# Usage of food industry by-products as raw materials in lactic acid fermentation

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# Beer production scheme


BREWER'S SPENT GRAIN

- ✓ **Brewer's spent grain (BSG) is the most abundant brewing by-product, corresponding to around 85% of the total by-products generated**

- ✓ **Per 100 L of beer produced 20 kg of brewer's spent grain are obtained which results in an annual production of over 38 million tonnes of BSG worldwide**

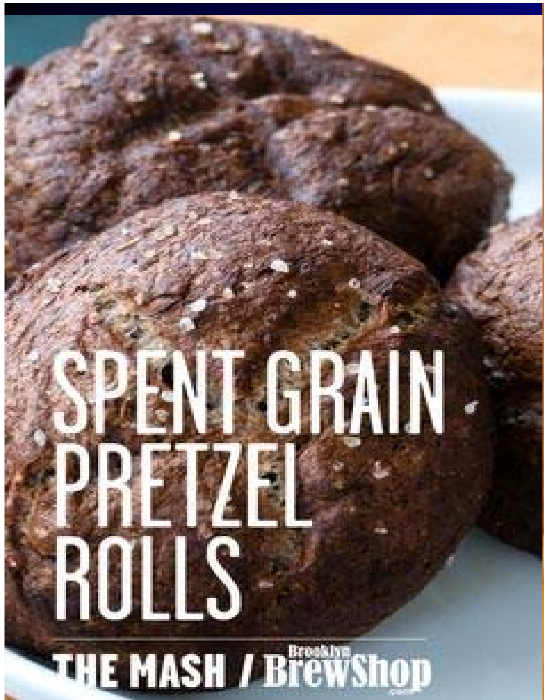
**The chemical composition of brewer’s spent grain varies according:**

- ✓**to barley variety,**
- ✓**harvest time,**
- ✓**malting and mashing conditions, and**
- ✓**the quality and type of unmalted raw materials used in the brewing process.**

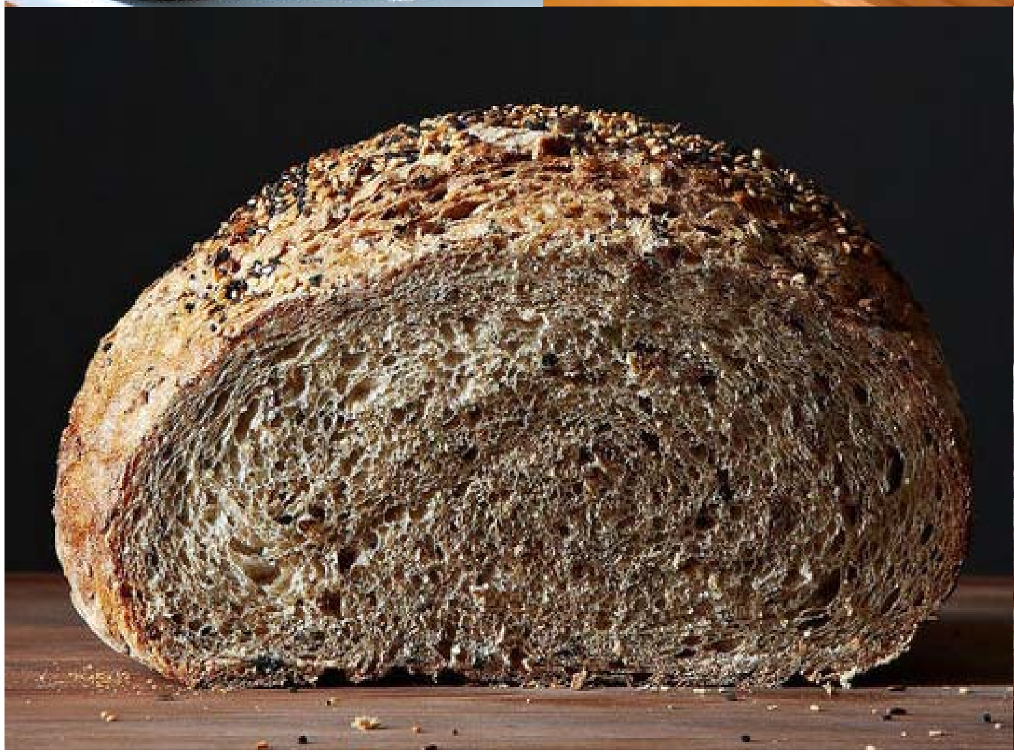
## Brewer's spent grain is a lignocellulosic material rich in protein and fibre, which account for around 20 and 70% of its composition, respectively.

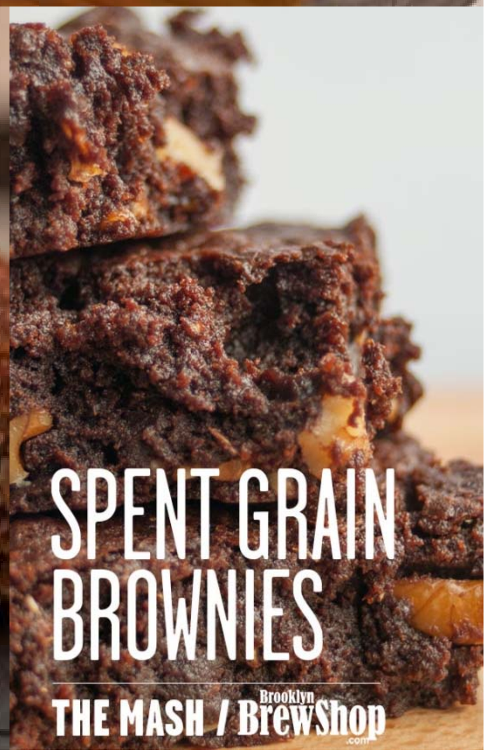
| Component (% dry matter) | Bogar et al. (2002) | Mussatto and Roberto (2005) | Serena and Knudsen (2007) | Dehnavi (2009) |
|---|---|---|---|---|
| Cellulose | 15 | 16.8 | 14.7±2.2 | 15.1 |
| Hemicellulose | 23 | 28.4 | - | 32.5 |
| Lignin | 22 | 27.8 | 12.6±0.6 | 13.4±1.9 |
| Proteins | 18 | 15.25 | 21.5±2.1 | - |
| Ash | - | 4.6 | 4.8±0.5 | 3.4±0.1 |
| Carbohydrates | - | - | 52.5±4.0 | - |
| Lipids | - | - | 11.7±0.5 | - |
| Starch | 12 | - | 6.0±1.4 | 12.5 |


