SUMMARY OF THE MAIN RESULTS AND SCIENTIFIC CONTRIBUTIONS
of Chief Assistant Professor Stoyko Petrin, PhD, Eng., submitted for participation in the competition for the academic position of "Associate Professor" in scientific specialty 5.11. Biotechnology (Technology of Biologically Active Substances), announced by the University of Chemical Technology and Metallurgy in State Gazette, issue 71/07.08.2026.

The report on the scientific contributions of Chief Assistant Professor Stoyko Petrin has been prepared on the basis of 36 publications, of which 27 were published in scientific journals referenced and indexed in the internationally recognized Web of Science and Scopus databases, with an impact factor and/or SJR, while 9 were published in non-indexed peer-reviewed journals or edited proceedings of national and international scientific conferences. In addition, the candidate participates in the competition with 58 citations. Chief Assistant Professor Stoyko Petrin, PhD, has actively participated in 10 research projects, 6 of which were funded by the Bulgarian National Science Fund. He has been the principal investigator of 4 research contracts with the Research and Development Sector at UCTM. His average annual teaching workload during the last five academic years has exceeded 360 hours. Under his supervision, 17 diploma theses have been prepared and successfully defended, including 11 Bachelor's and 6 Master's theses.

The research activity of Chief Assistant Professor Stoyko Petrin is focused primarily on the kinetics of enzymatic processes, pretreatment and bioconversion of various lignocellulosic biomasses, production of high-value-added cellulose products and fermentable sugars, production of biocomposites, valorization of plant residues and natural bioactive compounds, as well as the synthesis and characterization of bioactive peptides.

The scientific and applied scientific contributions can be systematized into the following sections:

1. **Physicochemical and kinetic regularities of the enzymatic hydrolysis of different types of lignocellulosic biomass**
2. **Pretreatment and bioconversion of lignocellulosic biomass into fermentable sugars**
3. **Acid hydrolysis and modification of cellulose for the production of value-added products**
4. **Production of dry-process medium- and high-density wood-fiber boards (MDF and HDF), recycling and utilization of secondary fibrous raw materials, and use of additives to improve board properties**

5. **Production, modification, and characterization of natural and synthetic biologically active compounds**
6. **Spectroscopic approaches for monitoring and characterizing chemical transformations and technological studies in chemical pulp production**

1. Physicochemical and kinetic regularities of the enzymatic hydrolysis of cellulosic and lignocellulosic materials

This section combines studies on the adsorption and kinetics of enzymatic hydrolysis, the influence of feedstock structure, different pretreatment methods, and the subsequent production of fermentable sugars from woody and plant biomass, including industrial hemp residues. The initial stage of the interaction between cellulase enzymes and the cellulosic substrate was analyzed. Cellulase adsorption onto cellulose fibers was shown to be most adequately described by the Temkin isotherm. The thermodynamic relationships obtained allow the process to be interpreted as the combined result of energetic and entropic factors. Depletion of accessible adsorption sites, restrictions within the adsorption layer, and conformational changes in the enzyme molecule were found to exert a substantial influence on the enzyme–substrate interaction. This approach provides a physicochemical basis for a more detailed interpretation of subsequent enzymatic hydrolysis *(publication № I.1)*.

Kinetic studies of steam-explosion pretreatment of wheat straw showed that the process is heterogeneous in nature and differs from that described by classical models of homogeneous enzyme kinetics. An exponential kinetic model was developed that accounts for the effects of temperature, enzyme concentration, and degree of hydrolysis. The activation energy was found to remain practically constant, whereas the pre-exponential factor decreases as hydrolysis proceeds and plays a determining role in the gradual decrease in reaction rate. A generalized relationship was derived that enables prediction of the degree of hydrolysis at predefined temperature, enzyme concentration, and time, giving the model direct relevance to process control and optimization *(publication № I.2)*.

A model for reactions on uniformly heterogeneous surfaces was applied to steam-explosion-treated Paulownia tomentosa. A simultaneous increase in activation energy and the pre-exponential factor with increasing conversion was established, together with a pronounced

compensation effect between the two kinetic parameters. The increase in activation energy is associated with the progressively restricted access of the enzyme to less accessible regions of the cell wall, whereas the increase in the pre-exponential factor reflects changes in the accessible reaction surface. Energetic limitations were shown to play a leading role in the decrease in hydrolysis rate *(publication № I.3)*.

