EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology, 2025, 26(11), 139–145
https://doi.org/10.12912/27197050/211807
ISSN 2719–7050, License CC-BY 4.0

Received: 2025.09.23
Accepted: 2025.10.20
Published: 2025.11.01

From plant waste to substrate for second-generation biofuels

Vita Halysh[1,2*], Inna Trus[2], Olha Yashchenko[1], Maryna Hromnadska[3], Pavlo Zubik[3], Irina Deykun[1], Romaniia Cheropkina[1], Tamara Krysenko[1], Viktoria Ovsyankina[1]

[1] Department of Ecology and Technology of Plant Polymers, Faculty of Automation, Industrial Engineering and Ecology, Igor Sikorsky Kyiv Polytechnic Institute, Beresteysky Avenue 37/4, 03056 Kyiv, Ukraine
[2] Laboratory of Kinetics and Mechanisms of Chemical Transformations on the Surface of Solids, Department of Physico-Chemistry of Carbon Nanomaterials, Chuiko Institute of Surface Chemistry, National Academy of Sciences of Ukraine, Oleg Mudrak St. 17, 03164 Kyiv, Ukraine
[3] Department of Industrial Biotechnology and Biopharmacy, Faculty of Biotechnology and Biotechnics, Igor Sikorsky Kyiv Polytechnic Institute, Beresteysky Avenue 37/4, 03056 Kyiv, Ukraine
* Corresponding author's e-mail: v.galysh@gmail.com

ABSTRACT

Pretreatment of plant biomass is a key stage in the production of second-generation biofuels, as it ensures the destruction of the lignocellulosic structure and increases the availability of polysaccharides for further conversion. The paper investigates the effectiveness of various methods of processing oat straw for the isolation of the polysaccharide component. Mechanical, aqueous, alkaline and organic methods, as well as combined organosolvent processing using acetic acid and hydrogen peroxide, are considered. It was found that the highest yield of solid product is provided by processing with ethanol (98.7%) and alcohol-benzene mixture (96.8%), however, the obtained materials retain the original structure of biomass. Instead, hot water (82.7%) and alkali (58.0%) lead to partial extraction of soluble substances and hemicelluloses, while the use of peracetic acid gives the lowest yield (53.1%), but provides deep delignification, ash reduction and formation of a porous structure suitable for enzymatic hydrolysis. Thus, it is the peroxide-acetic acid treatment that has proven to be the most promising for increasing cellulose availability and preparing oat straw for further biotechnological use in biofuel production.

Keywords: cellulose, lignin, biofuel, bioethanol, substrate.

INTRODUCTION

Today, as energy security and climate change issues become increasingly pressing, biofuels are emerging as a key element of a sustainable energy future (Sikiru et al., 2024). The relevance of biofuels is determined by three main factors. First, it is climate change, caused by high levels of greenhouse gas emissions from the combustion of oil, gas and coal. Biofuels, which are a renewable energy source, contribute to a significant reduction in net carbon dioxide emissions into the atmosphere (Clauser et al., 2025). Second, it is the depletion of fossil fuel reserves. Since the world's oil and gas resources are limited, the transition to alternative sources is inevitable (Malik et al., 2024). Third, it is energy independence, since the production of biofuels on a local raw material base allows countries to reduce their dependence on energy imports (Pryshliak et al., 2022, Esonye et al., 2023).

The first generation of biofuels is derived from food crops such as corn, wheat, sugarcane, rapeseed, soybeans and palm oil, with bioethanol and biodiesel as the main products, but it competes with food production and creates socio-economic risks (Cavelius et al., 2023, Deora et al., 2022, Singh et al., 2021). The second generation uses non-food lignocellulosic biomass (straw, wood waste, corn stalks, sunflower husks, pulp and paper residues) through hydrolysis, fermentation and thermochemical methods, thus avoiding competition with the food sector, utilizing waste and reducing greenhouse gas emissions (Giertl et al., 2024, Kumar et al., 2023, Pant et al., 2023). The third generation is based on algae, which offer high productivity without agricultural land use but face high costs and technical challenges (Chowdhury and Loganathan, 2019), while the fourth relies on genetically modified microorganisms and synthetic biology, promising negative emissions but limited by complexity, costs and regulatory issues (Mat Aron et al., 2020, Sing et al., 2024, Torkashvand et al., 2022).

