REPORT

Tobacco as a promising crop for low-carbon biorefinery

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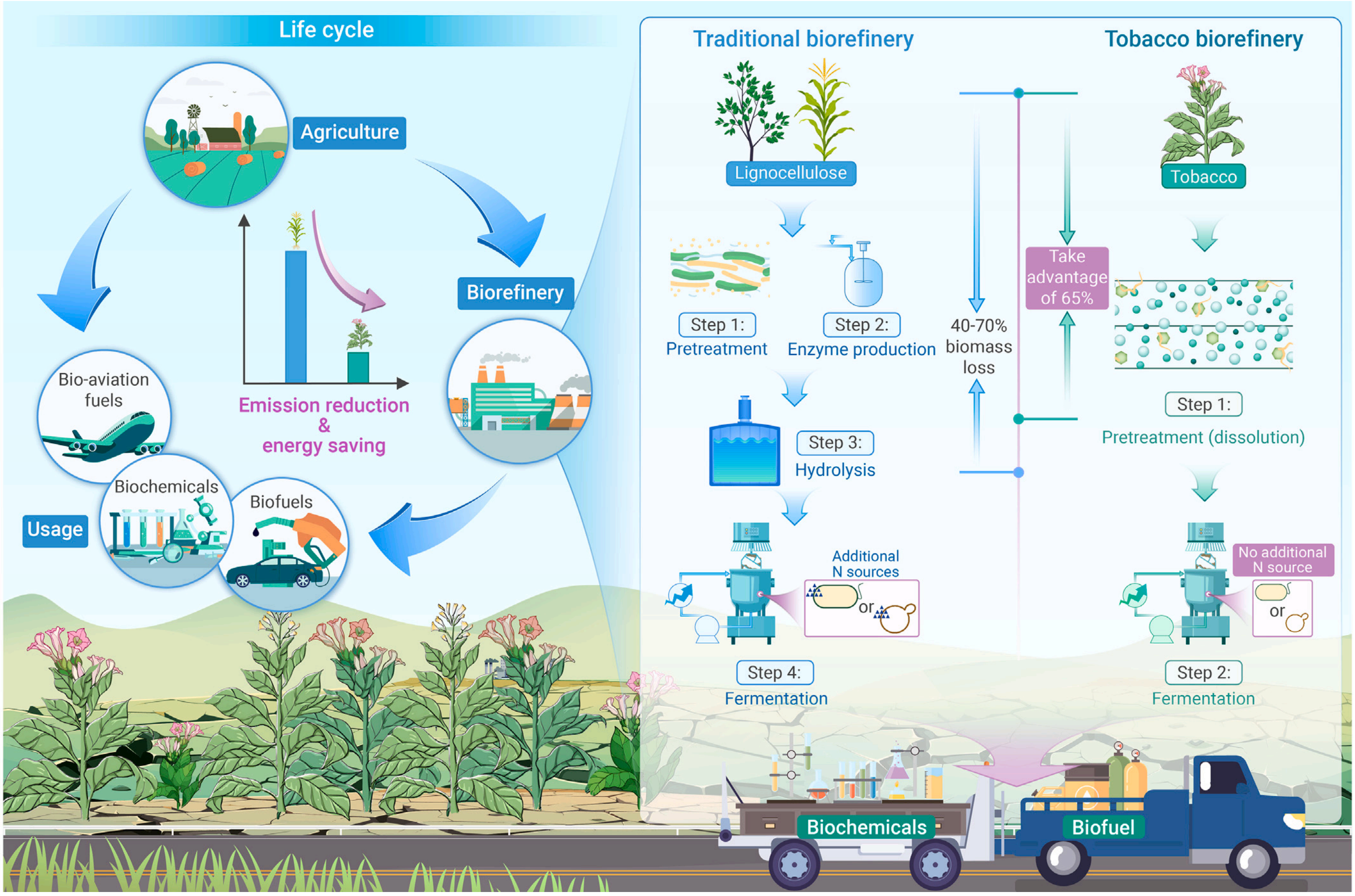
GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Cultivating tobacco on barren lands presents an excellent solution for sustainable bioenergy production.
- More than 65% of tobacco can be dissolved in water through simple autoclaving.
- The autoclaved solution of tobacco effectively supports the growth of microorganisms and the production of bioproducts.
- Tobacco-derived bioethanol significantly contributes to reducing carbon emissions.

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Energy crops play a vital role in meeting future energy and chemical demands while addressing climate change. However, the idealization of low-carbon workflows and careful consideration of cost-benefit equations are crucial for their more sustainable implementation. Here, we propose tobacco as a promising energy crop because of its exceptional water solubility, mainly attributed to a high proportion of water-soluble carbohydrates and nitrogen, less lignocellulose, and the presence of acids. We then designed a strategy that maximizes biomass conversion into bio-based products while minimizing energy and material inputs. By autoclaving tobacco leaves in water, we obtained a nutrient-rich medium capable of supporting the growth of microorganisms and the production of bioproducts without the need for extensive pretreatment, hydrolysis, or additional supplements. Additionally, cultivating tobacco on barren lands can generate sufficient biomass to produce approximately 573 billion gallons of ethanol per year. This approach also leads to a reduction of greenhouse gas emissions by approximately 76% compared to traditional corn stover during biorefinery processes. Therefore, our study presents a novel and direct strategy that could significantly contribute to the goal of reducing carbon emissions and global sustainable development compared to traditional methods.

INTRODUCTION

High global demand for fuels and chemicals, coupled with an unstable and uncertain petroleum supply and concerns regarding global climate change, has sparked a renewed interest in renewable alternatives.[1] Energy crops have emerged as sustainable substitutes for petroleum, capable of being converted into various chemicals and fuels that are compatible with existing infrastructure and do not compete with food production.[2] However, the large-scale production of chemicals and fuels from energy crop biomass, rich in cellulose and hemicellulose, necessitates costly processes to break down the complex polymers into their constituent sugars.[3] These processes often involve high temperatures, high pressures, massive chemicals, and expensive enzymes. Harsh processing conditions frequently result in the production of toxic byproducts and the loss of a significant portion of biomass.[4] Furthermore, the total protein content, which accounts for 10%–15% of energy crop biomass, is typically considered waste or requires additional processing for recovery and separate utilization.[5] Additionally, since the hydrolysis products of biomass are rich in sugars but lack other essential elements, additional nitrogen and phosphorus are necessary to support fermentation.[6] Consequently, the widespread use of energy crops as fermentation feedstocks is currently impeded by the cumbersome process and high costs associated with biomass feedstocks and the conversion of biomass into sugars, including pretreatment and enzyme hydrolysis operations, which can account for up to 35% of total production costs.[7,8] Therefore, it is imperative to identify suitable energy crops and develop sustainable, cost-effective methods for pretreatment and hydrolysis to achieve maximum sugar conversion yields with minimal environmental impact.[9]

Tobacco is a globally cultivated major commercial crop primarily used in the controversial smoking industry. It also serves as a model system in plant research, with its genome sequence published in 2014 and an extensive body of knowledge and engineering tools accumulated.[10] Genetic engineering has been utilized to enhance drought resistance,[11,12] increase leaf biomass production,[13] boost fermentable sugar content, and promote seed oil production in tobacco plants.[14] When grown for more biomass production, coppicing can be employed to stimulate re-sprouting and allow for multiple harvests.[15] Tobacco seed oil has been proposed for biodiesel production, while tobacco biomass has been tested for biogas production.[14,15] However, there have been limited studies examining the full potential of tobacco as an energy crop for its use as a renewable raw material in biorefineries for the production of chemicals and fuels.

