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Lactic acid fermentation of a combined agro-food waste substrate

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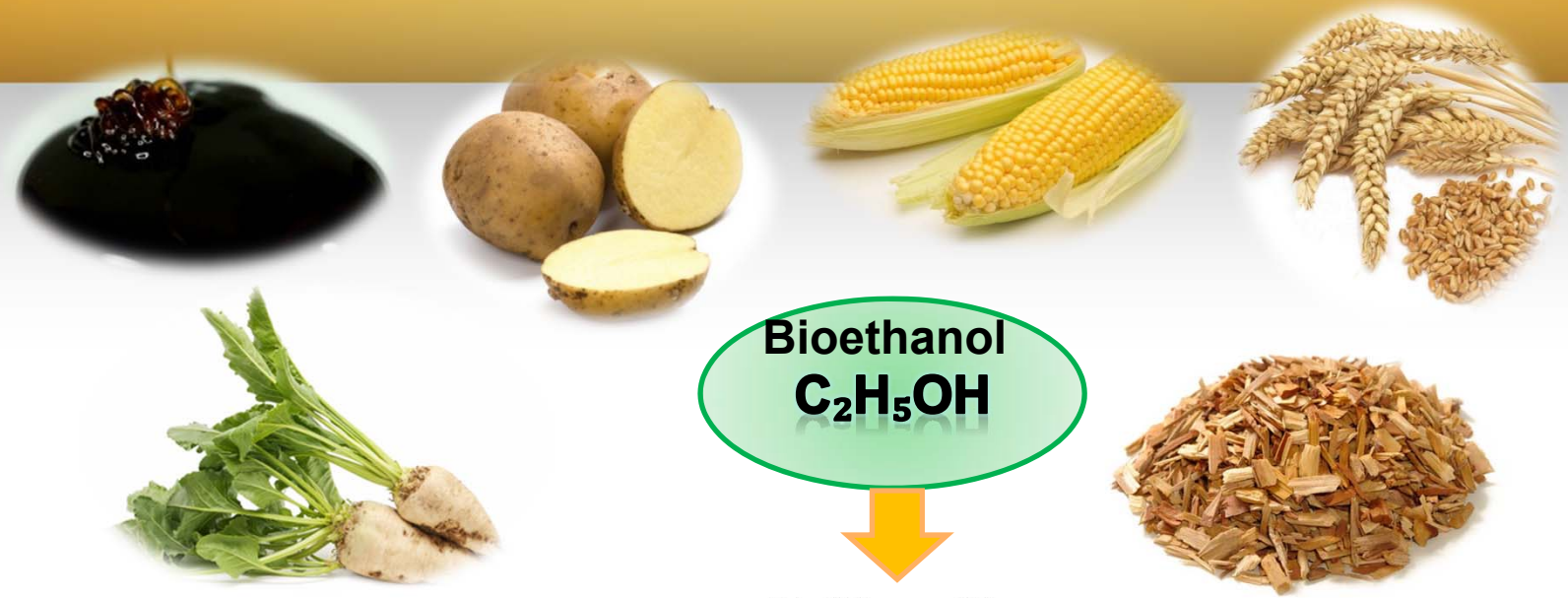
DISTILLERY STILLAGE FROM BIOETHANOL PRODUCTION



Bioethanol
C_2H_5OH

Distillery stillage

Waste water from bioethanol production

- **Distillery stillage – a serious ecological problem, approximately 13 L per L of bioethanol, low pH, high BOD_5 (15-340 g L^{-1})**
- **By its utilization the enhancement of economy of bioethanol production and solving environmental issues are performed**

Current stillage utilization:

- **As animal feed, as dried distillers' grains with solubles - DDGS (preferably in USA)**
- **As wet distillers' grains - WDG (mostly in Europe due to high costs of energy for complete drying).**
- **As a substrate for production of hydrogen, butanol, acetic acid and biogas, fertilizer**

