Report regarding January-December 2015 Research Phase

1. Ongoing study of biomass production capacity of sweet sorghum

Sorghum crop experimental plots were established by two technologies: sorghum as main crops set in spring, and as catch crops set in the summer, after harvesting winter cereals.

In the case of main crops, established on May 1st 2015, field works were applied for maintenance, pest control and proper fertilization according to sorghum crops technologies. The climate conditions much different in 2015 comparing with the year 2014 led to lower production of biomass as those recorded in 2014. As in 2014 productions of around 100 tonnes fresh biomass per hectare were obtained with some hybrids, the maximum yields this year were located around 60 tonnes per hectare. Sorghum plants samples were harvested starting August 1st (from 90 days of cultivation) and continuing until November 1st (180 days of cultivation). Collected plants were analyzed in terms of height, weight, dry matter content, the leaves and panicles were removed and the strains were fragmented and chopped using Retch MS 100 laboratory mill. The chopped biomass was then pressed with the laboratory press, sweet juice was harvested, and the resulting bagasse undergo to the process of simultaneous conservation and extraction of sugars left un-extracted by pressing. The process of ***simultaneous conservation and extraction of sugars*** from biomass sorghum is an original concept developed upon the results obtained in this project and is described briefly below, at paragraph 3 (Further research regarding the possibilities for storage and pretreatment of harvested sorghum biomass, for renewable energy and other biocompounds production). Regarding the yields obtained in 2015, in conditions of very low rainfall, which resulted in a shortage of water in the soil after 90 days from seeding, the plants reached heights between 120 cm and 180 cm, stalks weights between 130 and 235 grams, dry mater concentration of around 37% and the total sugar concentration of 6.6-6.8 Brix determined by refractometer, varying according with the hybrid type and culture conditions. After other 15 days (105 days after seeding) plants exceeded 250 cm, the strains reached 280 grams and refractometer determined concentrations of total sugars ranged between 8 and 11.2 Brix. Maximum values: maximum height of 320 cm, the maximum weight of 794 grams and the maximum dry matter concentration of 76.05% were reached after 150 days of cultivation (samples taken on October 1st). In terms of production of sugar, the highest concentration of total sugars in the juice (17 Brix) is reached after 135 days of culture and remained constant until the last samples harvest after 180 days from seeding (November 1st). Other analyzed parameters were concentrations of glucose, sucrose, reducing sugars during the whole period from 90 to 180 days after seeding, with sampling every 15 days during this period. This way the optimal moment to harvest sweet sorghum have been determined depending on the genetic variety, phenophase, temperature and rainfall, aiming maximum amounts of sugars harvested by pressing the juice from sorghum stems. The results were published in scientific papers and presented at conferences: ***Sugar extraction and saccharification of sweet sorghum biomass for biorefining***, authors: Vintila T., Sumalan R., Iosif G., Pop G., Kovacs K., in Journal of Biotechnology, Volume 208, Supplement Issue ISSN 0168-1656 and presented at European

Biotechnology Congress 2015, București; ***Sweet sorghum as feedstock for fermentation industry***, authors: Teodor Vintilă, Kornel Kovacs, Nicolae Popa, Iosif Gergen, Radu Șumălan, Abstract Book, vol. 1 of Animal Science and Biotechnologies Symposium Bioengineering of Animal Resources, 28-29 Mai 2015, ISSN 2343-9459. The research works in this package of activities have been performed using equipments and materials existing in the Laboratory of Industrial and Microbial Biotechnology from the USAMVB Timisoara and using equipments and materials purchased with funds from the grant for this project, such as mill laboratory Retsch SM100, laboratory press Ferrari Group, refractometer, spectrophotometer, etc. Different services have also been made for establishment and maintenance experimental plots of sorghum, sampling, participation at conferences, salaries for staff involved in these activities.