Energy crop plantations have traditionally occupied cropland, raising concerns regarding competition with food production, global food security, and environmental sustainability.[16] An alternative approach is to cultivate energy crops on barren lands, optimizing management strategies, utilizing genetic modifications, and developing new agricultural technologies.[17,18] Estimations of indirect carbon costs for corn grain ethanol range from 25 to 200 gCO_2e/MJ, diminishing the environmental benefits of energy crops grown on arable land for biofuels.[19] Accordingly, energy crop biomass production on barren lands has been identified as a strategy that offers climate-related advantages. Furthermore, life cycle assessment (LCA) provides a method for evaluating the potential environmental impacts of a product system throughout its life cycle,[9] which helps in concluding the environmental benefits of biofuels derived from a specific material. Therefore, we aim to assess the environmental impact of tobacco cultivation and utilization, providing insights for policy-making related to sustainability that promotes its optimal use in bioenergy applications.

RESULTS

Autoclaving tobacco leaves to dissolve significant biomass portion

Lignocellulosic bioconversion faces challenges related to process costs, biomass recalcitrance, and product conversion efficiency, prompting us to explore the direct utilization potential of tobacco for biochemicals and biofuels production. Our experimental results demonstrated that more than 65% of tobacco leaves can be dissolved through autoclaving at 115°C for 30 min (Figure 1A), a process easily implementable in industrial fermentation using steam sterilization at 121°C (15 psi) for 15 min. Comparative analysis of chemical components revealed that tobacco leaves and whole tobacco outperform other feedstocks in terms of water-soluble components (SCM), water-soluble carbohydrates (WSC), and total nitrogen content (Figures 1A–1C). Additionally, tobacco leaves exhibited lower cellulose, hemicellulose, and lignin contents compared to other feedstocks (Figures 1D–1F). The SCM, WSC, and total nitrogen contents of whole tobacco were more than twice as high as those in switchgrass, rice straw, corn cob, and poplar wood, while its lignocellulosic content was approximately 60% lower. Following autoclaving at 115°C for 30 min, tobacco leaves showed superior chemical composition in terms of SCM (65.88%), WSC (27.74%), and total nitrogen (2.07%), with cellulose, hemicellulose, and lignin percentages at 8.66%, 2.73%, and 3.52% respectively. Two different tobacco leaf varieties displayed highly similar compositions

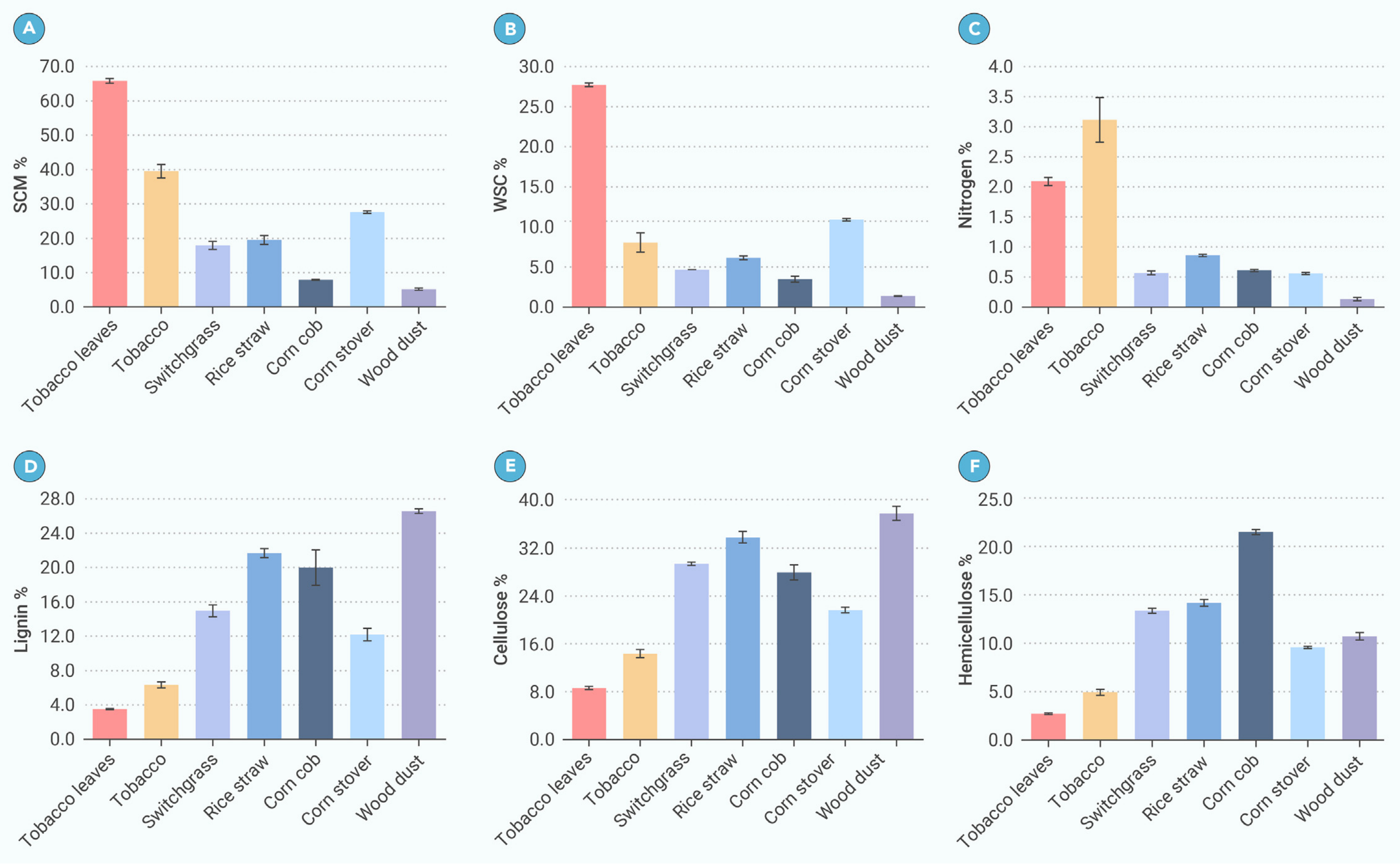

Figure 1. Main advantages of tobacco compared to five common biomasses (A–C) Analysis of the significant substances after being treated by autoclaving at 115°C for 30 min. (A) The water-soluble component (SCM) analysis. (B) The water-soluble carbohydrates (WSC) analysis. (C) The nitrogen analysis. (D–F) Analysis of the significant substances of biomass itself. (D) Lignin analysis. (E) Cellulose analysis. (F) Hemicellulose analysis. Tobacco leaves contained a substantially larger amount of SCM, WSC, and nitrogen and a smaller amount of lignocellulose compared to other biomasses. Error bars represent standard deviations (*n* = 3).

(Table S1). Furthermore, the resulting liquid from autoclaved tobacco leaves has a pH of approximately 5.0. When 0.8 mL 5 M NaOH was added, a neutral pH was formed, resulting in a lower solubility (~55%). Acid treatment with 1.00% H_2SO_4 or HCl proved to be the most effective in dissolving tobacco leaves (~67%), followed by water (~64%) and alkali (~57%). The high-water solubility of tobacco leaves is not solely attributed to high soluble matter contents but is also improved by the presence of various acids and acetic acid produced by autoclaving, which hydrolyze polysaccharides and proteins and form quaternary ammonium salts of alkaloids (Text S1).

The analysis of SCM in tobacco leaves indicates that it consists of sugars, proteins, ash, metal ions, nicotine, and other components (Figure 2A). We would like to point out that just like other semi-synthetic or natural media such as Luria-Bertani (LB) medium and potato dextrose broth (PDB), its nutrients can only be limited to a certain range rather than a precise concentration like M9 minimal medium (M9). Notably, the analysis results showed that fructose, glucose, and sucrose were the main sugars in the tobacco samples (Figures S1 and S2), and these are defined as fermentable sugars. The SCM includes over 20 g/L of fermentable sugars (containing 6.60 g/L fructose, 10.70 g/L glucose, and 6.85 g/L sucrose) and 1 g/L of total nitrogen (Figure 2C), making it suitable for use as a medium in growth and fermentation. While the autoclaving solution shows a high level of acetic acid, which likely results from the deacetylate of hemicellulose, and exhibits relatively low levels of fermentation inhibitors such as 22.81 mg/L 5-(hydroxymethyl)-2-furaldehyde (5-HMF) and 0.50 mg/L furfural (Figure 2D), likely due to the lower temperature used and the absence of chemicals in the process. Moreover, the nicotine is considered to affect the growth and metabolize metabolism of microorganisms. The nicotine in the solution (1,160.61 ± 13.49 mg/L) has little effect on the growth of *Escherichia coli* BL21(DE3) and *Saccharomyces cerevisiae* S288c (Figure S3).