Further development of the kinetic studies was associated with the use of fast-growing woody species (poplar, paulownia, willow, and black locust) for second-generation bioethanol production. The process is best described by the modified Prout–Tompkins topochemical equation. According to this model, the rate of enzymatic hydrolysis is governed simultaneously by the amount of unreacted substrate, inhibition, chemical interactions at the surface, and diffusion limitations. Relationships were established between the kinetic constant, the exponent, and wood density, together with a compensation effect between activation energy and the pre-exponential factor. The resulting temperature–time relationship can be used for simulation, control, and technological management of the process *(publication № II.3)*.

The proposed kinetic mechanism was further confirmed for the high-temperature hydrolysis of wheat straw and maize stalks. Enzymatic hydrolysis of both substrates is described by an exponential kinetic equation for processes occurring on uniformly heterogeneous surfaces. During the reaction, both activation energy and the pre-exponential factor increase, and a compensation effect is observed, indicating a common kinetic mechanism. The higher reaction rate for maize stalks is associated with lower energetic barriers, while increasing enzyme concentration raises the initial rate and the pre-exponential factor without substantially changing the activation energy *(publications № III.3 and III.5)*.

2. Pretreatment and bioconversion of lignocellulosic biomass into fermentable sugars

Comparative studies of fast-growing woody species (paulownia, poplar, willow, and black locust) showed that the morphological and anatomical characteristics of the feedstock are as important as its chemical composition. For paulownia, the highest glucose yield after steam-explosion pretreatment followed by enzymatic hydrolysis was 52%, approximately 24% higher than under the corresponding conditions for poplar and willow. The results show that the low density and specific fibrous structure of the wood facilitate steam-explosion pretreatment and enable efficient subsequent enzymatic hydrolysis. Poplar

and willow also show potential comparable to that of the most widely used agricultural lignocellulosic feedstocks—wheat straw and maize stalks *(publication № II.2)*.

The use of oxidizing reagents (oxygen, hydrogen peroxide, and peracetic acid) as an intermediate stage between pretreatment and enzymatic hydrolysis of wheat straw and maize stalks was investigated. Peracetic acid at low concentration was found to improve subsequent enzymatic hydrolysis through partial disruption and dissolution of lignin structures. At the same time, a purification effect with respect to phenolic compounds and inhibitors of enzymatic hydrolysis, including furfural, was established. The results demonstrate the potential application of oxidizing reagents as a means of increasing enzyme accessibility to cellulose *(publication № II.1)*.

The application of other annual plant species, such as ryegrass, for bioethanol production was also examined. Acid and alkaline pretreatments followed by enzymatic hydrolysis and fermentation were compared. The highest concentrations of reducing sugars were obtained after treatment with 1% HCl and 1% NaOH at 121°C. Acid treatment yielded 33.6 $mg.mL^{-1}$ reducing sugars and 5.55 g ethanol/100 g dry matter, whereas alkaline treatment yielded 29.0 $mg.mL^{-1}$ and 4.73 g ethanol/100 g dry matter, respectively *(publication № II.7)*.

A comparative study of steam-explosion, high-temperature, and acid hydrolysis of wheat straw and maize stalks showed that dilute-acid hydrolysis at 130°C provides the highest overall yield of reducing sugars after two-stage treatment. Acid hydrolysis favors conversion of dissolved oligosaccharides into monosaccharides with minimal furfural formation, whereas after high-temperature hydrolysis more oligosaccharides remain and the formation of furfural and hydroxymethylfurfural increases. For annual plant feedstocks (wheat straw and maize stalks), steam-explosion treatment was not an effective treatment method compared with the other pretreatment methods investigated (high-temperature and acid hydrolysis) *(publications № III.2 and III.4)*.

Following acid hydrolysis combined with subsequent enzymatic hydrolysis of Paulownia tomentosa, a substantial yield of reducing sugars was obtained. The total amount of reducing sugars reached approximately 31% and was markedly higher than that obtained after high-temperature hydrolysis followed by enzymatic hydrolysis. An additional advantage of the acid pretreatment method was the formation of smaller amounts of the inhibitors furfural and HMF. The results support the use of paulownia as a feedstock for second-generation bioethanol technologies *(publication № III.1)*.

