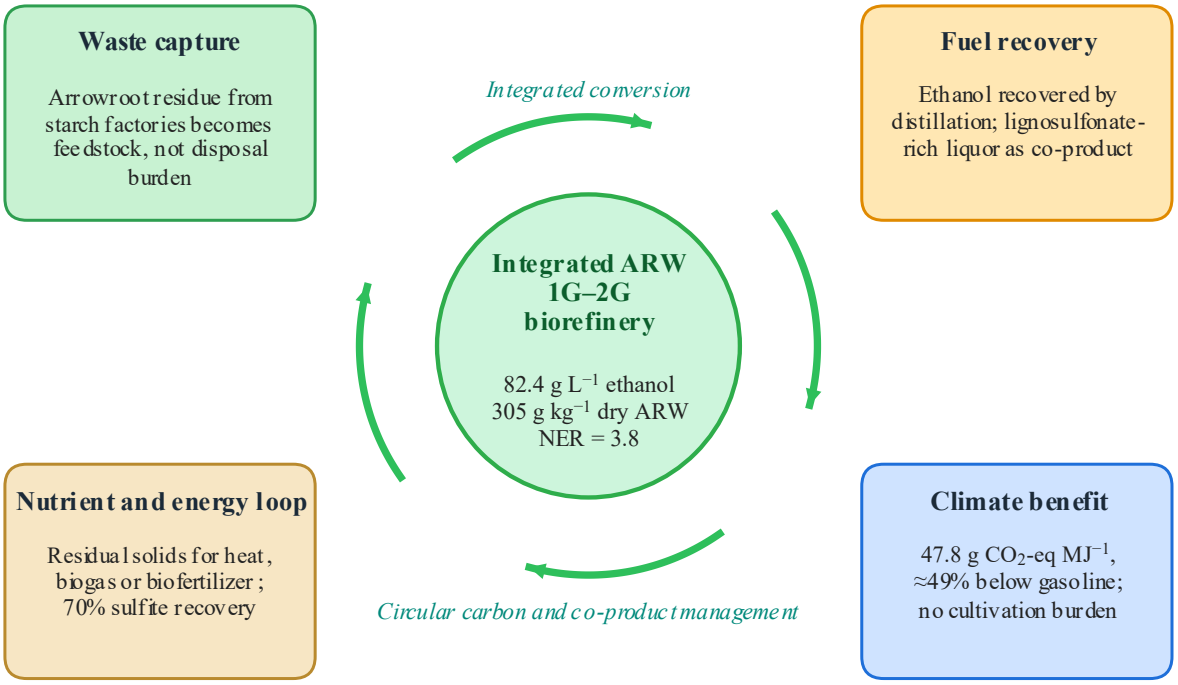

Fig. 8 Circular biorefinery concept linking ARW capture, integrated conversion, co-product utilization, and climate benefit.

Using a cradle-to-gate boundary with 1 MJ of anhydrous ethanol as the functional unit, a 35 km feedstock radius, and 70% sulfite recovery, the ARW route produced an estimated greenhouse-gas intensity of 47.8 g CO_2-eq MJ^{-1} ethanol and a net energy ratio of 3.8. Those values indicate a meaningful improvement over fossil gasoline and place the process within the range expected for waste-derived ethanol systems; well-to-wheels analyses likewise report large greenhouse-gas reductions for cellulosic ethanol relative to gasoline [31]. The carbon benefit is strengthened because ARW is treated here as an industrial residue, meaning cultivation burdens are not allocated to the ethanol pathway.

The environmental acceptability of sulfomethylation also requires consideration. Any scale-up of the process should incorporate chemical recovery, condensate scrubbing, and dedicated wastewater treatment to manage sulfite- and formaldehyde-containing streams. With these controls in place, sulfomethylation can remain a practical option for low-lignin residues such as ARW; without them, the environmental advantage of the downstream ethanol product would be undermined.

Finally, regional deployment in Thailand and Southeast Asia appears plausible because starch extraction plants are already distributed across agricultural belts where residue logistics, labor, and co-product utilization can be integrated locally. The most realistic near-term pathway is not a stand-alone megascale refinery but a modular biorefinery attached to or colocated with existing starch operations, where steam, wastewater treatment, and solid-residue management can be shared [18,21].

5. Conclusions

This study demonstrates that arrowroot waste can be converted into fuel ethanol through an internally integrated 1G–2G process that recovers both residual starch and pretreated cellulose from a single industrial residue. These findings are supported by triplicate-based statistics, NREL analytical methodology, pretreatment validation, mass–carbon closure, inhibitor profiling, sensitivity analysis, and an explicit environmental framework.

Among the tested configurations, the integrated SSCF route performed best, achieving 82.4 ± 1.2 g L^{-1} ethanol and 76.9 ± 1.0% overall yield within 60 h. CS hydrolysis was fast and nearly quantitative, while STFR provided a slower but highly productive second carbohydrate-release phase. The combined process achieved acceptable balance closure and favorable preliminary sustainability metrics.

Future work should prioritize pilot-scale validation, continuous or fed-batch SSCF operation, rigorous chemical-recovery experiments, and a full LCA linked to measured utility consumption. Even at the present stage, ARW emerges as a strong candidate feedstock for circular bioethanol production in tropical starch-processing industries.

Acknowledgements

The authors thank Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani,

Thailand and Maejo University, Chiang Mai, Thailand for laboratory facilities.

Data availability: The data supporting the findings of this study are included in this article. Raw data are available from the corresponding author upon reasonable request.

Conflict of interest: The authors declare no conflict of interest.

Author contributions: **T**akunrat Taksima: Conceptualization, methodology, Wattanee Boonwittaya: Conceptualization, methodology, investigation, formal analysis, supervision, and project administration, writing—original draft, writing—review and editing; Yuwalee Unpaprom: Conceptualization, methodology, and project administration, writing—original draft, writing—review and editing; Rameshprabu Ramaraj: Conceptualization, methodology, investigation, formal analysis, writing—original draft, writing—review and editing., Piyaphong Yongphet: Conceptualization, methodology, investigation, formal analysis, supervision, and project administration, writing—original draft, writing—review and editing.

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