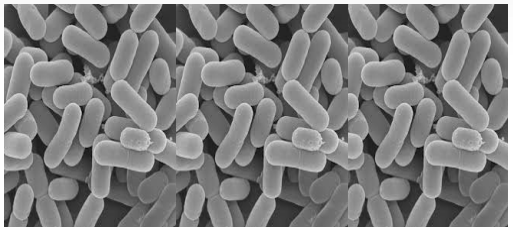
POTATO STILLAGE AND SUGAR BEET MOLASSES AS A SUBSTRATE FOR LACTIC ACID AND BIOMASS PRODUCTION

Lactic acid

- Important chemical with use in chemical, food, pharmaceutical, cosmetic and textile industries, increase
- For the production of polymers –**poly-lactides** (implants and grafts in medicine, controled release, environmental friendly packaging material for food)
- Serbia is importing lactic acid –production on the stillage would be a good strategy

Probiotic biomass

- Lactic acid bacteria - probiotic potential



- Fermentation by-product (the remains after the fermentation)– feed additive

THE MAIN OBJECTIVES:

- ***To assess utilization of industrial distillery stillage from bioethanol production on wasted potato and sugar beet molasses together as a potential substrate for lactic acid (LA) and biomass production***

- ***To select the most promising microorganism for lactic acid (LA)and biomass production on the potato distillery stillage and sugar beet molasses.***

- ***To determine main fermentation parameters, especially the effect of initial sugar concentration on the LA and biomass production in batch system.***

POTATO STILLAGE AND SUGAR BEET MOLASSES AS A SUBSTRATE FOR LACTIC ACID AND BIOMASS PRODUCTION

Potato stillage

- Potentially good substrate for lactic acid production
  significant amount of proteins, vitamins (B complex), minerals...

Sugar beet molasses

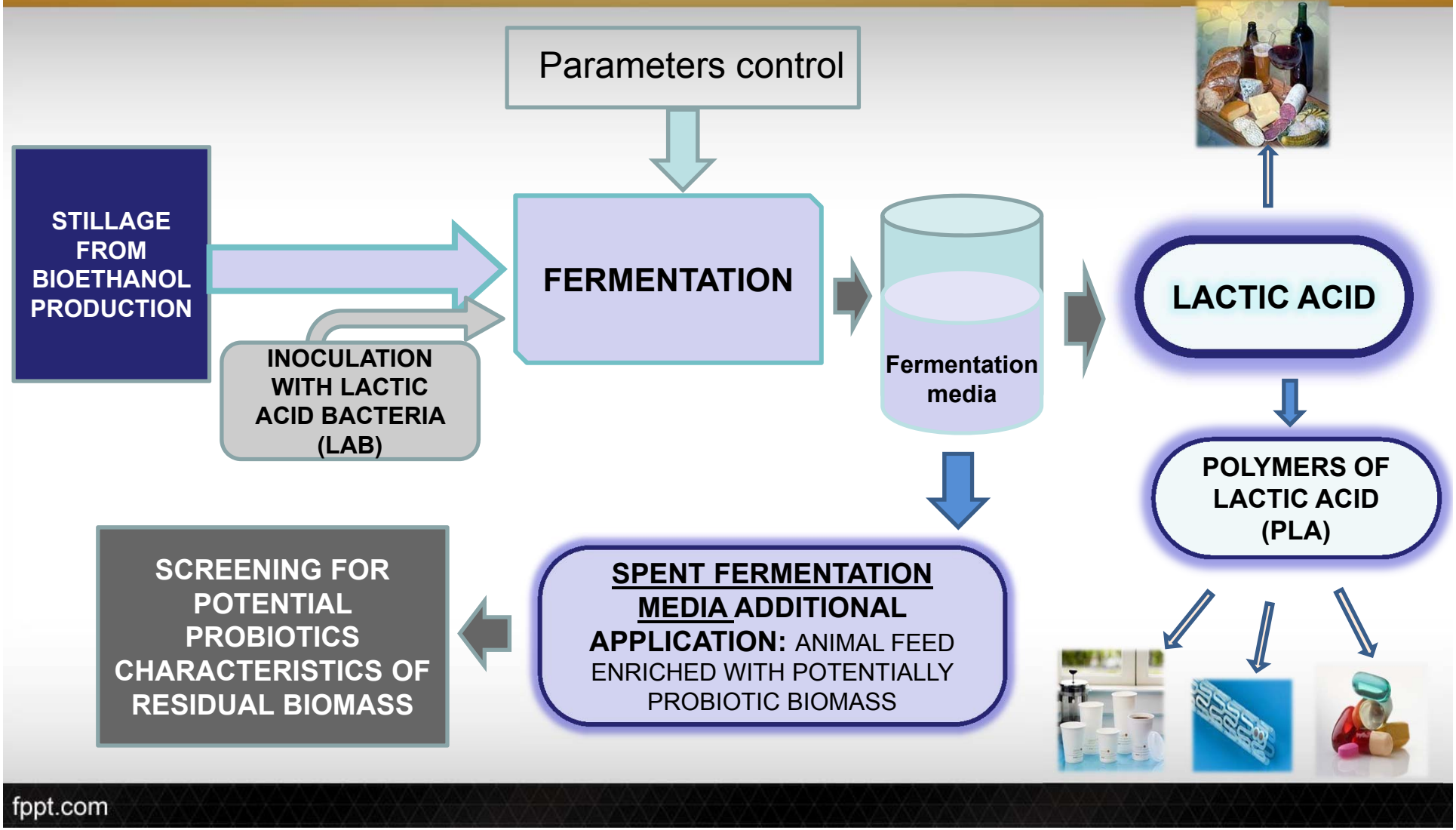
- High amount of sugars (sucrose, glucose, fructose and raffinose)
- Majority of N in molasses - betain