Further analysis of the solid residue from tobacco leaves reveals 22.95% cellulose, 9.11% hemicellulose, 11.96% lignin, and 20.13% ash (Figure 2B). The broken structure of tobacco leaves enhances enzyme efficiency (Figures S4–S8). Treatment with cellulase, xylanase, and β-glucosidase hydrolyzes approximately 70% of cellulose and hemicellulose into sugars (Table S2). Therefore, incorporating additional enzymatic hydrolysis could further increase the biomass utilization of tobacco leaves to approximately 85%. However, the percentages of cellulose and hemicellulose are low. To optimize biomass utilization, tobacco leaves could be mixed with traditional energy crop biomass for use in second-generation biorefineries or applied in anaerobic digestion to produce a biogas stream (methane) that can be fed to a combustor for heat.[8]

Direct utilization of the autoclaving solution for fermentation

To evaluate the performance of the tobacco medium (autoclaving solution or SCM), we conducted growth and fermentation experiments. Initially, we characterized the growth of three *E. coli* strains, BL21(DE3), BW25113, and JM109(DE3), and three yeast strains, *S. cerevisiae* S288c, *Pichia pastoris* GS115, and *Yarrowia lipolytica* Po1h, in the tobacco medium. The results indicated that *E. coli* BL21(DE3) achieved an optical density 600 (OD_{600}) of approximately 3.00 in the tobacco medium, similar to the OD_{600} in the M9 but lower that in LB (~5.00). While *E. coli* BW25113 exhibited slower growth in the early stage in tobacco medium, its growth rate became comparable to that in LB in the later stage and more favorable than that in the M9. Similarly, although *E. coli* JM109(DE3) showed slightly slower growth in the early stage in tobacco medium, its growth rate was superior to that in LB after 16 h (Figure 3A). *S. cerevisiae* S288c and *P. pastoris* GS115 showed robust growth in the tobacco medium compared to yeast extract peptone dextrose (YPD) and malt extract medium (MEM), whereas *Y. lipolytica* Po1h displayed unsatisfied growth (Figure 3B).

Next, we investigated whether tobacco leaves provided a superior environment for the growth of *S. cerevisiae* S288c compared to other natural materials such as potato and corn meal. When compared to potato dextrose medium (200.0 g/L potato) and corn meal medium (50.0 g/L corn meal), the tobacco

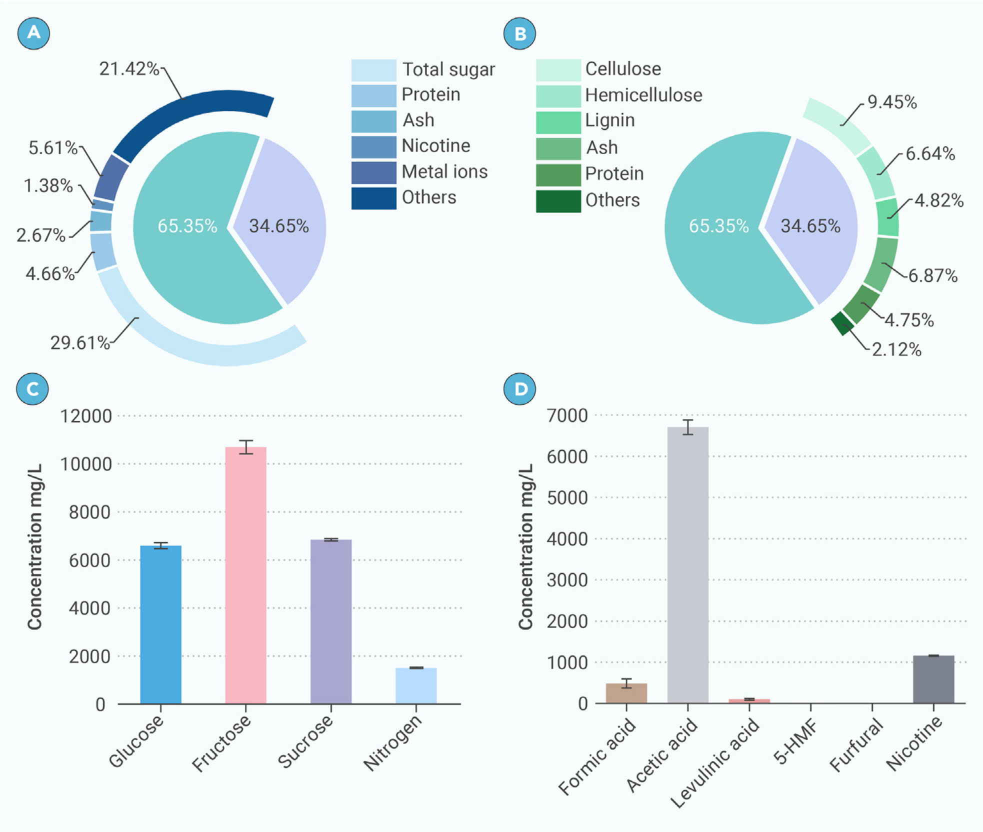

Figure 2. Main chemical components of tobacco leaves treated by autoclaving (A) The composition of the aqueous solution. Ash is calculated from the results of raw and treated tobacco leaves, which mainly includes some salts. In addition, others include a small amount of sugar, a relatively large proportion of acids and phenols, and amino acids, which can be used as helpful factors for the growth of microorganisms. (B) The composition of solid residue. Others include some sugars, acids, phenols, amino acids, etc., that are not dissolved in the aqueous solution. (C) Main sugars and nitrogen contents in SCM. (D) The fermentation inhibitors in SCM. Error bars represent standard deviations ($n = 3$).

medium (100.0 g/L tobacco leaves) yielded the highest OD_{600} of 21.19 (Figure S9), despite the control potato dextrose medium containing 20 g/L glucose.

Subsequently, we tested genetically engineered *E. coli* and *S. cerevisiae* strains developed in our laboratory for farnesene biosynthesis using the tobacco medium. Fermentation experiments were conducted using the control medium (synthetic medium), tobacco medium, and tobacco addition medium (where the tobacco medium replaced the carbon and nitrogen sources in the control medium). The production of farnesene after 48 h was approximately 2.19 ± 0.15, 0.69 ± 0.04, and 1.07 ± 0.10 g/L for the engineered *E. coli* in the control medium, tobacco medium, and tobacco addition medium, respectively (Figure 3C). These results indicate that the tobacco medium can partially replace the conventional high-nutrient medium. However, engineered *S. cerevisiae* fermentation in synthetic medium, tobacco medium, and tobacco addition medium resulted in farnesene production of approximately 27.24 ± 0.66, 50.59 ± 0.26, and 45.43 ± 0.58 mg/L, respectively, within 48 h (Figure 3D). This suggests that the tobacco medium could serve as a viable substitute for the synthetic medium in supporting eukaryotic growth and farnesene production. Similar findings were observed in our experiments on the production of (2R,3R)-(−)-2,3-butanediol (2,3-BD) using the tobacco medium (Figure 3F). For biofuel production from the tobacco medium, we conducted ethanol fermentation using wild *S. cerevisiae* S288c. The results demonstrated ethanol production of 7.48 ± 0.37, 8.47 ± 0.17, and 7.62 ± 0.32 g/L in 48 h using the synthetic medium, tobacco medium, and tobacco addition medium, respectively (Figure 3E). The successful growth and production of bioaviation fuel, biochemical, and biofuel confirm the significant potential of tobacco and provide a solution to the issue of costly nitrogen sources. We would like to emphasize that the presented data are intended to illustrate the superior performance and functionality of our tobacco leaves rather than showcase the current strain performance or yield.