The use of brewer's spent grain is still limited, being basically used as animal feed


# and in human nutrition



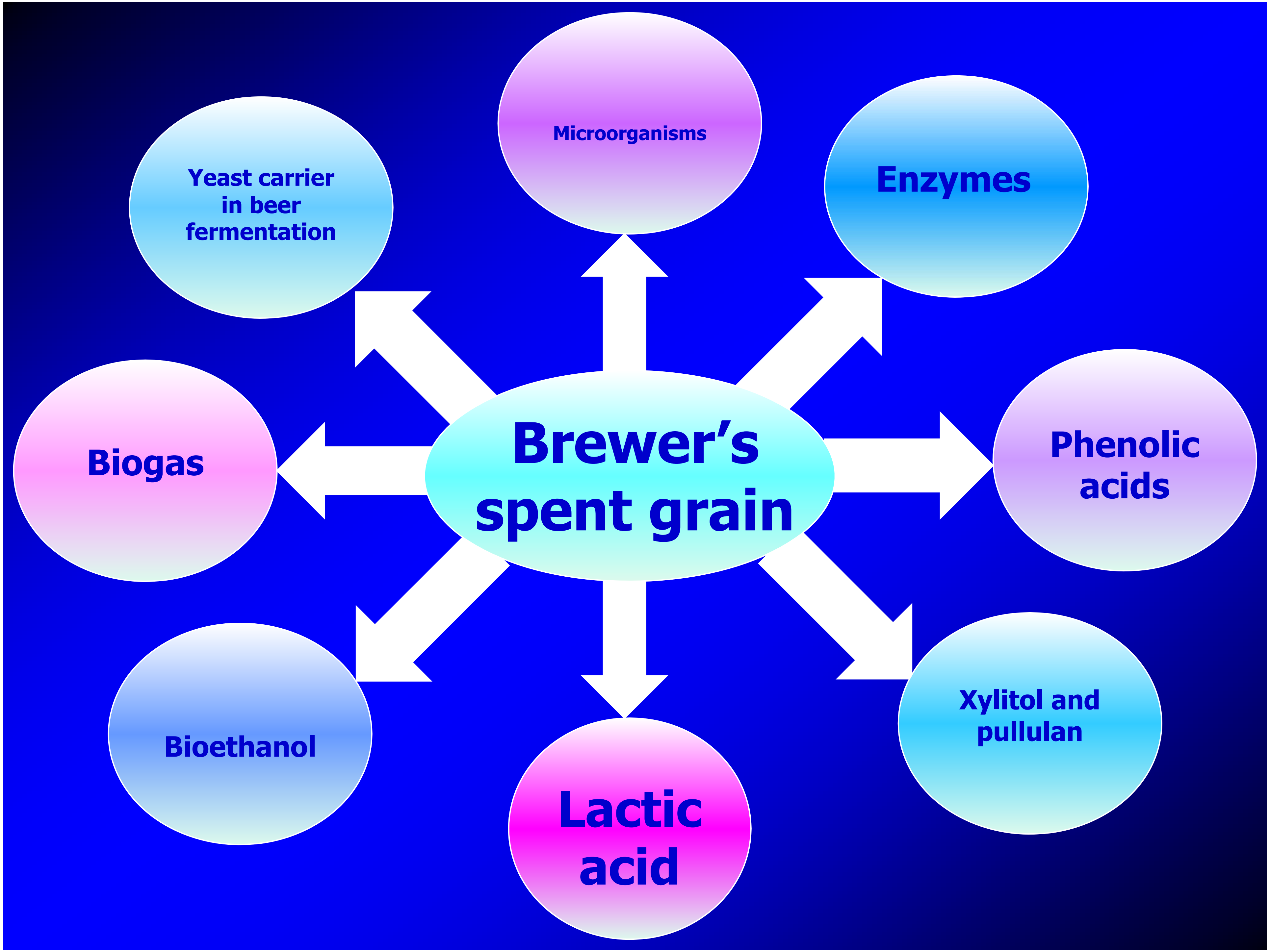



**Its possible applications are as a raw material in:**

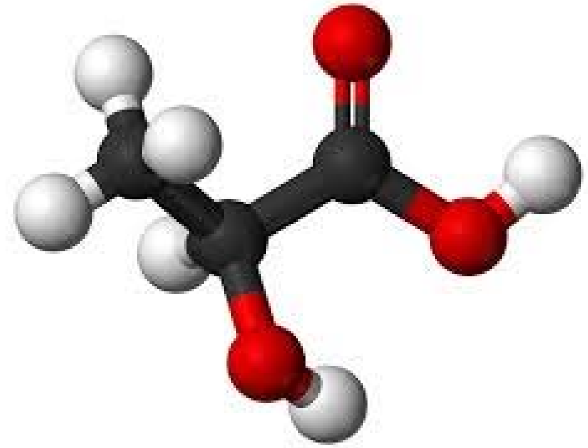
- ✓ **biotechnology,**
- ✓ **energy production,**
- ✓ **charcoal production,**
- ✓ **paper manufacture,**

**Or**

- ➢ **as a brick component,**
- ➢ **and adsorbent.**


Brewer's spent grain
Microorganisms
Enzymes
Phenolic acids
Xylitol and pullulan
Lactic acid
Bioethanol
Biogas
Yeast carrier in beer fermentation

# Lactic acid



**Food and food-related applications account for approximately 85% of the demand for lactic acid (LA).**

**The demand for lactic acid has grows yearly by 5–8%.**

**There are two optical isomers of lactic acid, L-(+)-lactic acid and D-(-)-lactic acid.**

**Lactic acid can be manufactured either by chemical synthesis or by microbial fermentations.**

**Presently, almost all lactic acid produced worldwide comes from the fermentative production route.**

**A desired isomer of lactic acid can be produced via fermentation using selected lactic acid-producing strains.**

**Besides this, microbial lactic acid fermentation offers an advantage in terms of the utilization of renewable carbohydrate biomass, low production temperature and energy consumption.**