N source

Supstrate for lactic acid production

C source

MATERIALS AND METHODS



MATERIALS AND METHODS

Microorganisms:

- ***Lactobacillus rhamnosus* ATCC 7469,**
- ***Lactobacillus salivarius* ATCC 11741**
- ***Lactobacillus paracasei* NRRL B-4564**

Selection of the most appropriate microorganism:

Three different substrates were used for LA fermentation:

- **stillage media,**
- **modified MRS media with sucrose**
- **and modified MRS media with molasses**

Fermentation substrates:

- **Stillage from bioethanol production on waste potato** (Reahem, Srbobran, Serbia)
- **Sugar beet molasses** (from ethanol plant Alpis, Kovin, Serbia)

RESULTS AND DISCUSSION

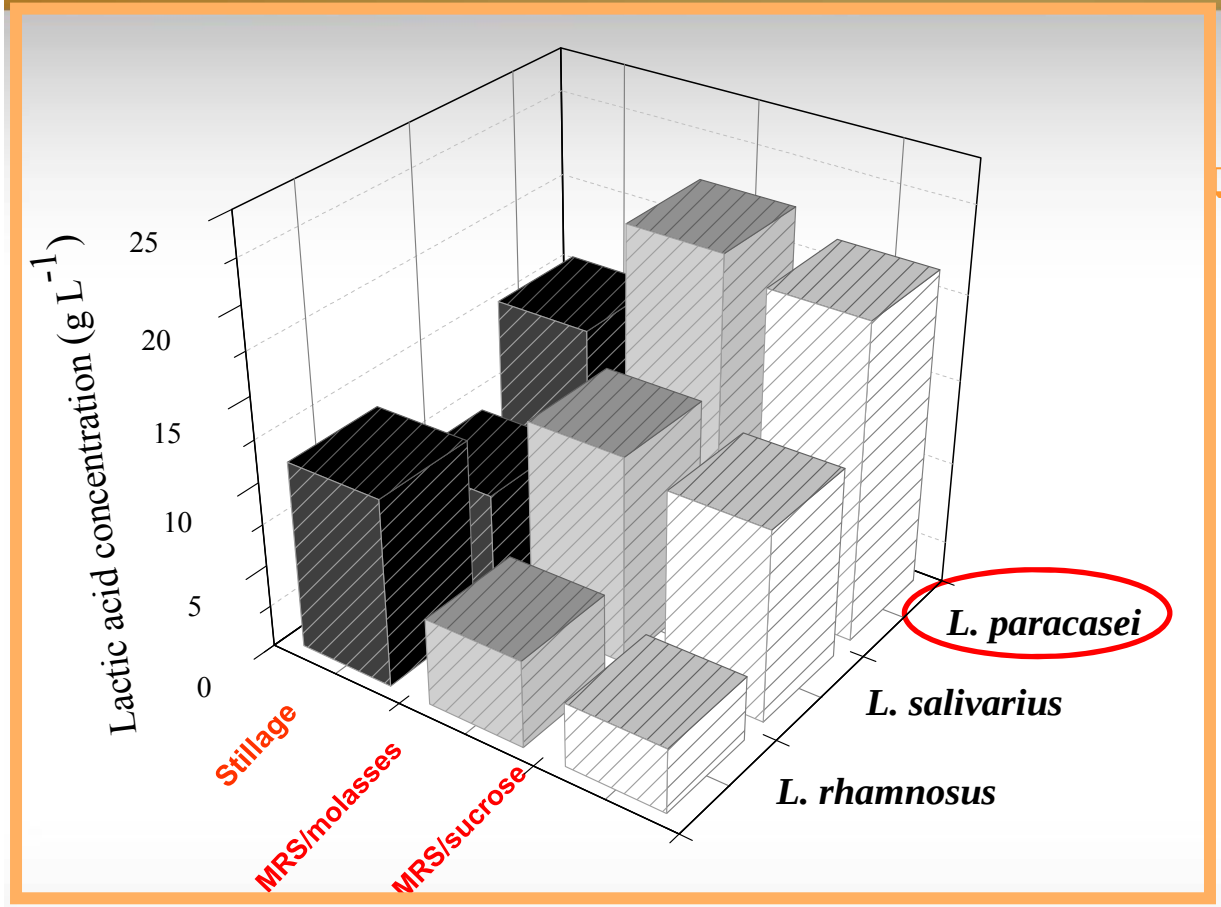
Chemical composition of distillery stillage and sugar beet molasses

Parameter	%(w/w) Potato stillage	%(w/w) Sugar beet molasses
Dry matter	5.74±0.14	83.62±0.89
Total reducing sugar	1.57±0.26	53.16±0.51
Total nitrogen	0.18±0.03	1.31±0.16
Lipids	0.31±0.05	0.28±0.07
Sulfated ash	0.89±0.09	11.97±0.67

- The nitrogen content needed for LAB of potato stillage is pretty high (0.18 %) It originates from residual yeast biomass, but also from the substrate used for ethanol fermentation (in standard media for LA fermentation it ranges from 0.01-0.21%).
- Assimilable N content of molasses low (mostly betaine)
- Lipids present in the stillage and molasses are also valuable for LAB fermentation
- Amount of fermentable sugars is low in the stillage but high in molasses
- The combination of this 2 substrates could be provided necessary nutrients for LA fermentation

RESULTS AND DISCUSSION

Selection of lactic acid bacteria for lactic acid production on sucrose rich substrate