Holistic solutions for a sustainable global tobacco-cultivating expansion

Recent empirical research has provided valuable insights into the utilization of marginal or barren lands for planting energy crops. These lands are currently not suitable for food production due to factors like low fertility and environmental sensitivity. By using such lands, we can potentially avoid conflicts between food and fuel production and minimize indirect land-use change effects, all while achieving significant climate benefits. To explore this possibility, we analyzed global data on barren/very sparsely vegetated land, including its extent and average annual temperature and rainfall (Figure 4; Tables S3–S5), using available databases.[20,21] Our findings reveal that the global distribution of barren/very sparsely vegetated land spans an area of 63,520,726 km^2. The mean annual air temperature in these areas is 17.4°C (1990–2010), with a range from −17.5°C in Greenland to 30.0°C in Burkina Faso. The average precipitation is 615.7 mm per year (1990–2010), varying from 25.3 mm in Egypt to 2,677.6 mm in Guadeloupe. There are sufficient methods to improve tobacco cultivation, including genetic strategies,[22] use of chemical fertilizers and agrochemicals, water management, and innovative cultivation techniques. Consequently, we propose that barren lands can be used for tobacco cultivation to meet energy demands without compromising empirical assessments. By considering non-cultivated lands on a global scale, we can develop truly sustainable solutions for the global energy plant system.

Tobacco exhibits greater agronomic stability in response to temperature fluctuations, requires less water, and displays higher tolerance to salinity, alkalinity, and drought compared to other crops.[23] Studies indicated that mean temperature significantly affects tobacco biomass yield but not with rainfalls.[24] Based on the biomass yield of a tobacco species by extensive farming, the overall tobacco yield is estimated to be around 9.00 Mg per hectare (Table S6). However, previous research has demonstrated that when tobacco is grown specifically for biomass production rather than tobacco products, the yield can reach 170 Mg per hectare, with an estimated tobacco leaves of 100 Mg per hectare.[25] Meanwhile, another study suggested that tobacco cultivated for biomass can achieve yields ranging from 44 to 70 Mg per hectare (dry basis), with tobacco leaf production ranging from 11 to 17.5 Mg per hectare.[26] These findings highlight the significant biomass potential of tobacco, making it advantageous for large-scale cultivation and manufacturing biofuel and biochemicals.[17]

When assessing the suitability of tobacco cultivation under traditional agricultural conditions, key factors to consider are soil type, temperature, and rainfall. Empirically, tobacco can grow in various soil types with a temperature range of 8°C–38°C and a daily water consumption of 3.5–8.5 mm. There is a lack of specific studies on tobacco cultivation in poor soils, but improvements have been achieved by increasing the productivity of other crops on marginal lands.[18,27] However, it is crucial to acquire knowledge that enables successful cultivation of highly productive tobacco species on barren lands. By implementing advanced cultivation techniques to enhance tobacco yield and utilize its by-products, tobacco can be transformed into a novel industrial crop that provides renewable sources for both biofuel and biomass. Concerns regarding the cultivation of biofuel crops, such as rising food prices and biodiversity loss, can be alleviated with the expansion of global tobacco cultivation. The production data used in this analysis are from the actual global production of tobacco leaves (unmanufactured) in 2019, which may represent the lowest yield, as these tobacco leaves were cultivated for high-quality cigarette products. Nevertheless, tobacco

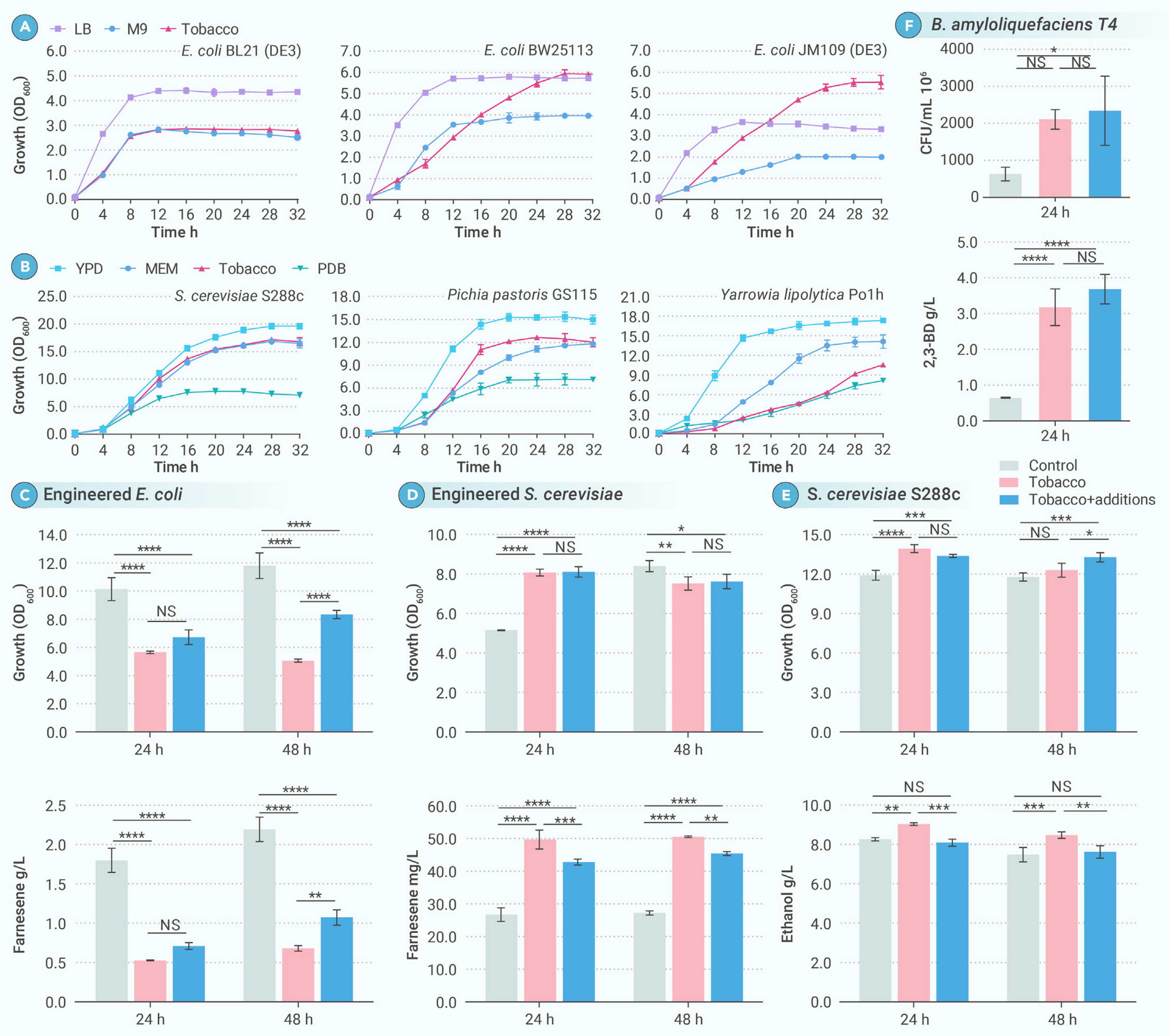

Figure 3. Tobacco medium for prokaryotic and eukaryotic growth and bioconversion (A) Growth of three *E. coli* strains, BL21(DE3), BW25113, and JM109(DE3), in the tobacco medium compared to other common media. (B) Growth of three yeast strains, *S. cerevisiae* S288c, *P. pastoris* GS115, and *Y. lipolytica* Po1h, in the tobacco medium compared to other common media. (C) Production of farnesene by engineered *E. coli* strain in control medium, tobacco medium, and tobacco addition medium. (D) Production of farnesene by engineered *S. cerevisiae* strain in control medium, tobacco medium, and tobacco addition medium. (E) Production of ethanol by *S. cerevisiae* S288c in control medium, tobacco medium, and tobacco addition medium. (F) Production of 2,3-BD by wild *Bacillus amyloliquefaciens* T4 in control medium, tobacco medium, and tobacco addition medium. Error bars represent standard deviations ($n = 3$). Statistical significance: *$p > 0.05$, **$p < 0.05$, ***$p < 0.001$, and ****$p < 0.0001$ based on two-way ANOVA.