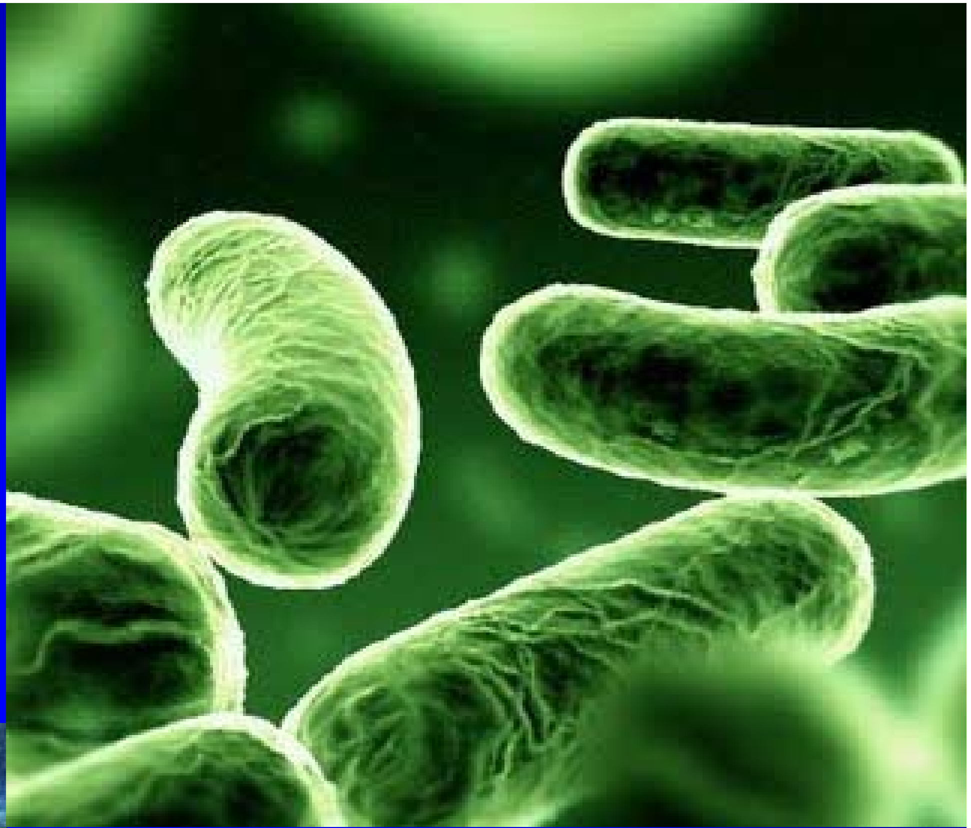
# BREWER'S SPENT GRAIN HYDROLYSIS OPTIMIZATION

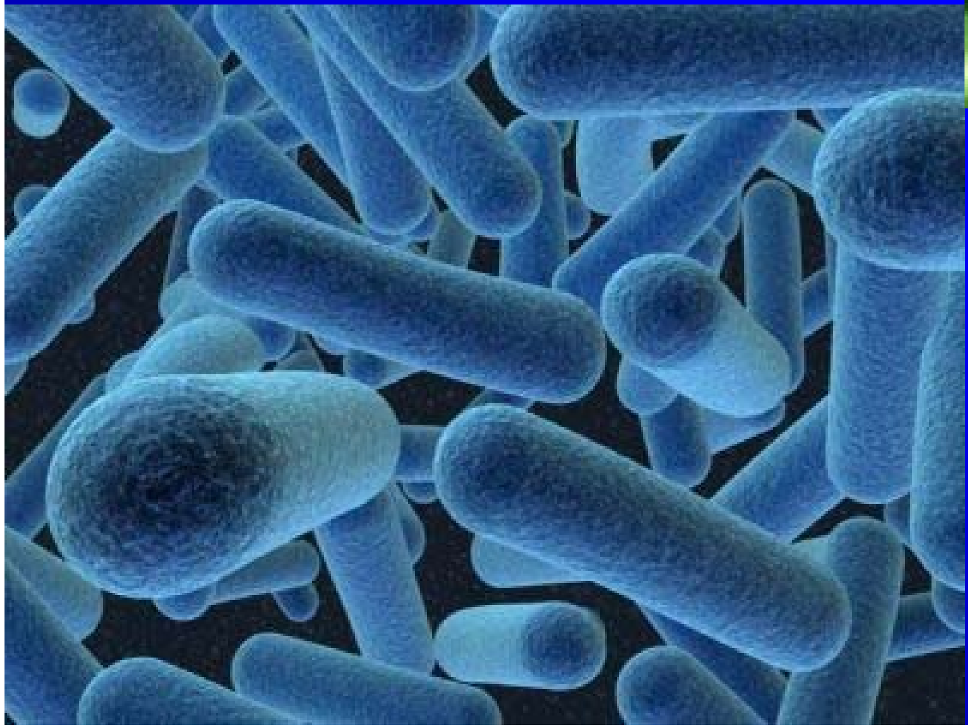
**Brewer's spent grain obtained in a lager beer production was dried at 40°C for 12 hours.**

**Brewer's spent grain hydrolysis was carried out under optimal conditions using the following enzymes:**

1. **Termamyl SC - α-amylase,**
2. **SAN Super 240 L – glucoamylase, and**
3. **Cellic Ctec2– cellulase (Novozymes, Denmark).**

**Most lactic acid bacteria require a wide range of growth factors including amino acids, vitamins, fatty acids, purines, and pyrimidines for their growth and biological activity.**





**Thus, the substrate composition and nutritional requirements of the strain considerably affect the overall performance of the fermentation.**

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# The influence of calcium-carbonate and yeast extract addition on lactic acid fermentation of brewer's spent grain hydrolysate

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- ✓ ***L. rhamnosus*** **produced mostly L-(+)-LA in all fermentations (98%).**
- ✓ **The highest L-(+)-LA yield (96%) and volumetric productivity (0.52 g/L·h$^{-1}$) were reached when 2% of yeast extract was added.**

Research article

Institute of Brewing & Distilling

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(wileyonlinelibrary.com) DOI 10.1002/jib.403

# Lactic acid fermentation of brewer's spent grain hydrolysate by *Lactobacillus rhamnosus* with yeast extract addition and pH control

Jelena Pejin,[1]* Miloš Radosavljević,[1] Sunčica Kocić-Tanackov,[1] Aleksandra Djukić-Vuković[2] and Ljiljana Mojović[2]