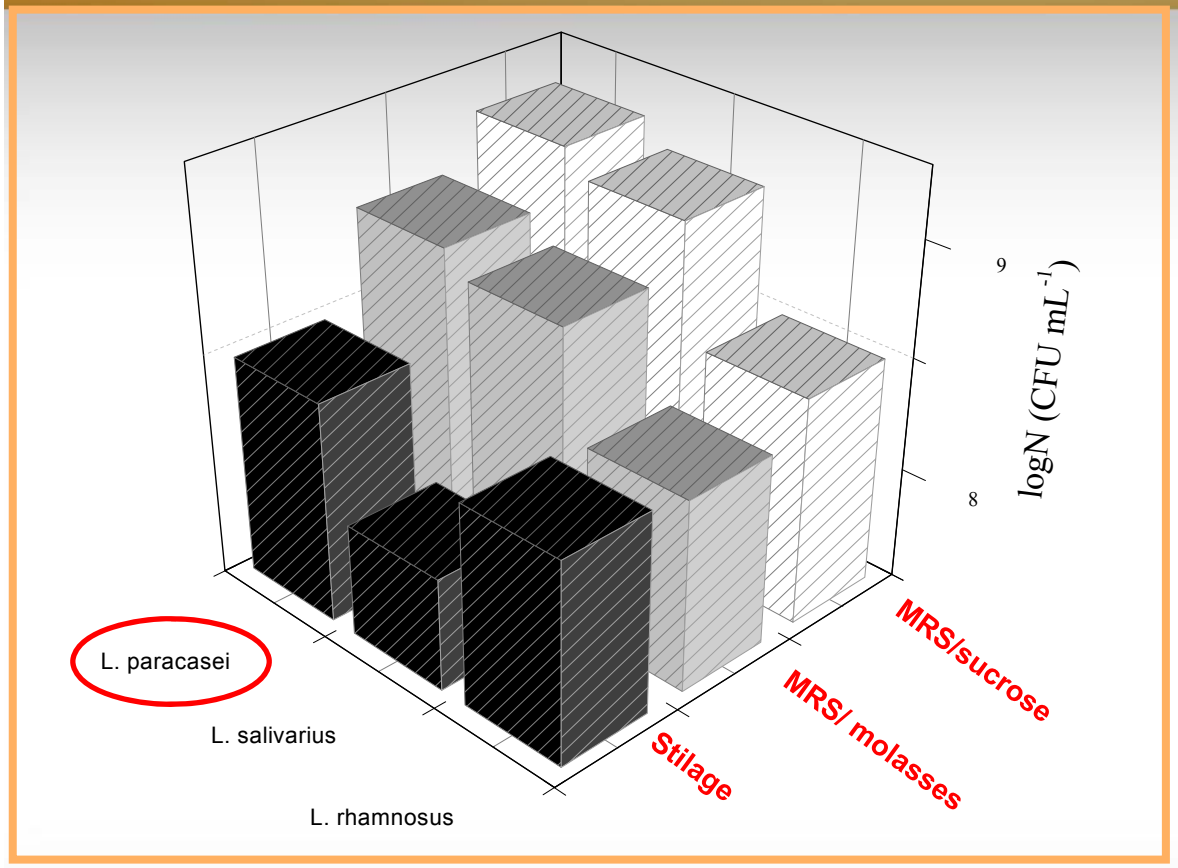


- The results showed that the *L. paracasei* utilized sucrose the best and produced high amount of LA on MRS/molasses and MRS/sucrose media.

Figure 1. Production of lactic acid by *L. paracasei* NRRL B-4564, *L. salivarius* ATCC 11741 and *L. rhamnosus* ATCC 7469 on