leaves harvested from barren lands have the potential to theoretically produce 573 billion gallons of bioethanol (Table S7), roughly 20 times more than the global ethanol production in 2019.[28]

LCA of tobacco bioethanol

Ethanol fuel is an economically viable, environmentally friendly, easily accessible, and renewable energy source.[29] Among plant-based biofuels, ethanol production stands out as the most widely used alternative fuel on a global scale.[30] With this in mind, we provided a conservative estimate of the environmental performance of tobacco in bioethanol production using an LCA approach. The system boundary encompassed tobacco planting, transportation of tobacco leaves to the biorefinery, preparation, and bioconversion, as depicted in Figure S10. The bioconversion was simulated using Aspen Plus v.12.1 software (Text S2). The findings revealed that our designed tobacco-to-bioethanol strategy had a life cycle energy consumption of 90.34 MJ/kg and carbon emissions of 1.84 kgCO$_2$e/kg. Compared to lignocellulosic bioethanol produced from corn stover, bioethanol derived from tobacco demonstrated a reduction of approximately 26% in energy consumption and about 27% in greenhouse gas (GHG) emissions (Figures 5A and 5C). These reductions primarily stem from the bioconversion stage, which involves energy and chemicals used in pretreatment and enzymatic hydrolysis.

Furthermore, we omitted two steps by directly sterilizing and utilizing tobacco biomass as a fermentation medium in the present study. This approach not only resulted in a reduction of approximately 81% in energy consumption but also led to a decrease of around 76% in CO$_2$ emissions during the bioconversion stage (Figures 5B and 5D), as indicated by process simulations using Aspen Plus v.12.1 software. Thus, the tobacco biomass has the potential to be a game changer, offering an alternative means to address CO$_2$ emission issues. However,

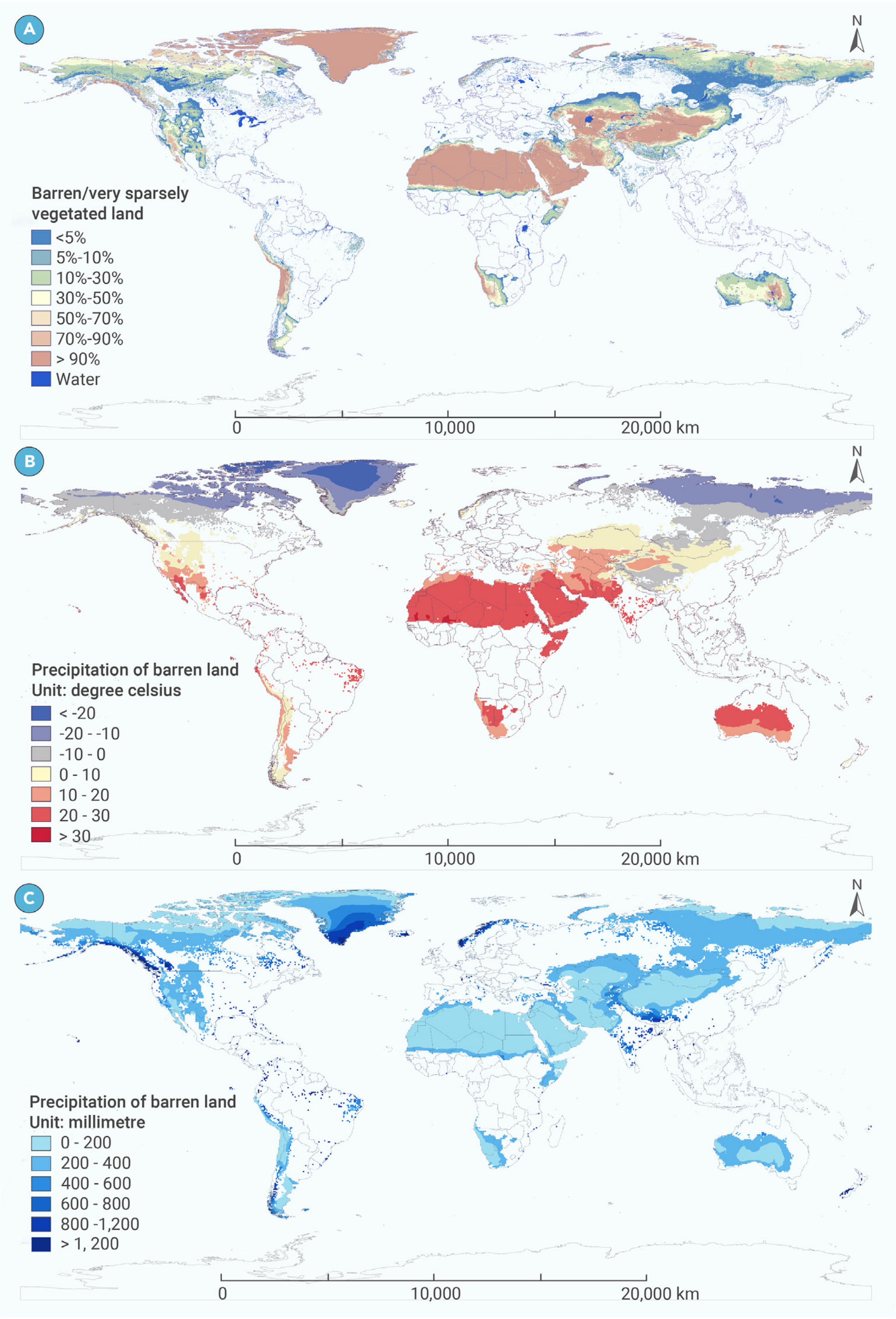

Figure 4. The global barren/very sparsely vegetated land for tobacco plants (A) The distribution of global barren/very sparsely vegetated land. (B) The annual average temperature of existing global barren/very sparsely vegetated land in 1990–2010. (C) The annual average precipitation of existing global barren/very sparsely vegetated land in 1990–2010.

empirical knowledge regarding soil GHG emissions, soil organic carbon (SOC) dynamics, and spatial variability in tobacco biomass yields on barren lands awaits further study.

DISCUSSION

Expanding the range of raw materials for next-generation biofuel production is crucial to enhance the effectiveness of biofuels and biochemicals. Initially, tobacco was identified as a potential candidate for biofuel due to its efficient oil biosynthesis mechanism in seeds. The mutagenized tobacco variety "Solaris" was subsequently found to possess an oil yield of 40%–60% of the seed's dry weight, making it suitable for biodiesel production.[31,32] Researchers from the United States, Italy, and Poland recognized the potential of tobacco plants as an energy crop for biofuel production,[15,24,33,34] emphasizing their economic and financial viability.[35] Moreover, the decline in subsidies and tobacco consumption, along with significant waste in the tobacco industry, has highlighted the alternative utilization of tobacco plants for biofuel production.[36] The high leafiness of tobacco plants and the impact of maturity and drying methods on water-soluble components and sugar accumulation[37] support the superior performance of cured tobacco leaves in our study. Furthermore, tobacco stems have been evaluated as a sustainable energy source,[38] serving purposes such as biochar,[39] biomass raw materials,[40] energy storage material,[41] and botanical pesticides.[42] The direct utilization of tobacco leaves represents a revolutionary approach to traditional biomass production strategies. Considering this, our focus primarily lies in researching tobacco leaves as a medium and optimizing their utilization strategy for the production of bioproducts. In addition, sweet sorghum is also considered to be directly utilized as a medium by juicing and heating. However, the major challenges associated with biofuel production using sweet sorghum juice are short harvest period and fast sugar degradation during storage.[43] Additionally, sweet sorghum juice lacks a nitrogen source when used directly as a medium. However, the use of tobacco leaves as a medium can almost perfectly solve the above shortcomings in storage, transportation, and application.