The potential of fast-growing woody species was further explored through the study of different Robinia pseudoacacia L. clones („Pordim, Zhaskiser, and Tsarevets“). A relationship was established between wood density and chemical composition in relation to the production of unbleached pulp and glucose. The „Tsarevets“ clone is characterized by lower density, higher cellulose content, and lower lignin content. The studies indicate its suitability for unbleached pulp production as well as for enzymatic hydrolysis. The glucose yield after steam-explosion treatment was higher than that obtained for the fast-growing woody species investigated previously (paulownia, poplar, willow, and black locust), demonstrating the applicability of plantation cultivation of these Robinia pseudoacacia L. clones *(publication № III.6)*.

The possibilities for valorizing residual industrial hemp biomass after mechanical removal of the long fibers under industrial conditions were studied. Steam-explosion pretreatment of the biomass with and without CO_2 addition was compared. CO_2 addition was found to create conditions similar to dilute-acid hydrolysis. The highest xylose yield during CO_2 steam-explosion hydrolysis was obtained at 190°C, whereas at 200°C furfural formation increased and undesirable carbohydrate degradation became more pronounced. Subsequent enzymatic hydrolysis showed a maximum glucose yield when pretreatment was conducted within the temperature range of 180-190°C *(publications № II.8 and II.9)*.

It was additionally established that the specific structure of residual hemp biomass, particularly the presence of long and coarse fibers after steam-explosion pretreatment, requires additional mechanical refining before enzymatic hydrolysis. Refining induces fibrillation and increases the accessibility of cellulase enzymes to cellulose, thereby increasing glucose yield. The results show a higher glucose yield than that obtained for wheat straw. For hemp feedstocks containing fewer long fibers and subjected to CO_2 steam-explosion pretreatment, the need for mechanical refining decreases and the process temperature can be reduced by 20°C *(publication № III8)*.

3. Acid hydrolysis and modification of cellulose for the production of value-added products

The conditions for acid hydrolysis of bleached hardwood pulp were investigated in relation to the potential utilization of the waste hydrolysate for subsequent bioconversion and biogas

production. Xylose was found to be released during the initial stage of the process, whereas glucose yield increased over time. At higher temperature and longer acid-hydrolysis duration, substantial furfural formation was observed. The determined biochemical methane potential of the resulting acid hydrolysate was 0.68 LCH_4/gCOD, indicating the possibility of utilizing the waste hydrolysate for biogas production *(publication № II.4)*.

Dilute-sulfuric-acid hydrolysis was also investigated for the production of microcrystalline cellulose (MCC) and modified cellulose intended for the production of microfibrillated cellulose (MFC). Both cellulose products have broad applications and strong current market demand. To obtain MCC with a degree of polymerization below 350, meeting the requirements of the European Pharmacopoeia, the hydrolysis temperature must be 130°C or higher. At 120°C and 2% H_2SO_4, modified cellulose with a degree of polymerization above 350 is obtained and can be used for MFC production. Kinetic investigation of acid hydrolysis showed that depolymerization of bleached hardwood pulp is most accurately described by the modified Prout–Tompkins topochemical equation. The established temperature–time relationship can be used to control and simulate the depolymerization of bleached pulp to MCC *(publications № II.16 and III.9)*.

4. Production of dry-process medium- and high-density wood-fiber boards (MDF and HDF), recycling and utilization of secondary fibrous raw materials, and use of additives to improve board properties

This section systematizes research on fiberboards and biocomposites, linking MDF recycling, utilization of technical lignins, reduction of formaldehyde emissions, and improvement of water resistance through functional additives. A research direction was developed to reduce the content of synthetic binders (resins) in MDF by using lignosulfonates and technical hydrolysis lignin.

Laboratory boards with different contents of phenol–formaldehyde resin (PF resin) and lignin additives were produced, enabling assessment of their effects on physical and mechanical properties. The studies demonstrate the technological feasibility of replacing part of the resin with renewable or waste-derived lignin products, while emphasizing the need to optimize the ratio between binder components and hot-pressing conditions *(publication № II.5)*.

This concept was further developed by using unmodified technical hydrolysis lignin as the principal binder and a small amount of phenol-formaldehyde resin as an auxiliary binder. Hot-pressing temperature was found to be decisive for lignin activation and board properties. Very good mechanical properties can be achieved at 160°C, whereas a temperature of at least 190°C is required to ensure the water resistance required by European standards *(publication № I.8)*.