Second-generation biofuels are considered the most promising, as they enable efficient waste use, reduce environmental impacts and add economic value without threatening food security. Agro-industrial waste is a particularly attractive raw material for various industries (Halysh et al., 2018, Shkliarenko et al., 2023, Trembus et al., 2018) and for bioenergy, providing renewable resources, enhancing energy security and addressing waste disposal issues, though challenges remain due to high costs, energy-intensive processes and incomplete optimization of current technologies (Halysh et al., 2023).

Pretreatment of plant raw materials is an important stage in the production of second-generation biofuels, as it destroys the lignocellulosic structure of plant biomass and makes cellulose and hemicellulose available for further conversion. Mechanical, thermal, chemical and combined methods are used, among which the most promising is organosolvent treatment with a mixture of acetic acid and hydrogen peroxide (Başar and Perendeci, 2021 It allows for the most efficient removal of lignin and the production of a substrate with a high cellulose content, which maximizes the yield of sugars for biofuels under relatively mild conditions.

Ukraine, as one of the leading agricultural countries, annually produces significant volumes of oats, which together with them is formed a large amount of straw, often unused and recognized as waste. The rational use of this biomass in bioenergy technologies can not only solve the problem of disposal, but also contribute to increasing the country's energy independence.

Therefore, the purpose of the work is to investigate the effectiveness of using various methods of oat straw processing to ensure a high yield of polysaccharide component for further use in obtaining second-generation biofuels

MATERIALS AND METHODS

For the isolation of the polysaccharide component, oat straw from the 2024 harvest, collected in the Chernihiv region, was used as the starting material. After sorting, the straw was cut into pieces of approximately 1 cm. Both the initial plant material and all obtained cellulose samples were stored in desiccators at room temperature to maintain a constant humidity and a stable chemical composition.

The content of structural components and extractives in the initial straw is shown in Figure 1.

To isolate the polysaccharide component, the biomass was treated using several different methods. The primary goal of these treatments was to remove extractive substances and other non-cellulosic structural components. The types of treatments and their parameters are detailed in Table 1. For each test, 40 g of oven-dry biomass was used.

Following each treatment, the resulting products were air-dried to a consistent moisture content of 7%. The efficiency of each process was comprehensively assessed based on two primary factors. First, the visual and structural changes in the material before and after treatment were visually observed. Second, qualitative indicators that reflect the effectiveness of the polysaccharide component's separation were analyzed (Trembus et al., 2022). These included the gravimetric determination of product yield and the quantification of residual lignin and ash content using the established TAPPI standards T 222 cm-02 (Technical Association of the Pulp and Paper Industry. 2002. Acid–insoluble lignin in wood and pulp) and T 211 om-02 (Technical Association of the Pulp and Paper Industry. 2002. Ash in wood, pulp, paper and paperboard: combustion at 525 °C, T 211 om-02), respectively.

All experiments related to biomass treatment and characterization were conducted in triplicate, and the average values were reported. The relative standard deviations were below 5% (shown as error bars in the figures).

RESULTS AND DISCUSSION

After treating biomass with each of the studied reagents, solid cellulose products were obtained. These residues were then labeled based on their specific processing scheme, and they showed significant differences in yield, as illustrated in Figure 2.