Nitrogen is indispensable for the growth of all living organisms, and its assimilation into various life-sustaining compounds has been extensively studied by microbiologists.[44] The nitrogen-rich tobacco makes it particularly attractive in nitrogen-rich compounds biosynthesis, such as guanidine (CH_5N_3) and hydrazine.[45] Although our results indicated that nicotine in tobacco leaf medium had no effect on yeast growth or product production, with only a slight impact on *E. coli*, this can be addressed through nicotine removal or genetic engineering techniques.[46,47] Moreover, ongoing research on nicotine-free tobacco plants offers even greater potential for widespread tobacco utilization. In addition, the production of recombinant protein therapeutics, vaccines, and plasma products heavily relies on various expression systems (*E. coli*, yeasts, mammalian cell culture, and insect cells) that are cultivated in media supplemented with animal-derived nitrogen components to support viability and productivity. These proteins are also commonly added as excipients and stabilizers in the final drug formulation. However, animal-derived raw materials carry a risk of viral contamination due to contact with viruses shed by animals.[48,49] Thus, the nutrient-rich tobacco, being a non-animal-derived component, can effectively mitigate the risk of virus transmission from animal sources, offering a more viable long-term solution. As a result, tobacco becomes an attractive alternative source for synthetic media in biorefineries.

Economic feasibility is a crucial factor in the biomass-derived fuel controversy.[50] The major cost components in bioethanol production from

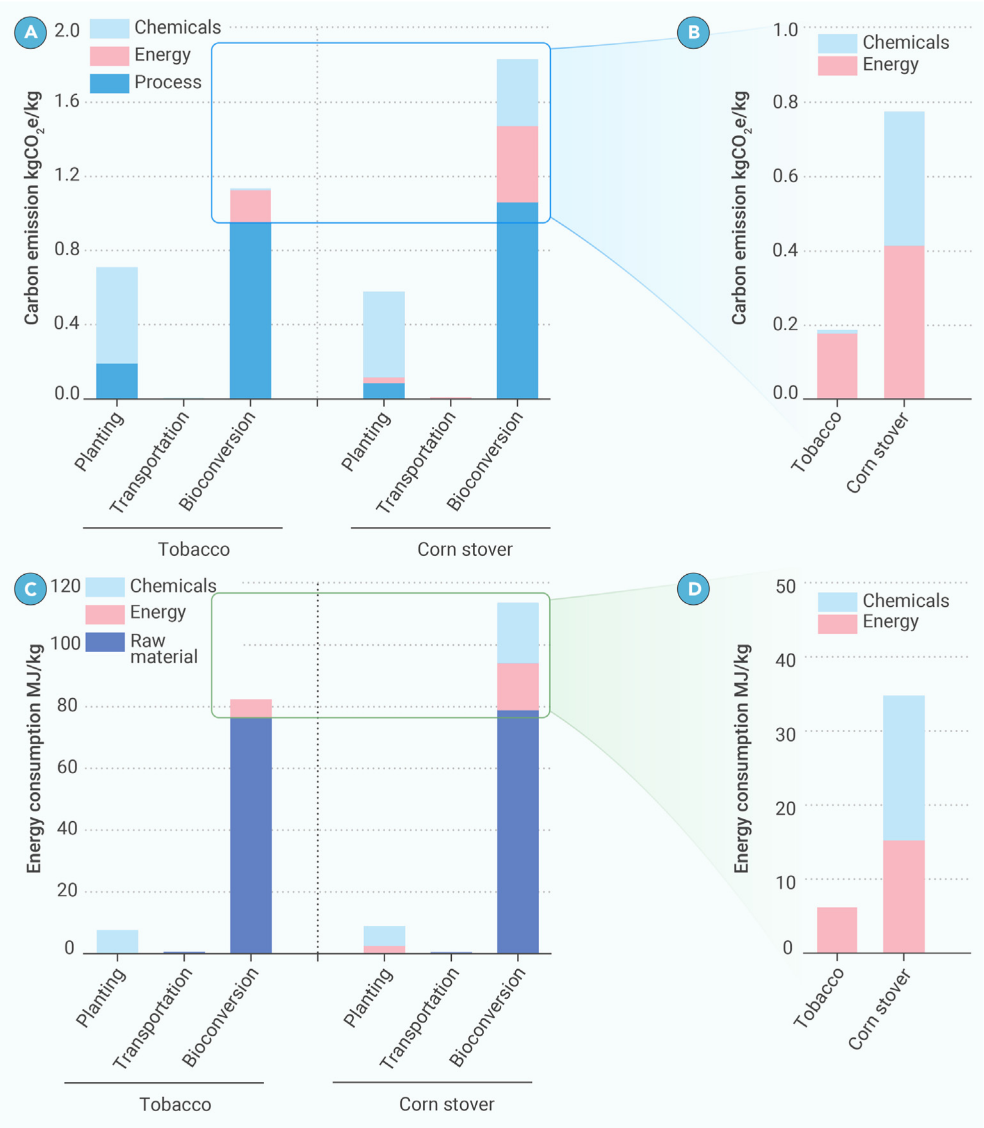

Figure 5. Life cycle assessment of tobacco and corn stover to bioethanol Life cycle greenhouse gas emissions (A and B) and energy consumption (C and D) of tobacco and corn stover to bioethanol. (B) and (D) represent the comparison of the greenhouse gas emissions and energy consumption from the chemicals and energy of bioconversion stage.

lignocellulosic biomass are pretreatment and enzymatic hydrolysis steps.[51–53] Efficient pretreatment strategies can lead to substantial enzyme savings, as these processes are interconnected. Therefore, optimizing these two critical steps, which collectively account for approximately 70% of the total processing cost, presents significant challenges for the commercialization of bioethanol from second-generation feedstocks.[54] Additionally, previous studies have shown that tobacco leaves alone may not be profitable for biofuel production, while the project can become economically viable when focusing on high-value products, particularly high-value squalene.[35] This conclusion stems from the fact that the concept of using tobacco as a feedstock was in its early stages at that time, with technology primarily focused on extracting biofuels from tobacco leaves and seeds. Incorporating approaches that modulate metabolic pathways in microbial bioconversion to synthesize higher-value products can enhance the economic viability of biomass-derived biofuels, especially as fossil fuels are currently produced at significantly lower prices than biofuels.[55] By combining the production of higher-value products with biofuels through our simple and efficient tobacco utilization method, their economic viability can be increased.

Bioenergy systems play a significant role in large-scale carbon dioxide removal (CDR), which is imperative for accomplishing climate goals by converting atmospheric CO_2 into carbohydrates.[56] Decades of research have led to a substantial knowledge base on enhancing CO_2 fixation and increasing dry matter productivity in tobacco, such as reducing the size of light-harvesting antenna in photosystems,[57] accelerating recovery from photoprotection,[58] and incorporating synthetic glycolate metabolic pathways into chloroplasts. Field experiments have validated that engineering photorespiratory pathways while inhibiting the native pathway can increase tobacco biomass by over 40%, benefiting from its ease of genetic transformation and robustness in the field.[13] Our analysis of actual global tobacco leaf production in 2019 reveals that even with tobacco leaves grown for high-quality cigarette products, the leaves harvested from barren lands have the potential to produce approximately 572 billion gallons of bioethanol, roughly 20 times the global ethanol production in 2019. While this framework analysis has limitations, such as the lack of real-world evidence for biomass production in barren areas, these outcomes have practical implications for the application and adoption of tobacco biofuels.

The climate benefits of cellulosic biofuels have faced challenges regarding technological feasibility and carbon debt from indirect land-use changes, leading to calls for reduced support for large-scale deployment.[17] Nevertheless, it has been demonstrated that second-generation biofuels have greater potential to reduce GHG emissions (around 50%) compared to first-generation biofuels when land-use changes are not considered in LCAs.[51] In this context, we propose the use of barren soils for tobacco cultivation to address concerns about carbon losses from land-use change. Our LCA results indicate that tobacco-based ethanol already achieves a negative carbon footprint, thereby promoting further utilization of tobacco biomass. Enhancing the efficiency and sustainability of the tobacco crop requires an integrated approach involving agronomists, engineers, and farmers. Modern genetic approaches like CRISPR offer insights into improving the carbon fixation ability of crops and can be explored for reducing atmospheric CO_2.[55] Restoring or enhancing the productivity of barren lands while utilizing them for biofuel cropping systems could contribute significantly to global energy and GHG mitigation goals, along with conservation benefits.