- ✓ **NaOH was used as neutralizing agent. The pH control with NaOH greatly increased reducing sugar utilization, L-(+)-LA concentration, yield and volumetric productivity.**

- ✓ **The highest L-(+)-LA concentration, yield, and volumetric productivity were achieved with the reducing sugar concentration of 5.4% and yeast extract content of 5% in BSG hydrolysate.**

Research article

Institute of Brewing & Distilling

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# Fed-batch L-(+)-lactic acid fermentation of brewer's spent grain hydrolysate

Jelena Pejin,[1] Miloš Radosavljević,[1]* Sunčica Kocić-Tanackov,[1] Dragana Mladenović,[2] Aleksandra Djukić-Vuković[2] and Ljiljana Mojović[2]

- ✓ **For further increase in efficiency of L-(+)-LA fermentation fed-batch fermentation was investigated.**
- ✓ **The addition of glucose, glucose and yeast extract, and wort during L-(+)-LA fermentation and its effect on fermentation parameters was investigated.**

- ✓ **In all fed-batch fermentations higher L-(+)-LA concentration, yield, and volumetric productivity were achieved compared with the batch fermentation.**
- ✓ **The highest L-(+)-LA yield and volumetric productivity of 93.3% and 2.04 g/L·h$^{-1}$, respectively, were achieved in fermentation with glucose and yeast extract addition during fermentation.**

# Lactic acid fermentation with the addition of renewable raw materials

**During the fermentation, determination of:**

- Lactic acid concentration (L-(+)-lactic acid assay, Megazyme®, Wicklow, Ireland)
- Reducing sugar concentration
- Cell viability (pour plate technique, MRS agar, 37°C)
- pH value

**Brewer`s spent grain hydrolysis**

**Centrifugation**

**Sterile liquid hydrolysate – fermentation media**

**pH control (30% NaOH)**

**Brewer`s yeast (0.5-5.0%)**

**Stillage (5-20%)**

**Thin stillage (5-50%)**

**Soybean meal (1-3%)**

**Malt rootlets (1-2%)**

**Soybean meal extract (10-50%)**

**Malt rootlets extract (10-50%)**

**Batch and fed-batch fermentation**

***L. rhamnosus* ATCC 7469 at 37°C**
**150 rpm**

**L-(+)-Lactic acid**

Research article

Institute of Brewing & Distilling

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# Brewers' spent grain and thin stillage as raw materials in L-(+)-lactic acid fermentation

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Dragana Mladenović,[2] Aleksandra Djukić-Vuković[2] and Ljiljana Mojović[2]

- ✓ **Effect of thin stillage (TS) and thin stillage (5–50%) and glucose addition in BSG hydrolysate on batch and fed-batch L-(+)-LA fermentation.**
- ✓ **TS was investigated as a nitrogen and mineral source.**

- ✓ **TS addition significantly increased free amino nitrogen concentration (by up to 209%) which is important for bacterial growth.**

- ✓ **A strong positive correlation between free amino nitrogen and L-(+)-LA concentration was determined.**

- ✓ **In fed-batch fermentation the highest L-(+)-LA concentration, yield and volumetric productivity of 48.02 g/L, 87.8% and 0.96 $g/L{\cdot}h^{-1}$, respectively, were achieved.**

# Brewer`s yeast

✓Spent yeast in the brewing industry is relatively inexpensive, it is utilized largely in the production of extracts to meet the needs of food and fermentation industries

✓Yeast cells contain plenty of protein, lipid, RNA, vitamins, and minerals

✓The brewer‘s yeast is an inexpensive nitrogen source and generally recognized as safe (GRAS)

✓Large quantities of brewer’s spent yeast are obtained after beer fermentation

Dry brewer's yeast

8€/kg

vs.