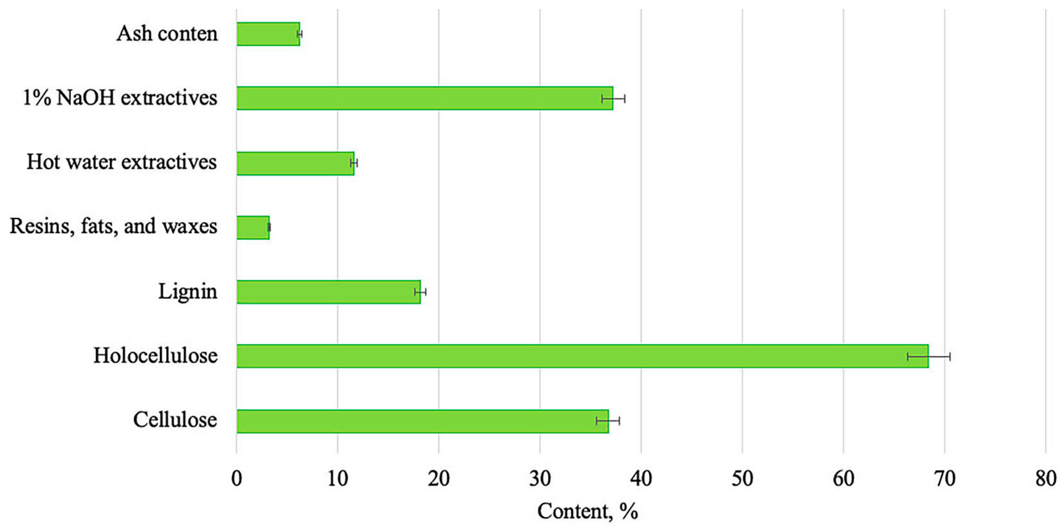

Figure 1. Chemical content of the biomass

Table 1. Treatment parameters for the isolation of the polysaccharide component

Method of treatment	Treatment conditions
Aqueous prehydrolysis (APH)	Biomass was treated with distilled water in a steel autoclave at a hydromodule of 10:1. The process involved heating the mixture in a glycerin bath at 120 °C for one hour. After treatment, the autoclave was cooled with tap water, and the resulting fibrous product was washed with distilled water until the filtrate ran clear.
Alkali treatment (AT)	Biomass was processed in glass heat-resistant flasks for one hour at 95 °C using a 3% NaOH solution at a hydromodule of 10:1. The flasks were heated in a water bath, and a reflux condenser was used to prevent the loss of solution components. The resulting product was then washed with distilled water until the wash water's pH was neutral.
Ethyl alcohol treatment (EAT)	The biomass was thoroughly extracted for 4 hours using a Soxhlet apparatus. For this process, a thimble containing the biomass was placed in the central chamber of the apparatus. A 100 ml volume of ethyl alcohol was added to a round-bottom flask, which was then heated in a water bath to initiate the extraction. Following the extraction, the biomass was air-dried to allow for complete evaporation of the residual solvent.
Alcohol-benzene mixture treatment (ABT)	The treatment method was implemented similarly to EAT, but a 1:2 alcohol-benzene mixture was used instead of ethyl alcohol.
Treatment with peracetic acid (PAT)	The biomass was treated for 120 minutes at 95 °C with a peracetic acid mixture, which was prepared by combining glacial acetic acid and 35% hydrogen peroxide in a 70:30 volume ratio. This process was carried out in glass heat-resistant flasks with a hydromodule of 10:1, using reflux condensers to prevent the loss of volatile components. Following the treatment, the resulting fibrous product was separated and washed with distilled water until its pH became neutral.

The highest yields were achieved by treating the biomass with ethyl alcohol (98.7% yield) and an alcohol-benzene mixture (96.8% yield). This high retention of material can be attributed to the fact that these solvents primarily remove non-structural, or extractive, substances (Zhao et al., 2022). These compounds are not part of the plant's cell wall and include materials like waxes, fats, and resins, which often provide a protective coating on the plant's surface. The slight difference in yield between the two is due to the properties of the solvents. Ethyl alcohol, as a polar solvent, effectively extracts other polar compounds. However, the alcohol-benzene mixture, which combines a polar solvent with a non-polar one, exhibits a synergistic effect. This combination allows it to remove a much broader spectrum of extractive substances from the raw material, accounting for the slightly lower final yield. In both cases, the resulting product is a fibrous material that still retains the initial structure of the original straw. This indicates that these treatments successfully isolated the main structural components of the plant without causing significant damage to the cell wall.