2. Selection of yeast strains and testing the ability of ethanol biosynthesis from sorghum juice

Several organisms able to ferment sugars to produce ethanol are known; also parameters used during the fermentation process directly affect the metabolic activity of organisms. Therefore, the selection of suitable strains of microorganisms and determining the optimum fermentation parameters are essential for optimizing the performance and efficiency of the fermentation process for the production of ethanol from sweet sorghum juice. Therefore, the project team tried to investigate the ability of microorganisms to ferment various sugars from sorghum juice, assessing the influence of inoculation rate, temperature, addition of nitrogen and phosphorus sources on productivity of fermentation process.

From a total of twelve microbial strains tested (yeasts of the genus *Saccharomyces* and *Kluyveromyces* and bacteria of the genus *Zymomonas*) yeast strains of *Saccharomyces cerevisiae* have performed the best in the processes of fermentation of the sorghum juice and yielded higher conversion yields of sugars to ethanol. Have been established the optimum inoculation rate that, concluding that should not exceed 5% of vegetative yeast culture, or 0.5 g / l of dry yeast. A particular yeast strain was selected as having the best performance with regard to fermentation of sugars from the sorghum juice and allowed obtaining of high yields of ethanol at 37°C from the sweet sorghum juice. Regarding supplementation of sorghum juice with exogenous sources of nitrogen and phosphorus, insignificant increasing of yields has resulted in conversion of sugars to ethanol. The results were published in scientific papers and presented at conferences:

Sweet sorghum, ideal raw material for biogas, biofuels and other bioproducts, presented by Teodor Vintilă, at International Workshop – Waste to Biogas, in 04.03.2015 at ENREG, Arad, România (http://www.enreg-expo.com/115.html?&L=1); ***Study on fermentation capacity of several microbial strains for bioethanol production from lactose***, Popa Nicolae, Skumpija Alen, Vintila Teodor, Gergen Iosif, presented at the conference: Research People and Actual Tasks on Multidisciplinary Sciences, 24 – 28 June 2015, Lozenec, Bulgaria. ***Evaluation of fermentation parameters and yeasts selection for ethanol production from sweet sorghum juice,*** authors Teodor Vintilă, Nicolae Popa, Georgeta Pop, Iosif Gergen, Radu Șumălan in Romanian Biotechnological Letters Vol. 20, No. 6, 2015.

The research work in this package of activities have been performed using equipment and materials existing in the laboratory and those purchased with funds from the grant for this project, such as strains of microorganisms, culture media, incubators, bioreactors, gas sensors, alcolyzer, spectrophotometer, etc. Travel expenses were also involved for participation to conferences, salaries for staff involved in these activities.

3. Further research regarding the possibilities for storage and pretreatment of harvested sorghum biomass, for renewable energy and other biocompounds production

Simultaneous conservation and extraction of sugars from biomass sorghum is an original concept developed upon the results obtained in this project and consists of ensilage sorghum biomass containing sugars with organic compounds additives that inhibit the growth of microorganisms in the silage and performing at the same time the extraction of the sugars from biomass in the liquid phase, this way obtaining much higher yields of sugar per hectare compared with extracting sorghum juice by pressing. Moreover, biomass sorghum harvested in late summer or autumn may be stored and processed throughout the whole year, allowing the use of equipment and facilities with much smaller capacities (and cheaper) than conventional technology which requires processing entire sorghum crop in a short time to prevent sugars lost from biomass. Studies concerning simultaneous conservation and extraction of sugars were conducted over a period of 1 year, starting from biomass harvested in the fall of 2014. The biomass was ensiled in October 2014, samples were harvested during the entire period and analyses were carried out regarding the sugars content of liquids extracted by pressing from biomass ensiled with organic additives. Another original aspect pursued in these studies is the possibility of degradation of lignocellulosic complex during conservation with organic additives. These findings will allow reducing or elimination of thermo-chemical pretreatments before the enzymatic hydrolysis of sorghum bagasse to obtain carbohydrates from lignocellulosic biomass. Therefore, by applying this process, three main processes can take place simultaneously: preservation of sugars for long periods of time, the extraction of sugars from the tissues of biomass in the liquid phase and pre-treatment of lignocellulosic material to enhance the rate of enzymatic hydrolysis of cellulose to glucose. At the end of October 2015 the last samples were analyzed and in November 2015 began drafting the patent application at Romanian Office of Inventions and Marks (OSIM). The patent application will be filed at the end of 2015 - beginning of 2016. We aim to transfer these results into practical application, as we are convinced that developing industrial technologies based on processes developed in this research will have remarkable technological, economic and social impact.