CONCLUSION

In summary, we identified a simple and viable technology of tobacco utilization to increase the effect of biofuels and bioenergy. By simply autoclaving tobacco leaves in water, a nutrient medium was obtained that readily supports microorganism's growth and biofuel and biochemical production without additional medium supplements. In addition, our work proposes using global barren/very sparsely vegetated land for growing tobacco to provide a holistic solution for a sustainable global tobacco cultivating expand and provides a reference for policies that address the best use of it for bioenergy by employing LCA to evaluate the environmental impact of tobacco cultivation and industrial utilization.

MATERIALS AND METHODS

See the supplemental information for details.

REFERENCES

1. Michael, E.H., Shi, Y.D., David, K.J., et al. (2007). Biomass recalcitrance: Engineering plants and enzymes for biofuels production. Science ***315***(5813): 804–807. https://doi.org/10.1126/science.1137016.

2. Wang, J., Fu, J., Zhao, Z., et al. (2023). Benefit analysis of multi-approach biomass energy utilization toward carbon neutrality. Innovation **4**(3): 100423. https://doi.org/10.1016/j.xinn.2023.100423.
3. Fierer, J.O., Tovar-Herrera, O.E., Weinstein, J.Y., et al. (2023). Affinity-induced covalent protein-protein ligation via the SpyCatcher-SpyTag interaction. Green Carbon **1**(1): 33–42. https://doi.org/10.1016/j.greenca.2023.07.001.
4. Queneau, Y., and Han, B. (2022). Biomass: Renewable carbon resource for chemical and energy industry. Innovation **3**(1): 100184. https://doi.org/10.1016/j.xinn.2021.100184.
5. Kim, T.H., Yoo, C.G., and Lamsal, B.P. (2013). Front-end recovery of protein from lignocellulosic biomass and its effects on chemical pretreatment and enzymatic saccharification. Bioprocess Biosyst. Eng. **36**(6): 687–694. https://doi.org/10.1007/s00449-013-0892-8.
6. Wu, L., Feng, S., Deng, J., et al. (2019). Altered carbon assimilation and cellulose accessibility to maximize bioethanol yield under low-cost biomass processing in corn brittle stalk. Green Chem. **21**(16): 4388–4399. https://doi.org/10.1039/C9GC01237K.
7. Hamelinck, C.N., Hooijdonk, G.V., and Faaij, A.P.C. (2005). Ethanol from lignocellulosic biomass: Techno-economic performance in short-middle- and long-term. Biomass Bioenergy **28**(4): 384–410. https://doi.org/10.1016/j.biombioe.2004.09.002.
8. Humbird, D., Davis, R., Tao, L., et al. (2011). Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol. REL/TP-5100-47764.
9. Wiloso, E.I., Heijungs, R., and de Snoo, G.R. (2012). LCA of second generation bioethanol: A review and some issues to be resolved for good LCA practice. Renew. Sustain. Energy Rev. **16**(7): 5295–5308. https://doi.org/10.1016/j.rser.2012.04.035.
10. Sierro, N., Battey, J.N.D., Ouadi, S., et al. (2014). The tobacco genome sequence and its comparison with those of tomato and potato. Nat. Commun. **5**: 3833. https://doi.org/10.1038/ncomms4833.
11. Holmström, K.O., Mäntylä, E., Welin, B., et al. (1996). Drought tolerance in tobacco. Nature **379**(6567): 683–684. https://doi.org/10.1038/379683a0.
12. Li, M., Liu, Z., Liu, C., et al. (2022). Drought resistance of tobacco overexpressing the *AfNAC1* gene of *Amorpha fruticosa* Linn. Front. Plant Sci. **13**: 980171. https://doi.org/10.3389/fpls.2022.980171.
13. South, P.F., Cavanagh, A.P., Liu, H.W., et al. (2019). Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. Science **363**(6422): eaat9077. https://doi.org/10.1126/science.aat9077.
14. Andrianov, V., Borisjuk, N., Pogrebnyak, N., et al. (2010). Tobacco as a production platform for biofuel: overexpression of *Arabidopsis DGAT* and *LEC2* genes increases accumulation and shifts the composition of lipids in green biomass. Plant Biotechnol. J. **8**(3): 277–287. https://doi.org/10.1111/j.1467-7652.2009.00458.x.
15. Barla, F.G., and Kumar, S. (2016). Tobacco biomass as a source of advanced biofuels. Biofuels **10**(3): 335–346. https://doi.org/10.1080/17597269.2016.1242684.
16. Robertson, G.P., Hamilton, S.K., Barham, B.L., et al. (2017). Cellulosic biofuel contributions to a sustainable energy future: Choices and outcomes. Science **356**(6245): 1349. https://doi.org/10.1126/science.aal2324.
17. Gelfand, I., Sahajpal, R., Zhang, X., et al. (2013). Sustainable bioenergy production from marginal lands in the US Midwest. Nature **493**: 514–517. https://doi.org/10.1038/nature11811.
18. Shi, D. (2023). Morning twilight of crop breeding for sodic land. Innovat. Life **1**(2): 100020. https://doi.org/10.59717/j.xinn-life.2023.100020.
19. Pu, Y., Hallac, B., and Ragauskas, A.J. (2013). Plant biomass characterization: Application of solution- and solid-state NMR spectroscopy. In Aqueous Pretreatment of Plant Biomass for Biological and Chemical Conversion to Fuels and Chemicals (John Wiley & Sons, Ltd.). https://doi.org/10.1002/9780470975831.ch18.
20. Climatic research unit data sets. https://sites.uea.ac.uk/cru/.
21. Fischer, G.F., Nachtergaele, S., Prieler, H.T., et al. (2008). Global Agro-ecological Zones Assessment for Agriculture (GAEZ 2008) (IIASA, Laxenburg, Austria and FAO).
22. Bailey-Serres, J., Parker, J.E., Ainsworth, E.A., et al. (2019). Genetic strategies for improving crop yields. Nature **575**(7781): 109–118. https://doi.org/10.1038/s41586-019-1679-0.
23. Zhao, C., Zhang, H., Song, C., et al. (2020). Mechanisms of plant responses and adaptation to soil salinity. Innovation **1**(1): 100017. https://doi.org/10.1016/j.xinn.2020.100017.
24. Fatica, A., Di Lucia, F., Marino, S., et al. (2019). Study on analytical characteristics of *Nicotiana tabacum* L., cv. *Solaris* biomass for potential uses in nutrition and biomethane production. Sci. Rep. **9**(1): 16828. https://doi.org/10.1038/s41598-019-53237-8.
25. Schillberg, S., Fischer, R., and Emans, N. (2003). 'Molecular farming' of antibodies in plants. Naturwissenschaften **90**(4): 145–155. https://doi.org/10.1007/s00114-002-0400-5.
26. Sheen, S.J. (1983). Biomass and chemical composition of tobacco plants under high density growth. Beitrage zur Tabakforschung International **12**(1): 35–42. https://doi.org/10.2478/CTTR-2013-0523.
27. Liu, Q., and Fu, X. (2023). Can heterotrimeric G proteins improve sustainable crop production and promote a more sustainable Green Revolution? Innovat. Life **1**(2): 100024. https://doi.org/10.59717/j.xinn-life.2023.100024.
28. (2021). Quantity of ethanol produced by country/region from 2007 to 2020. https://afdc.energy.gov/data/10331.
29. Ayas, N. (2018). Solvent Materials. In Comprehensive Energy Systems, I. Dincer, ed. (Elsevier), pp. 368–395. https://doi.org/10.1016/B978-0-12-809597-3.00226-1.
30. Farrell, A.E., Plevin, R.J., Turner, B.T., et al. (2006). Ethanol can contribute to energy and environmental goals. Science **311**(5760): 506–508. https://doi.org/10.1126/science.1121416.