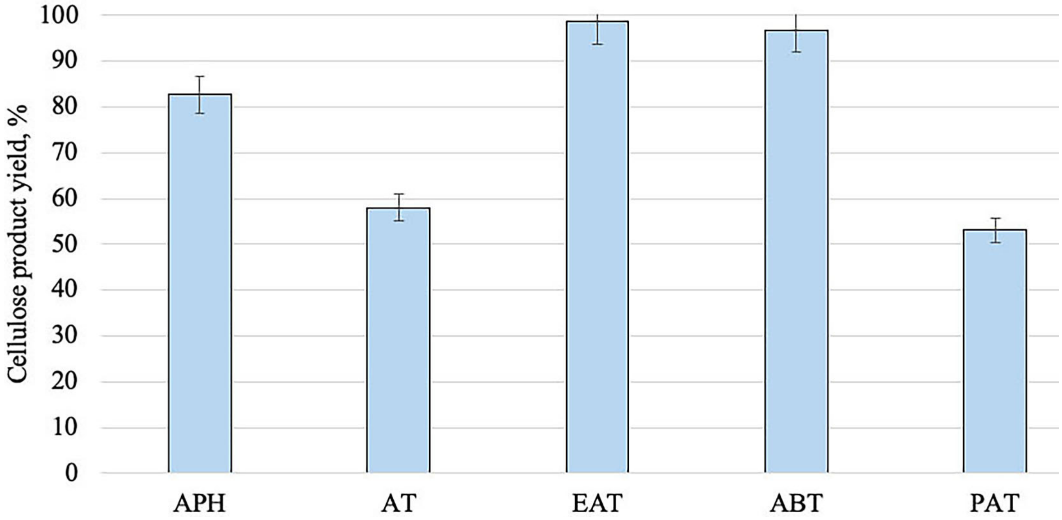

Figure 2. Yield of cellulose product from oat straw as a result of different types of treatment, %

The treatment of biomass with hot water during 1 h at 120 °C resulted in a solid cellulose product with a high yield of approximately 82.7%. This method is considered relatively gentle because it primarily removes water-soluble extractives from the raw material. These include compounds such as dyes, tannins, inorganic salts, and other substances that dissolve easily in an aqueous solution. Since this process does not significantly affect the primary structural polymers of the plant, such as cellulose, hemicelluloses, and lignin, the high yield of the solid residue is to be expected.

In contrast, treatment with a 3% alkali solution leads to a more notable decrease in the cellulosic product's yield, down to 58.0%. This is because the alkali solution removes more than just water-soluble components; it also dissolves lignin, low-molecular hemicelluloses and hemicelluloses (Oriez et al., 2020). The removal of these additional structural components accounts for the higher material loss compared to the hot water treatment.

Importantly, neither the hot water or the other solvent-based methods – has a profound or destructive effect on the core lignocellulosic structure of the biomass. As a result, the yields for these products remain high, and the obtained lignocellulosic fibers largely retain the fundamental structure of the original biomass.

The application of hydrogen peroxide to acetic acid fundamentally alters the outcome of the treatment. This combination leads to a notable delignification effect, resulting in a significant decrease in product yield, which falls to 53.1%. This dramatic change occurs because the hydrogen peroxide and acetic acid react to form peracetic acid, which serves as an effective agent for removing lignin from the biomass (Mayta et al., 2024).

The successful removal of lignin is visually confirmed by the product's much lighter color, as shown in Figure 3. Beyond the visual change, the treatment transforms the material into a fibrous product with a loose and more fiber-divided structure. This physical characteristic is highly beneficial for subsequent biochemical conversion processes.

A material with a looser structure offers a greater surface area, which allows enzymes to more efficiently access and break down the cellulose fibers. Therefore, this treatment not only purifies the biomass by removing lignin but also optimizes its physical properties for more effective downstream applications.

The ash content of biomass, which refers to the inorganic mineral components left after combustion, can significantly influence the efficiency of the biochemical conversion of polysaccharides into sugars (Park et al. 2022). The presence of these mineral components may act as an inhibitor, hindering the conversion process by interfering with the enzymatic activity of cellulases and hemicellulases. High mineral content can also disrupt the optimal pH of the reaction environment, further reducing overall sugar yield.

Consequently, a critical objective of biomass pretreatment is not only to expose the polysaccharides but also to reduce the mineral load. This makes it essential to investigate how various treatments affect the ash content of the resulting products. Figure 4 presents the results of the

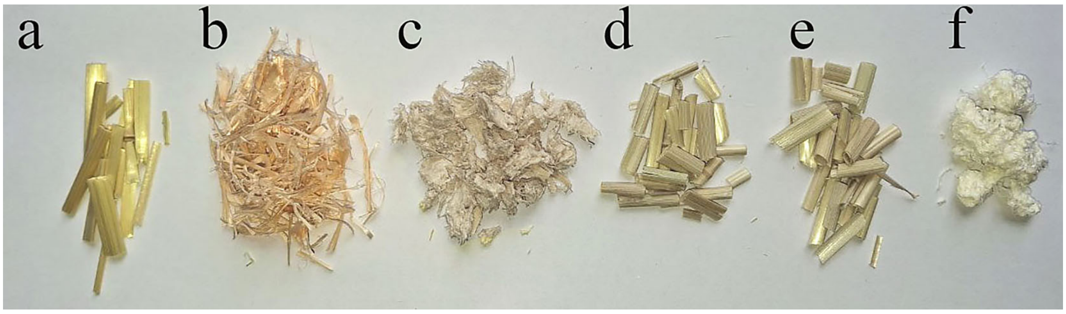

Figure 3. Visual changes in the structure of biomass before treatment (a) and after AHP (b), AT (c), EAT (d), ABT (e) and PAT (f)