The results were published in scientific papers and presented at conferences:

1. ***Sugar extraction and saccharification of sweet sorghum biomass for biorefining***, Vintila T., Sumalan R., Iosif G., Pop G., Kovacs K., in Journal of Biotechnology, Volume 208, Supplement Issue ISSN 0168-1656 and presented at European Biotechnology Congress 2015, Bucharest.
2. ***Tracking the path of metals in the process of lignocellulosic ethanol production from polluted biomass,*** Vintilă T., Sumalan R., Negrea P, Barbu C.H., Vintila D., Kovacs K.,

presented at the conference and published in Book of Abstracts of the 6th European Bioremediation Conference, Chania, Crete, Greece, June 29-July 2, 2015, ISBN 978-960-8475-23-6.

3. ***Cascade Processing of Sweet Sorghum Biomass for Advance Biofuels Production***, Teodor VINTILĂ, Nicolae POPA, Adrian TRULEA, presented at VII-a International Conference: Bioenergy in Romania, part of the Fair RENEXPO South-East Europe – București, România in November 17 2015.

4. In October 2015 has been sent for publication the manuscript: ***Metal distribution in the process of lignocellulosic ethanol production from heavy metal contaminated sorghum biomass***, authors Teodor Vintila, Radu Sumalan, Adina Negrea, Horia Barbu, Kornel Kovacs, in *Journal of Chemical Technology and Biotechnology* (indexed on web of science).

5. Production of lactic acid and other biocompounds from sorghum juice.

In the first stage 13 strains of lactic acid bacteria from the Collection of Industrial Microorganisms of USAMVBT were tested for the ability of lactic acid production in synthetic media containing sugar (a mixture of sucrose and glucose like in sorghum juice). It was analyzed the production of the lactic acid enantiomers (D and L forms) and screening was done for the production of acetic acid to select homofermentative and heterofermentative strains. Five strains of lactic acid bacteria of the genus *Lactobacillus* have been selected according to several criteria: the strains showed high efficiency of conversion of carbohydrates to lactic acid and lactic acid was obtained in high concentrations, strains which produced predominantly one of the two optical isomers, strains producing racemic mixtures and homofermentative strains. Important is to eliminate the presence of acetic acid in the fermentation medium, which is a contaminant with negative effects in the stage of extraction and purification of lactic acid. In the second phase of research lactic bacteria have been cultivated in sorghum juice to assess the production ability of lactic acid from such materials. D-isomer is used for the production of high molecular weight PLA, and the L-isomer of lactic acid is used for the production of PLA with low molecular weight. Both polymers are applied in the production of biodegradable plastics. Also in this work package was requested and obtained from UEFISCDI approval to build an installation for anaerobic microbial cultures using the funds allocated to this grant.

The results were published in scientific papers and presented at conferences:

Sorgul zaharat, materie primă ideală pentru biogaz, alți combustibili și bioproduse, prezentată de Teodor Vintilă, la Workshopul Internațional – Waste to Biogas, organized in 04.03.2015 at ENREG, Arad, România (http://www.enreg-expo.com/115.html?&L=1)

Cascade Processing of Sweet Sorghum Biomass for Advance Biofuels Production, Teodor VINTILĂ, Nicolae POPA, Adrian TRULEA, presented at VII-a International Conference: Bioenergy in Romania, part of the Fair RENEXPO South-East Europe – București, România în 17 Noiembrie 2015.

In the current year we managed to purchase a fermentation system with multiple bioreactors (15 bioreactors), which will be attached at the anaerobic microbial cultures installation, which will be used for the activities planned in the next year in this project.