RESULTS AND DISCUSSION

The biomass production of lactic acid bacteria on sucrose rich substrate

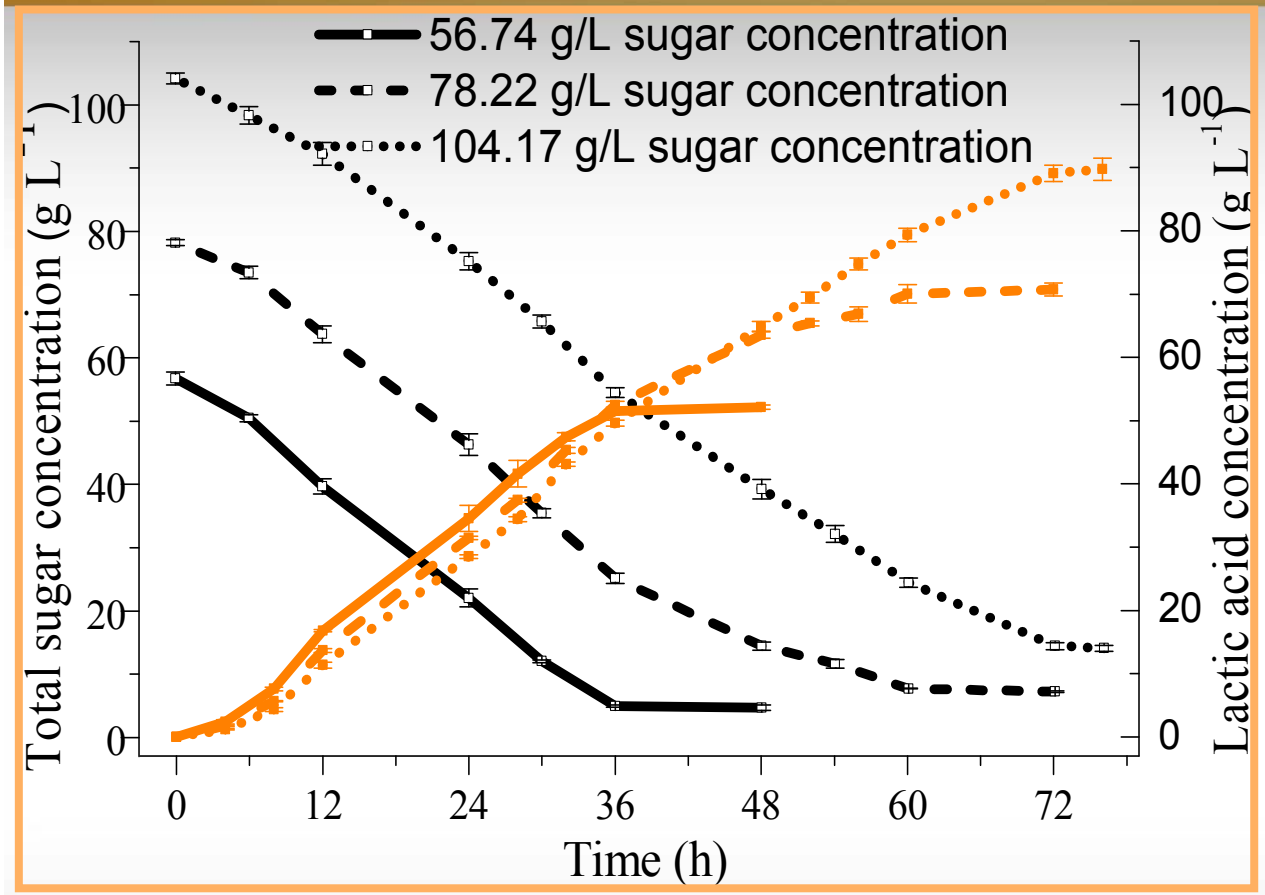


- *L. paracasei* showed good growth on MRS/sucrose and on MRS/molasses media while *L rhamnosus* grew well on the sole potato stillage.
- The most promising strain of *L. paracasei* NRRL B-4564 was chosen for further studied on a combined stillage/molasses media

Figure 2. Biomass production by *L. paracasei* NRRL B-4564, *L. salivarius* ATCC 11741 and *L. rhamnosus* ATCC 7469 on

RESULTS AND DISCUSSION

The effect of sugar concentration



- Lactic acid concentration was increasing with increase of the initial sugar concentration, but also the time of fermentation

Figure 3. Time course of lactic acid production and sugar consumption on stillage/molasses media by *L. paracasei* NRRL B-4564 at different initial sugar concentrations.

RESULTS AND DISCUSSION

The effect of sugar concentration

Fermentation time (h)	Initial sugar concentration (g L^{-1})	LA concentration (g L^{-1})	Number of viable cells (10^9 CFU ml^{-1})	LA yield (g g^{-1})	Volumetric LA productivity (g L^{-1} h^{-1})
48	56.74±1.03	51.55±0.54	3.9±0.14[a]	0.91±0.03	1.42±0.01
60	78.22±0.49	70.03±1.44	5.3±1.13[b]	0.90±0.01	1.14±0.01
72	104.17±0.86	89.19±1.27	4.5±0.85[b]	0.85±0.02	1.23±0.02

- **The high LA concentration obtained in this combined waste medium is probably additionally supported by high content of vitamins and minerals present in molasses and stillage**