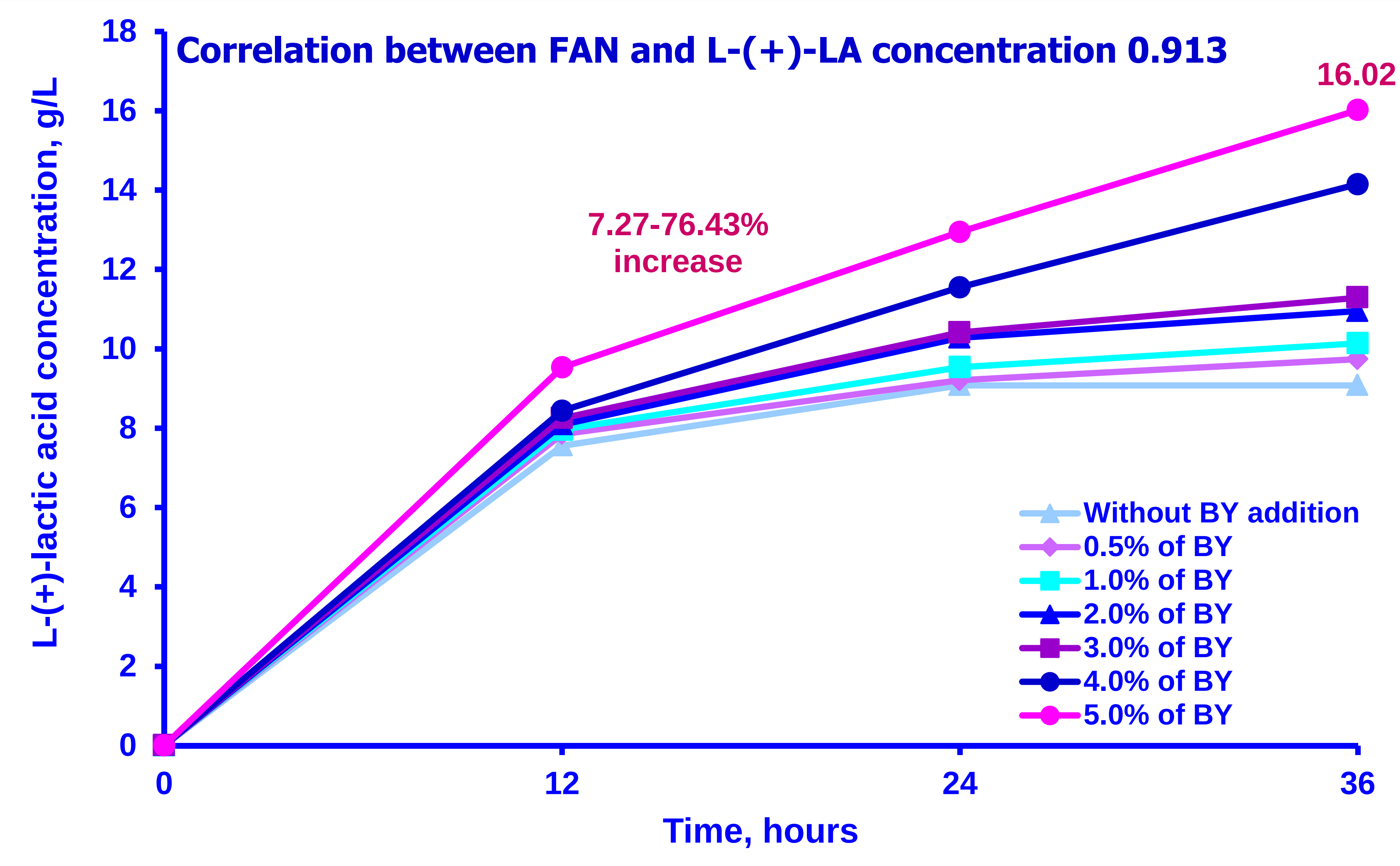
Yeast extract

51€/kg

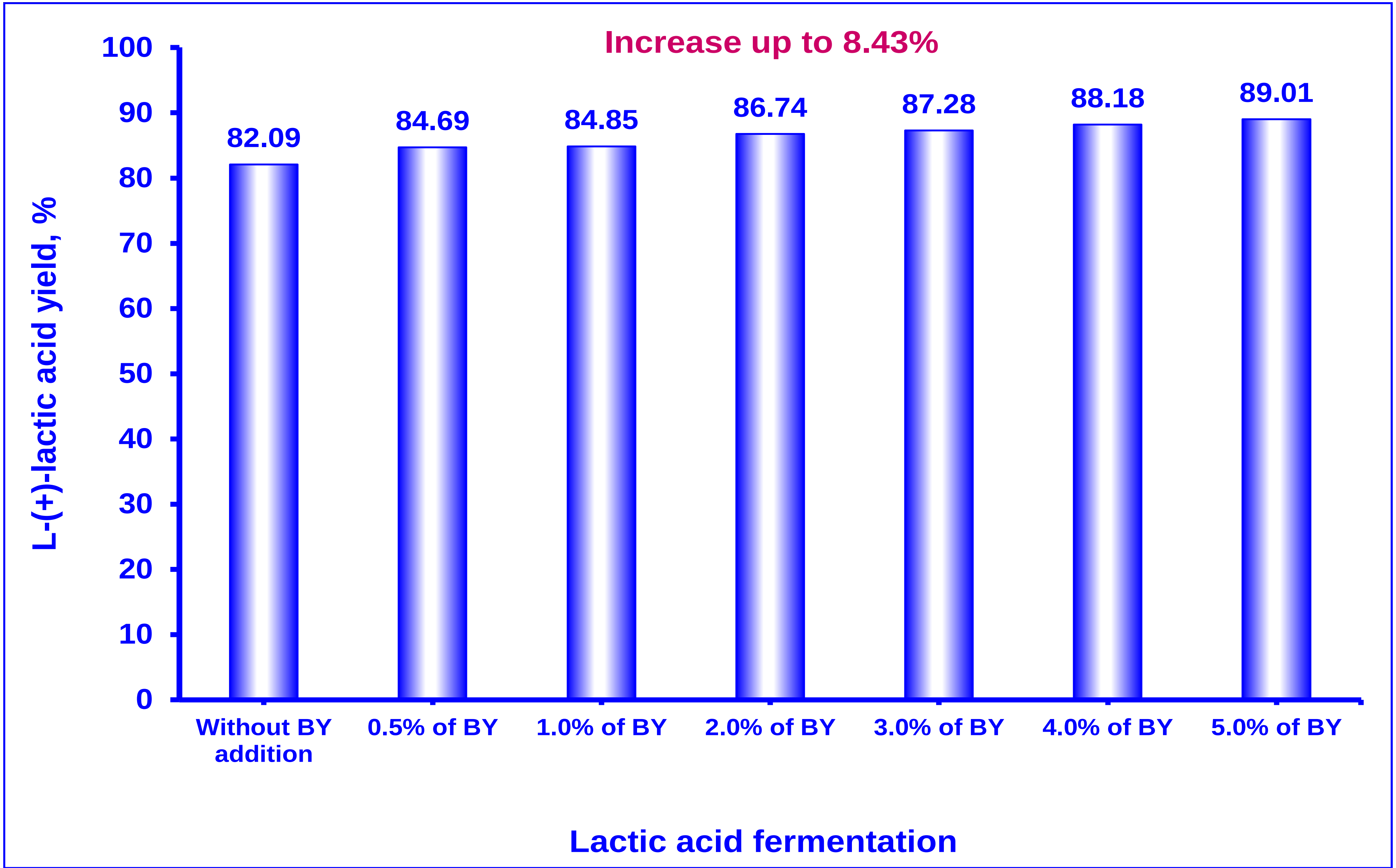
## Free amino nitrogen (FAN) concentration in brewer`s spent grain (BSG) hydrolysate without and with brewer`s yeast (BY) addition

| BY content (%) in BSG hydrolysate | FAN concentration (mg/L) |
|---|---|
| 0 | 54.98 |
| 0.5 | 68.86 |
| 1.0 | 147.54 |
| 2.0 | 249.22 |
| 3.0 | 296.45 |
| 4.0 | 376.91 |
| 5.0 | 393.61 |

**Increase 25-616%**




**L-(+)-lactic acid concentration in batch fermentations without and with brewer`s yeast (BY) addition**




**L-(+)-lactic acid yield in batch fermentations without and with brewer`s yeast (BY) addition**

## L-(+)-lactic acid volumetric productivity in batch fermentations without and with brewer`s yeast (BY) addition and with reducing sugar concentration correction to 5.0%

| Time, hours | Volumetric productivity ($g/L \cdot h^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Without BY addition | 0.5% of BY | 1.0% of BY | 2.0% of BY | 3.0% of BY | 4.0% of BY | 5.0% of BY |
| 12 | 0.60 | 0.67 | 0.71 | 0.79 | 0.83 | 0.87 | 0.89 |
| 24 | 0.57 | 0.66 | 0.69 | 0.77 | 0.79 | 0.81 | 0.83 |
| 36 | 0.56 | 0.65 | 0.67 | 0.71 | 0.73 | 0.74 | 0.75 |