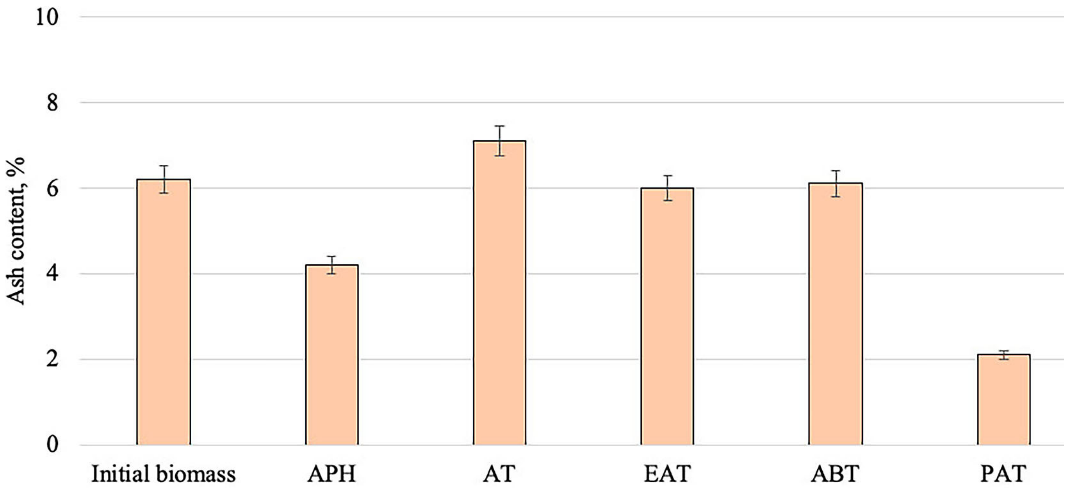

Figure 4. Ash content in cellulose product from oat straw as a result of different types of treatment, %

mineral component analysis, providing valuable insight into which specific treatment methods are most effective at removing these inhibitory minerals and improving the material's suitability for biochemical conversion.

The presented data suggest that treating biomass with hot water results in the removal of approximately 25% of its mineral components. This is likely due to the diffusion of soluble salts from the biomass structure into the water during the treatment. However, a portion of the minerals remains in the final product. It is presumed that these remaining components are those that are chemically bound to the functional groups of the biomass's structural components. Following alkali treatment, the ash content of the final product exhibits a slight increase. This phenomenon is directly related to the adsorption of the alkali onto the surface of the biomass during processing. Treatment with ethyl alcohol and an alcohol-benzene mixture has a minimal impact on the content of mineral components in the biomass. This is because these organic solvents are highly effective at removing organic extractive substances, such as waxes, fats, and resins, but are largely ineffective at dissolving inorganic minerals.

Unlike the other methods investigated, peracetic acid treatment is exceptionally effective at reducing the content of mineral components in the final product. This method's success is due to its powerful oxidizing nature, which is strong enough to not only remove lignin but also to disrupt the chemical bonds that hold many of the mineral components to the plant's structural polymers. While the treatment is highly effective, it does not lead to the complete removal of all minerals. This suggests that a portion of the mineral content is very tightly integrated into the biomass structure, resisting even this aggressive form of chemical treatment.

Based on the presented data, it's clear that each processing method has a distinct impact on the structure of the oat straw biomass. The different treatments selectively alter the material's composition, removing various components while leaving the core structure intact to varying degrees. However, a complete assessment of the feasibility of using these products for biofuel production requires further investigation. While the current results characterize the physical and chemical changes to the biomass, additional studies are necessary to evaluate crucial factors such as enzymatic digestibility and fermentation efficiency. Only after conducting these specific trials, it will be possible to confirm the true potential of the obtained products.