RESULTS AND DISCUSSION

The growth of *L. paracasei* NRRL B-4564

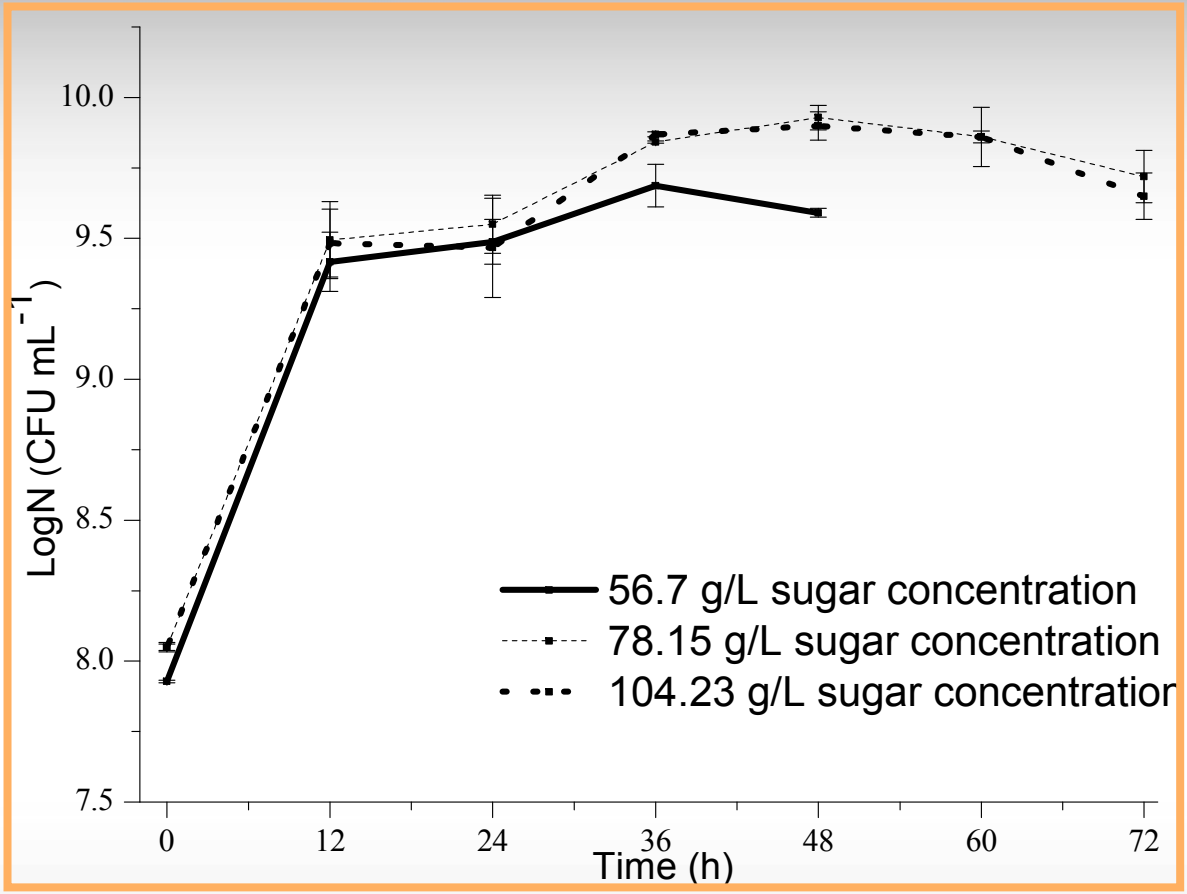


Figure 4. Effect of different initial sugar concentrations on growth of *L. paracasei* NRRL B-4564 on stillage/molasses media.