# Soybean meal and malt rootlets

- ✓ Soybeans are by far the most popular oilseed crop produced in the world today.
- ✓ Soybean meal consists of protein, carbohydrates (sucrose, oligosaccharides, and fibrous components), minerals, and vitamins.

| Parameter | % |
|---|---|
| Moisture | 11 |
| Crude protein (N × 6.25) | 50.0 |
| Fat (oil) | 1.0 |
| Minerals | 7.0 |
| Crude fiber | 6.0 |

- ✓ **Dried rootlets are separated from malt after kilning and used for feeding cattle.**
- ✓ **Rootlets are rich in B vitamins, peptides, amino acids, and protein.**

| Parameter | % |
|---|---|
| Moisture | <7.0 |
| Crude protein (N × 6.25) | 25-34 |
| Fat (oil) | 1.6-2.2 |
| Minerals | 6-7 |
| Nitrogen – free extractives | 35-44 (or even 50) |
| Pentosan | 15.6-18.9 |
| Cellulose | 6-10 |

**During the fermentation, determination of:**

- Lactic acid concentration (L-(+)-lactic acid assay, Megazyme®, Wicklow, Ireland)
- Reducing sugar concentration
- Cell viability (pour plate technique, MRS agar, 37°C)
- pH value

**Brewer`s spent grain hydrolysis** → **Centrifugation** → **Sterile liquid hydrolysate – fermentation media**

**pH control**

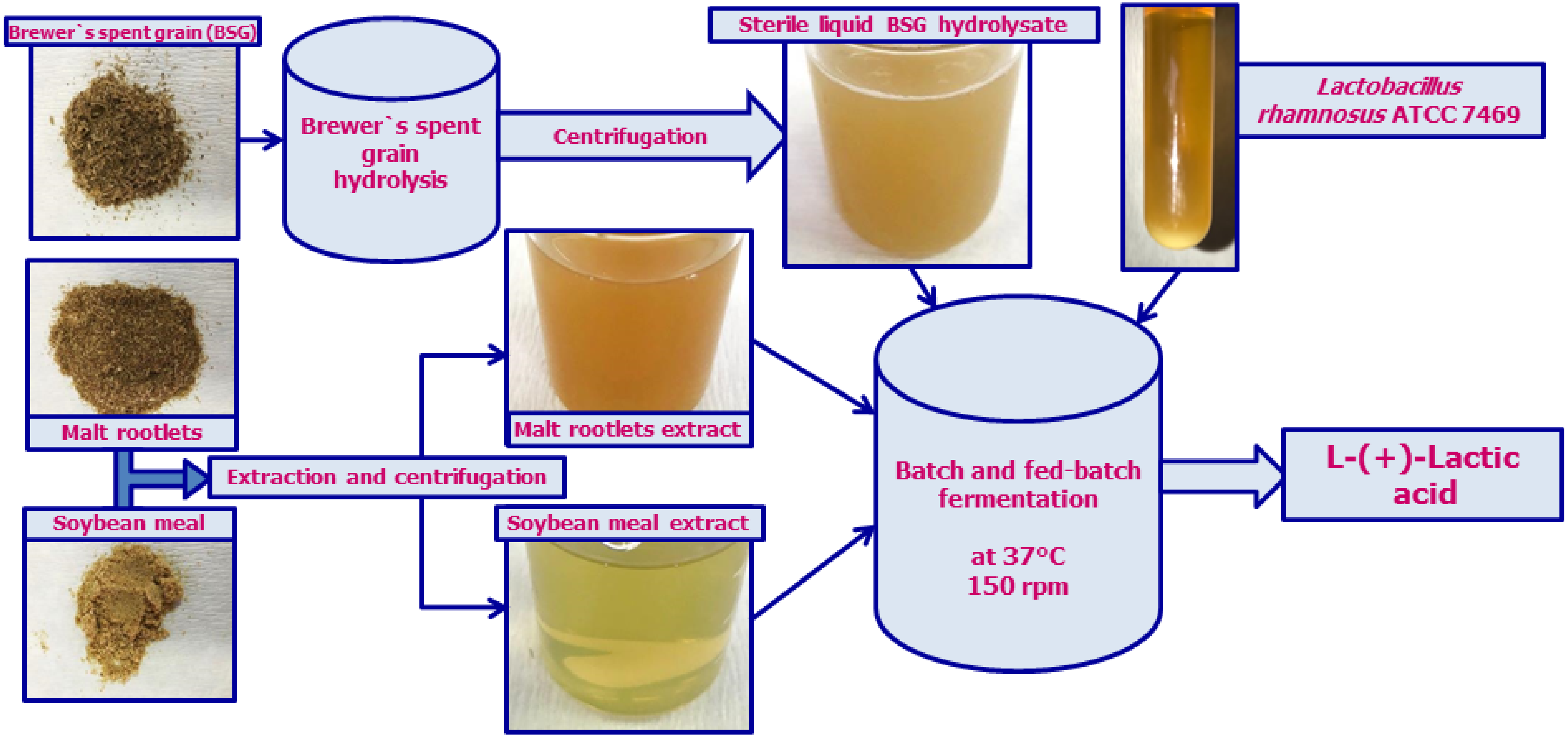
**Soybean meal extract (10-50%)**

**Malt rootlets extract (10-50%)**

→ **Batch and fed-batch fermentation**

***L. rhamnosus* ATCC 7469 at 37°C 150 rpm**

→ **L-(+)-Lactic acid**


Brewer`s spent grain (BSG)
Brewer`s spent grain hydrolysis
Centrifugation
Sterile liquid BSG hydrolysate
*Lactobacillus rhamnosus* ATCC 7469
Malt rootlets
Malt rootlets extract
Extraction and centrifugation
Soybean meal
Soybean meal extract
Batch and fed-batch fermentation
at 37°C
150 rpm
L-(+)-Lactic acid

## Free amino nitrogen (FAN) concentration in brewer`s spent grain (BSG) hydrolysate without and with malt rootlets extract addition