31. Corrado, F. (2008). In Mutagenized tobacco plant as seed culture for production of oil for energetic, industrial and alimentary uses, S.A. GmbH, ed.
32. Fogher, C., Norscia, N., and Tommasini, S. (2011). Developing tobacco's potential as a novel, high-yielding, renewable energy plant. Inform **22**(10): 631–634.
33. Grisan, S., Polizzotto, R., Raiola, P., et al. (2016). Alternative use of tobacco as a sustainable crop for seed oil, biofuel, and biomass. Agron. Sustain. Dev. **36**(4): 55. https://doi.org/10.1007/s13593-016-0395-5.
34. Berbeć, A.K., and Matyka, M. (2020). Biomass characteristics and energy yields of tobacco (*Nicotiana tabacum* L.) cultivated in eastern poland. Agriculture **10**(11): 551. https://doi.org/10.3390/agriculture10110551.
35. Maisashvili, A., Bryant, H.L., and Richardson, J.W. (2016). Economic feasibility of tobacco leaves for biofuel production and high value squalene. Int. Food Agribus. Manag. Rev. **19**(4): 145–162. https://doi.org/10.22434/ifamr2015.0179.
36. Qiu, H.L., Fan, S., Zhou, K., et al. (2023). Global burden and drivers of hyperglycemia: Estimates and predictions from 1990 to 2050. Innovation **4**(4): 100450. https://doi.org/10.1016/j.xinn.2023.100450.
37. Zong, J., He, X., Lin, Z., et al. (2020). Effect of two drying methods on chemical transformations in flue-cured tobacco. Dry. Technol. **40**: 188–196. https://doi.org/10.1080/07373937.2020.1779287.
38. Bareschino, P., Marrasso, E., and Roselli, C. (2021). Tobacco stalks as a sustainable energy source in civil sector: Assessment of techno-economic and environmental potential. Renew. Energy **175**: 373–390. https://doi.org/10.1016/j.renene.2021.04.101.
39. Zhang, J., Zhang, J., Wang, M., et al. (2019). Effect of tobacco stem-derived biochar on soil metal immobilization and the cultivation of tobacco plant. J. Soils Sediments **19**(5): 2313–2321. https://doi.org/10.1007/s11368-018-02226-x.
40. Palupi, B., Fachri, B.A., Fadilah, S.N., et al. (2021). Hydrolysis optimization of tobacco stems with ultrasonic-assisted hydrolysis method. IOP Conf. Ser. Mater. Sci. Eng. **1053**(1): 012045. https://doi.org/10.1088/1757-899X/1053/1/012045.
41. Xia, X., Liu, H., Shi, L., et al. (2012). Tobacco stem-based activated carbons for high performance supercapacitors. J. Mater. Eng. Perform. **21**(9): 1956–1961. https://doi.org/10.1007/s11665-011-0101-3.
42. Prabowo, H., Handoko, S., Miswarti, et al. (2021). Bio oil of waste tobacco stem: Extraction, physicochemical properties, and its biological activities. J. Drug Alcohol Res. **10**(3): 236120.
43. Umakanth, A.V., Bhargavi, H.A., Keerthi, L., et al. (2020). Sweet sorghum as first-generation biofuel feedstock and its commercialization. In Sorghum in the 21st Century: Food – Fodder – Feed – Fuel for a Rapidly Changing World (Springer US), pp. 705–721. https://doi.org/10.1007/978-981-15-8249-3_28.
44. Morrison, M., and Mackie, R.I. (1997). Gastrointestinal Microbiology: Volume 1 Gastrointestinal Ecosystems and Fermentations (Springer US). https://doi.org/10.1007/2F978-1-4615-4111-0.
45. Le Goff, G., and Ouazzani, J. (2014). Natural hydrazine-containing compounds: Biosynthesis, isolation, biological activities and synthesis. Bioorg. Med. Chem. **22**(23): 6529–6544. https://doi.org/10.1016/j.bmc.2014.10.011.
46. Chen, B., Sun, L., Zeng, G., et al. (2022). Gut bacteria alleviate smoking-related NASH by degrading gut nicotine. Nature **610**(7932): 562–568. https://doi.org/10.1038/s41586-022-05299-4.
47. Liu, Y., and Chen, J. (2024). Orthogonal DNA replication system accelerates evolution and cell factory construction in *Escherichia coli*. Innovat. Life **2**(1): 100055. https://doi.org/10.59717/j.xinn-life.2024.100055.
48. Grillberger, L., Kreil, T.R., Nasr, S., et al. (2009). Emerging trends in plasma-free manufacturing of recombinant protein therapeutics expressed in mammalian cells. Biotechnol. J. **4**(2): 186–201. https://doi.org/10.1002/biot.200800241.
49. Barone, P.W., Wiebe, M.E., Leung, J.C., et al. (2020). Viral contamination in biologic manufacture and implications for emerging therapies. Nat. Biotechnol. **38**(5): 563–572. https://doi.org/10.1038/s41587-020-0507-2.
50. Wang, F., Harindintwali, J.D., Yuan, Z., et al. (2021). Technologies and perspectives for achieving carbon neutrality. Innovation **2**(4): 100180. https://doi.org/10.1016/j.xinn.2021.100180.
51. Kargbo, H., Harris, J.S., and Phan, A.N. (2021). "Drop-in" fuel production from biomass: Critical review on techno-economic feasibility and sustainability. Renew. Sustain. Energy Rev. **135**: 110168. https://doi.org/10.1016/j.rser.2020.110168.
52. Satari, B., Karimi, K., and Kumar, R. (2019). Cellulose solvent-based pretreatment for enhanced second-generation biofuel production: a review. Sustain. Energy Fuels **3**(1): 11–62. https://doi.org/10.1039/C8SE00287H.
53. Jiang, W., Hernández Villamor, D., Peng, H., et al. (2021). Metabolic engineering strategies to enable microbial utilization of C1 feedstocks. Nat. Chem. Biol. **17**(8): 845–855. https://doi.org/10.1038/s41589-021-00836-0.
54. Zafar, S. (2021). Ethanol production from lignocellulosic biomass. https://www.bioenergyconsult.com/production-cellulosic-ethanol/.
55. Liu, Y., Cruz-Morales, P., Zargar, A., et al. (2021). Biofuels for a sustainable future. Cell **184**(6): 1636–1647. https://doi.org/10.1016/j.cell.2021.01.052.
56. Fu, C., and Xu, M. (2023). Achieving carbon neutrality through ecological carbon sinks: A systems perspective. Green Carbon **1**(1): 43–46. https://doi.org/10.1016/j.greenca.2023.08.005.
57. Kirst, H., Gabilly, S.T., Niyogi, K.K., et al. (2017). Photosynthetic antenna engineering to improve crop yields. Planta **245**(5): 1009–1020. https://doi.org/10.1007/s00425-017-2659-y.
58. Kromdijk, J., Głowacka, K., Leonelli, L., et al. (2016). Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. Science **354**(6314): 857. https://doi.org/10.1126/science.aai8878.

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AUTHOR CONTRIBUTIONS

All the chemical composition analysis experiments in this work were conducted by F.W. and Y.L., assisted by Y.J. and Y.Z.; growth analysis and fermentation verification experiments and related discussion were done by X.J. and F.W., assisted by Y.Z., Y.J., X.M., L.L., and X.C.; the data of current global tobacco distribution and global barren/very sparsely vegetated land were collected and analyzed by G.Z.; Y.L. and F.W. estimated the potential climate benefits of the tobacco biomass, assisted by L.W. and W.L.; S.S. and H.L. planted and collected the control whole-tobacco candidates; M.A. and C.F. helped analyze the data and revise the manuscript; and S.Y.L., H.Z., and Q.W. conceived the research, supervised and directed this project, and fully revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

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