CONCLUSIONS

The present study successfully achieved its goal of identifying an effective pretreatment method for oat straw to enhance its suitability as a raw material for second-generation biofuel production. The results demonstrated that peroxide–acetic acid treatment is the most efficient approach, providing deep delignification and mineral removal, while producing a structurally modified substrate that facilitates subsequent enzymatic hydrolysis. This work revealed, for the first time, a comprehensive comparison of different pretreatment agents applied to oat straw, clarifying how each solvent system influences lignocellulosic composition, structural integrity, and ash removal. The findings fill a key research gap concerning the optimization of pretreatment strategies specifically for oat straw, an abundant yet underutilized agricultural residue. By establishing peroxide–acetic acid treatment as a promising and relatively mild delignification route, this study opens new prospects for cost-effective bioconversion of agricultural waste into fermentable sugars and biofuels, contributing to the development of sustainable bioenergy technologies. Further investigations should focus on quantifying enzymatic hydrolysis efficiency and sugar yields to confirm the practical applicability of this method in large-scale biofuel production.

REFERENCES

1. Sikiru, S., Abioye, K. J., Adedayo, H. B., Adebukola, S. Y., Soleimani, H., Anar, M. (2024). Technology projection in biofuel production using agricultural waste materials as a source of energy sustainability: A comprehensive review. *Renewable and Sustainable Energy Reviews*, 200, 114535. https://doi.org/10.1016/j.rser.2024.114535
2. Clauser, N., Scown, C. D., Pett-Ridge, J., Sagues, W. J. (2025). A techno-economic assessment of carbon dioxide removal pathways via biochemical conversion of lignocellulose to biofuels and bioplastics. *Renewable and Sustainable Energy Reviews*, 216, 115714. https://doi.org/10.1016/j.rser.2025.115714
3. Malik, K., Capareda, S. C., Kamboj, B. R., Malik, S., Singh, K., Arya, S., Bishnoi, D. K. (2024). Biofuels production: a review on sustainable alternatives to traditional fuels and energy sources. *Fuels*, 5(2), 157–175. https://doi.org/10.3390/fuels5020010
4. Pryshliak, N., Zdyrko, N., Shynkovych, A., Koval, N. (2022). The Study on Energy Independence (Autonomy) of an Agricultural Enterprise Assurance. *TEM Journal*, 11(1), 403–410. https://doi.org/10.18421/TEM111-51
5. Esonye, C., Esonye, C. O., Agha, E. O., Ume, C. S., Njemanze, C. V., Eyisi, C. E., Adepoju, T. F. (2023). The 2022 Russia invasion on Ukraine: The biofuel energy security challenge on Ukraine and some related countries. *Heliyon*, 9(11), e21483. https://doi.org/10.1016/j.heliyon.2023.e21483
6. Cavelius, P., Engelhart-Straub, S., Mehlmer, N., Lercher, J., Awad, D., Brück, T. (2023). The potential of biofuels from first to fourth generation. *PLoS Biology*, 21(3), e3002063. https://doi.org/10.1371/journal.pbio.3002063
7. Deora, P. S., Verma, Y., Muhal, R. A., Goswami, C., Singh, T. (2022). Biofuels: An alternative to conventional fuel and energy source. *Materials Today: Proceedings*, 48, 1178–1184. https://doi.org/10.1016/j.matpr.2021.08.227
8. Singh, D., Sharma, D., Soni, S. L., Inda, C. S., Sharma, S., Sharma, P. K., Jhalani, A. (2021). A comprehensive review on 1st-generation biodiesel feedstock palm oil: production, engine performance, and exhaust emissions. *Bioenergy Research*, 14(1), 1–22. https://doi.org/10.1007/s12155-020-10171-2
9. Giertl, T., Vitázek, I., Gaduš, J., Kollárik, R., Przydatek, G. (2024). Thermochemical Conversion of Biomass into 2nd Generation Biofuel. *Processes*, 12(12), 2658. https://doi.org/10.3390/pr12122658
10. Pant, R., Gupta, A., Pant, G., Chaubey, K. K., Kumar, G., Patrick, N. (2023). Second-generation biofuels: Facts and future. *In Relationship Between Microbes and the Environment for Sustainable Ecosystem Services* 97–115. Elsevier. https://doi.org/10.1016/B978-0-323-89936-9.00011-4
11. Kumar, P., Sharma, P. K., Tripathi, S., Kumar, D., Nannaware, A. D., Chaturvedi, S., Rout, P. K. (2023). Second generation biofuels and extraction techniques. *Biofuel Extraction Techniques*, 207–255. https://doi.org/10.1002/9781119829522.ch8
12. Chowdhury, H., Loganathan, B. (2019). Third-generation biofuels from microalgae: a review. *Current*

opinion in green and sustainable chemistry, 20, 39–44. https://doi.org/10.1016/j.cogsc.2019.09.003