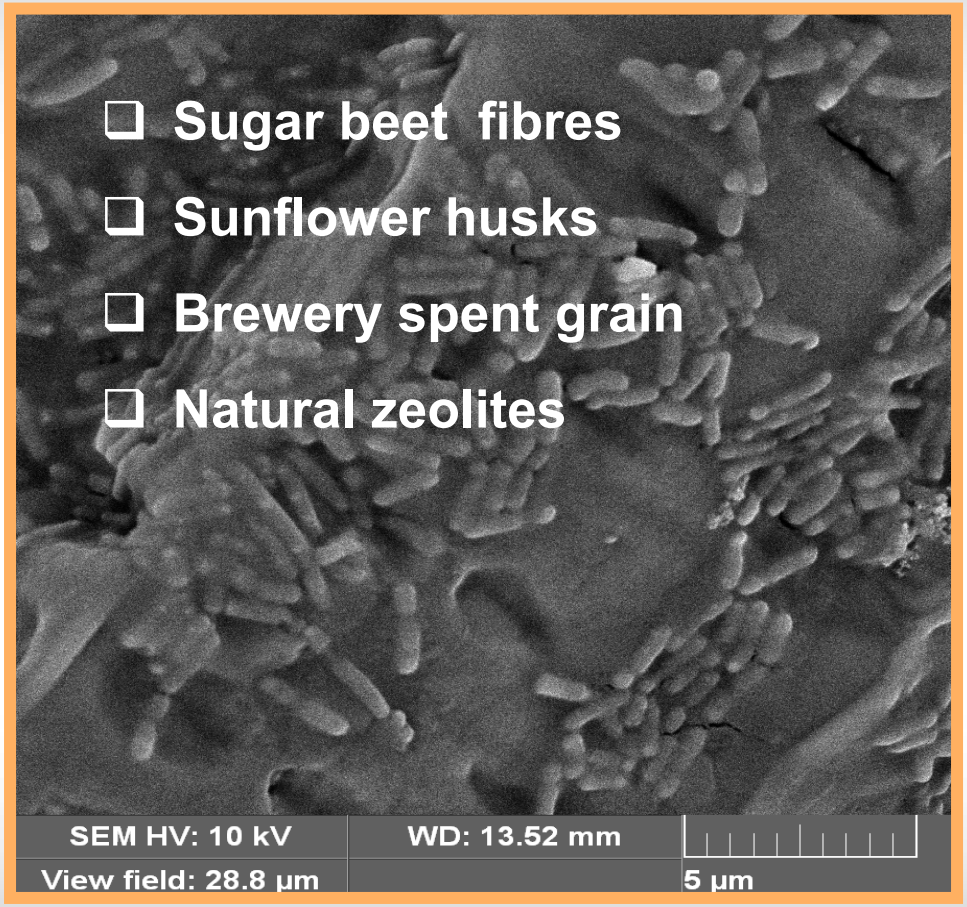
- The highest biomass production was obtained in a sample with initial sugar concentration of 78.22 g L^{-1} at the 48th hour of fermentation.
- The growth was in the range of 3.9–5.3 × 10^9 CFU mL^{-1}

FURTHER INVESTIGATIONS

Further research goals:

- Improvements in productivity by using fed batch fermentation mode or by utilization of immobilized biomass.
- Various immobilization materials such as zeolite, and natural and waste support materials such as sugar beet fibres, sunflower husks and brewery spent grain…
- Currently, we are also assessing the quality of the remains after LA fermentation together with the produced biomass as a feed, mostly for monogastric animals

Immobilization materials:



CONCLUSIONS

LACTIC ACID FERMENTATION OF A COMBINED AGRO-FOOD WASTE SUBSTRATE

- Among three assayed strains, *L. paracasei* NRRL B-4564 gave the highest LA production and good growth on the cheap and complex waste substrates.
- The highest LA productivity of 1.42 g L^{-1} h^{-1} and yield of 0.91 g g^{-1} were obtained at an initial sugar concentration of 56.74 g L^{-1} on stillage/molasses media.
- Also, the intense growth of *L. paracasei* during fermentation resulted in a high number of viable cells at the end of fermentation time (3.9–5.3 × 10^9 CFU mL^{-1}).
- Wasted potato distillery stillage and sugar beet molasses are a good combination of complementary industrial wastes that could be an economically and environmentally favorable for the production of LA and biomass

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LACTIC ACID FERMENTATION OF A COMBINED AGRO-FOOD WASTE SUBSTRATE

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Thank you for your attention!

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