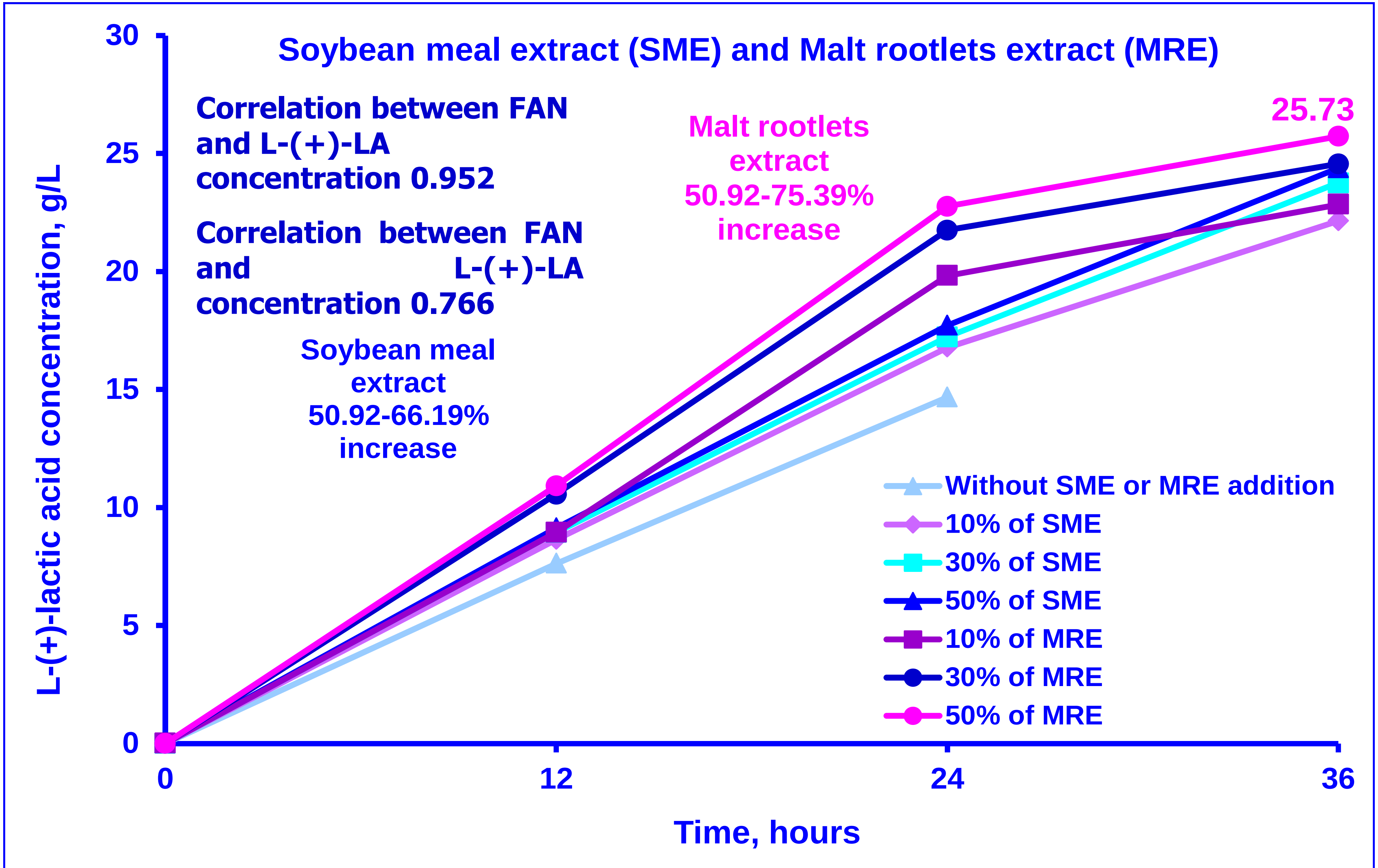
| | Brewer's spent grain hydrolysate | Malt rootlet extract | 10% malt rootlets extract | 30% malt rootlets extract | 50% malt rootlets extract |
|---|---|---|---|---|---|
| Free amino nitrogen concentration (mg/l) | 68.71 | 585.44 | 147.75 | 254.44 | 320.55 |

Increase 115-366%

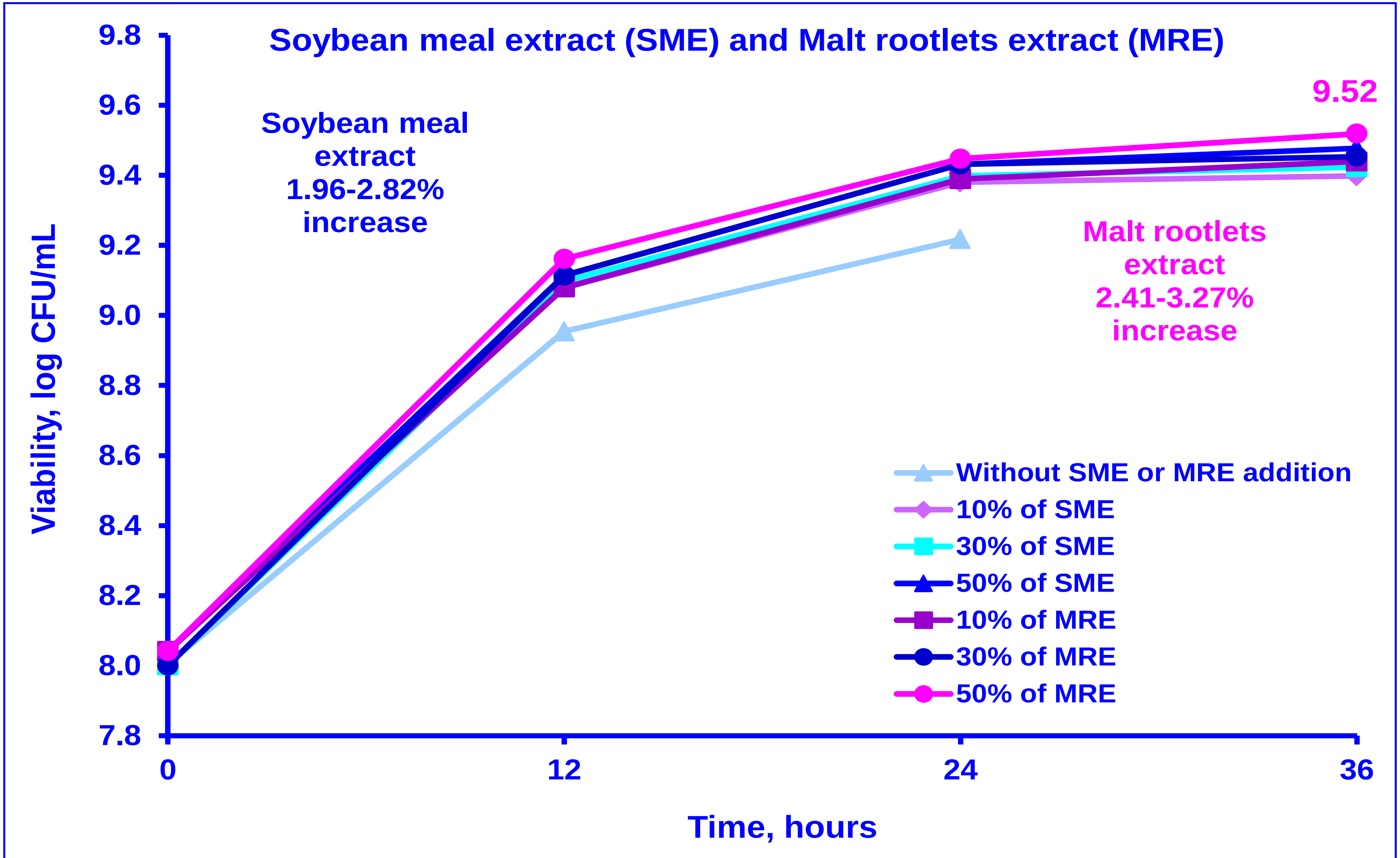
## Free amino nitrogen (FAN) concentration in brewer`s spent grain (BSG) hydrolysate without and with soybean meal extract addition