13. Mat Aron, N. S., Khoo, K. S., Chew, K. W., Show, P. L., Chen, W. H., Nguyen, T. H. P. (2020). Sustainability of the four generations of biofuels–a review. *International Journal of Energy Research*, 44(12), 9266–9282. https://doi.org/10.1002/er.5557
14. Torkashvand, M., Hasan-Zadeh, A., Torkashvand, A. (2022). Mini review on importance, application, advantages and disadvantages of biofuels. *Journal of Materials and Environmental Science*, 13(6), 612–630.
15. Sing, N., Mahali, K., Roy, S. (2024). *An Overview on Biofuels: Advantages and Disadvantages. Recent Developments in Chemistry and Biochemistry.* BP International. London, England, 98–116. https://doi.org/10.9734/bpi/rdcbr/v7/2778
16. Halysh, V., Trembus, I., Deykun, I., Ostapenko, A., Nikolaichuk, A., Ilnitska, G. (2018). Development of effective technique for the disposal of the Prunus Armeniaca seed shells. *Eastern-European Journal of Enterprise Technologies,* 1, 4–9. https://doi.org/10.15587/1729-4061.2018.123979
17. Shkliarenko, Y., Halysh, V., Nesterenko, A. (2023). Adsorptive performance of walnut shells modified with urea and surfactant for cationic dye removal. *Water*, 15(8), 1536. https://doi.org/10.3390/w15081536
18. Trembus, I. V., Trophimchuk, J. S., Galysh, V. V. (2018). Preparation of pulp from sunflower stalks using peroxy acids. *Voprosy Khimii i Khimicheskoi Tekhnologii*, (2), 122–127.
19. Halysh, V., Romero-García, J. M., Vidal, A. M., Kulik, T., Palianytsia, B., García, M., Castro, E. (2023). Apricot seed shells and walnut shells as unconventional sugars and lignin sources. *Molecules*, 28(3), 1455. https://doi.org/10.3390/molecules28031455
20. Başar, İ. A., Perendeci, N. A. (2021). Optimization of zero-waste hydrogen peroxide-Acetic acid pretreatment for sequential ethanol and methane production. *Energy*, 225, 120324. https://doi.org/10.1016/j.energy.2021.120324
21. Trembus, I., Hondovska, A., Halysh, V., Deykun, I., Cheropkina, R. (2022). Feasible technology for agricultural residues utilization for the obtaining of value-added products. *Ecological Engineering & Environmental Technology*, 23(2), 1–8. https://doi.org/10.12912/27197050/145732
22. Zhao, B., Wang, H., Xu, S., Qian, L., Li, H., Gao, J., Zhao, G., Ray M. B., Xu, C. C. (2022). Influence of extraction solvents on the recovery yields and properties of bio-oils from woody biomass liquefaction in sub-critical water, ethanol or water–ethanol mixed solvent. *Fuel*, 307, 121930. https://doi.org/10.1016/j.fuel.2021.121930
23. Oriez, V., Peydecastaing, J., Pontalier, P. Y. (2020). Lignocellulosic biomass mild alkaline fractionation and resulting extract purification processes: conditions, yields, and purities. *Clean Technologies*, 2(1), 91–115. https://doi.org/10.3390/cleantechnol2010007
24. Mayta, S., Huamani-Palomino, R. G., Córdova, B. M., Rivera, E., Quintana, M. (2024). Utilizing peracetic acid as an eco-friendly bleaching agent: Investigating whiteness levels of cellulose microfibers from corn husk waste. *Biomass Conversion and Biorefinery*, 1–13. https://doi.org/10.1007/s13399-024-05373-z
25. Park, H., Cruz, D., Tiller, P., Johnson, D. K., Mittal, A., Jameel, H., Venditti, R., Park, S. (2022). Effect of ash in paper sludge on enzymatic hydrolysis. *Biomass and Bioenergy*, 165, 106567. https://doi.org/10.1016/j.biombioe.2022.106567