For the recycling of waste MDF, high-temperature treatment with saturated steam at 121 and 134°C for 30-60 min. was investigated. Within the studied range, no substantial difference in the chemical composition of the wood fibers was observed. Residual products from hydrolysis of the urea-formaldehyde resin (UF resin), together with unaffected resin, remain associated with the recycled fibers. Increasing the temperature had a stronger influence on the generation of organic compounds in the wastewater than extending the treatment time. COD increased from 1838 $mg.L^{-1}$ at 121 °C for 30 min to 3020 $mg.L^{-1}$ at 134°C for 60 min. The results enable selection of an optimal high-temperature treatment regime for producing recycled defibrated MDF material in which the wood fibers remain unchanged while pollution of the resulting wastewater streams is limited *(publication № I.4)*.

Biocomposites based on wood fibers with the addition of spent coffee grounds and ammonium lignosulfonate were developed with the aim of reducing the free-formaldehyde content of the resulting MDF. A clear optimum was established at 50% of a mixture of coffee grounds and lignosulfonate relative to the dry mass of the fibers. At this composition, the modulus of elasticity reached 2707 $N.mm^{-2}$ and the bending strength 22.6 $N.mm^{-2}$, while the modulus of elasticity was practically comparable to that of control MDF samples produced using only urea-formaldehyde resin. At contents of the coffee-ground/lignosulfonate mixture above 50%, the mechanical properties decreased and water absorption increased. The results demonstrate the possibility of simultaneously valorizing both waste products - coffee grounds and lignosulfonate for the production of MDF without formaldehyde-containing synthetic resins *(publication № II.10)*.

Studies with technical hydrolysis lignin showed that at 190°C and a pressing factor of 1 $min.mm^{-1}$, lignin is not sufficiently activated to act as an independent binder and predominantly functions as a filler. Nevertheless, the possibility of adding 5% technical hydrolysis lignin together with 10% or more phenol-formaldehyde resin was established without substantial loss of the principal physicochemical properties. These results provide a basis for further experimental work

on optimization and activation of technical hydrolysis lignin in MDF production *(publication № III.7)*.

Further investigation of the applicability of technical hydrolysis lignin in MDF production established the substantial role of board density in the activation capacity of technical hydrolysis lignin. With a modified binder system containing a low amount of phenol–formaldehyde resin, boards with very good mechanical properties can be obtained at hot-pressing temperatures of at least 160°C. To meet the requirements of the European standard for use in dry conditions, a hot-pressing temperature of 190 °C is required, while applications in humid conditions require 200°C. The optimal board density at which the adhesive potential of technical hydrolysis lignin is manifested is 900 $kg.m^{-3}$ *(publication № II.11)*.

The production of MDF with very low free-formaldehyde content was investigated using ammonium bisulfite and urea-metabisulfite as formaldehyde-scavenging additives to a resin with an ultra-low F/U molar ratio of 1.06. Both additives modify the curing profile and effectively reduce the free-formaldehyde content. With 3% ammonium bisulfite and 5% urea-metabisulfite, free-formaldehyde values of 1.36 and 1.26 mg/100 g oven-dry board, respectively, corresponding to the super E0 level (according to the new European standard for free-formaldehyde content from June 2026), were achieved without significant deterioration of the physical and mechanical properties of the MDF (density, water absorption, swelling, modulus of elasticity, and tensile strength) *(publication № II.12)*.

The application of alkenyl succinic anhydride (ASA) as a water-repellent additive in board production was investigated. At additions of 2–8% ASA relative to the oven-dry fiber mass, the modulus of elasticity decreased by 17–35%, whereas at 10% ASA the decrease was practically eliminated, indicating a different interaction at the higher additive content. At the same time, water absorption decreased by up to threefold and thickness swelling by up to fivefold. These results demonstrate the potential use of ASA for substantially improving board water resistance *(publication № II.17)*.

5. Production, modification, and characterization of natural and synthetic biologically active compounds

The possibilities for valorizing spent lavender biomass through solid-liquid extraction followed by membrane filtration and concentration, spray drying, and low-temperature precipitation were investigated. An eightfold concentration of the resulting extracts was achieved using an X201 membrane. During spray drying at 85°C, the loss of antioxidant activity was below 6%, demonstrating the applicability of this drying method to the extracts. Low-temperature precipitation additionally resulted in fractionation and enrichment of the polyphenolic fraction. The results demonstrate the possibility of valorizing waste lavender biomass and converting it into value-added products *(publication № II.13)*.

Hydrodistillation of clove essential oil was optimized by simultaneously considering yield, eugenol content, antioxidant and antibacterial activity, and specific energy consumption. The optimal technological process was established at a power of 200 W and a collected distillate volume of 500 mL, yielding 1.219 g of oil from 25 g of dried clove buds, corresponding to a yield of 4.88%. The obtained oil was evaluated for antioxidant activity and the results were compared with those for a commercially purchased oil. A threefold higher antioxidant activity than that of the commercial sample was observed *(publication № II.14)*.

Analogues of the somatostatin analogue BIM-23052 were synthesized and characterized, in which phenylalanine residues were successively replaced by tyrosine. Lipophilicity, cytotoxicity, and antiproliferative activity were monitored, and relatively small changes in the amino-acid sequence were found to substantially alter biological behavior. The investigated analogues showed no toxic effects at concentrations below 200 μM, while individual structures exhibited more pronounced antiproliferative activity *(publication № I.5)*.

For analogues of the natural tetrapeptide FELL, the role of hydrophobic amino-acid residues at the third and fourth positions and the effect of modification of the C-terminal group were investigated. Compounds containing Leu, Nle, Ile, and Val were synthesized, and the relationship between structural modification, lipophilicity, and analgesic activity was examined. The results highlight the importance of leucine residues and the C-terminal amide group in determining activity and stability *(publication № I.6)*.

Bioconjugates of FELL with pyrrole acids were developed. All synthesized structures exhibited analgesic activity; the free pyrrole acids showed higher initial activity but were hydrolytically unstable. Their conjugation with the peptide moiety resulted in a pronounced increase in hydrolytic stability while preserving biological activity. Bioconjugation was thus shown to be a useful tool for balancing activity and stability in low-molecular-weight bioactive structures *(publication № I.7)*.

At the next stage, FELL analogues containing D-Phe at the first position were synthesized. Stereochemical modification combined with an appropriate selection of hydrophobic residues was found to improve the duration of analgesic action. The most favorable combination in terms of analgesic activity was observed for D-Phe at the first position and Leu at the third and fourth positions. Extension of the peptide chain with an additional hydrophobic residue also had a positive effect. The resulting molecules were characterized by low cytotoxicity and high hydrolytic stability *(publication № I.9)*.

The studies were extended to analogues of the antimicrobial peptide Temporin A with modifications at positions 1 and 10. Replacement of Phe with Tyr or fluorinated Phe, and of Ser with Tyr or Thr, enabled evaluation of the effects of hydrophobicity, spatial structure, and halogenation on antibacterial, antiproliferative, and cytotoxic activity. The analogue containing Tyr at position 10 showed the highest selectivity toward MCF-7 cells, whereas the fluorinated analogue DT4F exhibited the strongest antibacterial activity among the new compounds, with a minimum inhibitory concentration of 80 $\mu g.mL^{-1}$, albeit with trade-offs in cytotoxicity, phototoxicity, and stability at pH 9 *(publication № I.10)*.

6. Spectroscopic approaches for monitoring and characterizing chemical transformations and technological studies in chemical pulp production

The applicability of infrared spectroscopy for monitoring chemical transformations was demonstrated. Acetylation of p-aminophenol to paracetamol was used as a model system. Through preliminary selection of an appropriate solvent and characteristic IR bands, changes in the reaction system over time were monitored. The information obtained on conversion dynamics without the need to isolate intermediates demonstrates the potential of IR spectroscopy as an analytical tool for monitoring reactions in solution *(publication № II.6)*.

A kinetic study of kraft pulping of softwood was conducted with the aim of producing pulp suitable for subsequent bleaching under the industrial conditions of Svilosa AD. The active-alkali charge (expressed as sodium hydroxide) and pulping duration were investigated. Total and screened yield, reject content, residual active alkali in the black liquor, and kappa number were evaluated. The results show that the optimal active-alkali charge, expressed as sodium hydroxide, is 23% for the production of pulp intended for bleaching. At a 24% alkali charge, unbleached pulp with a lower kappa number is obtained without a practical decrease in screened yield. At alkali charges below 23%, unbleached pulp with a kappa number above 50 is obtained, suitable for the production of kraft papers and kraftliner *(publication № II.15)*.