| | Brewer's spent grain hydrolysate | Soybean meal extract | 10% soybean meal extract | 30% soybean meal extract | 50% soybean meal extract |
|---|---|---|---|---|---|
| Free amino nitrogen concentration (mg/l) | 68.71 | 118.53 | 70.36 | 73.21 | 77.50 |

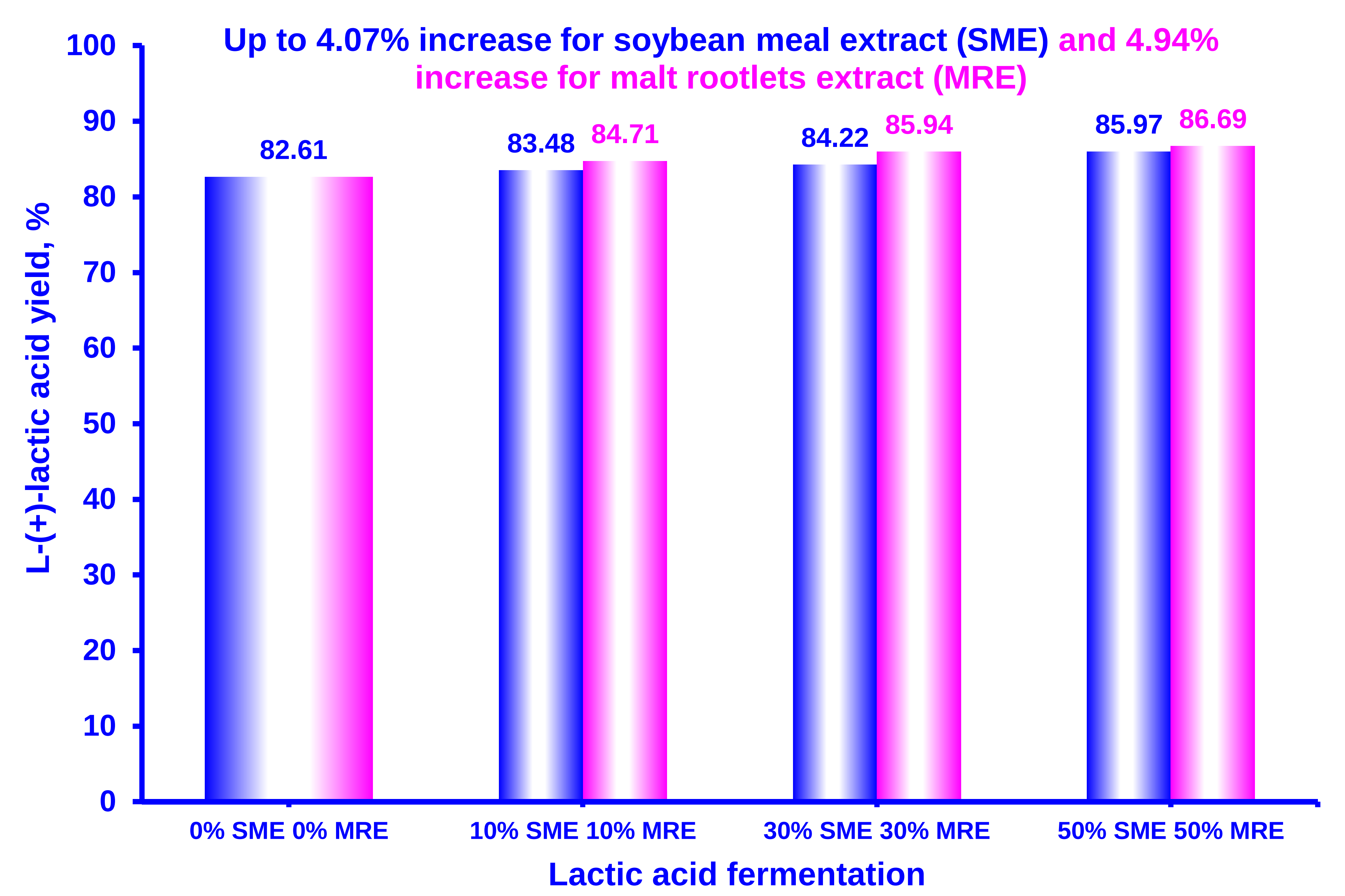
Increase 2-14%




**L-(+)-lactic acid concentration in batch fermentations with soybean meal extract (SME) or malt rootlets extract (MRE) addition**



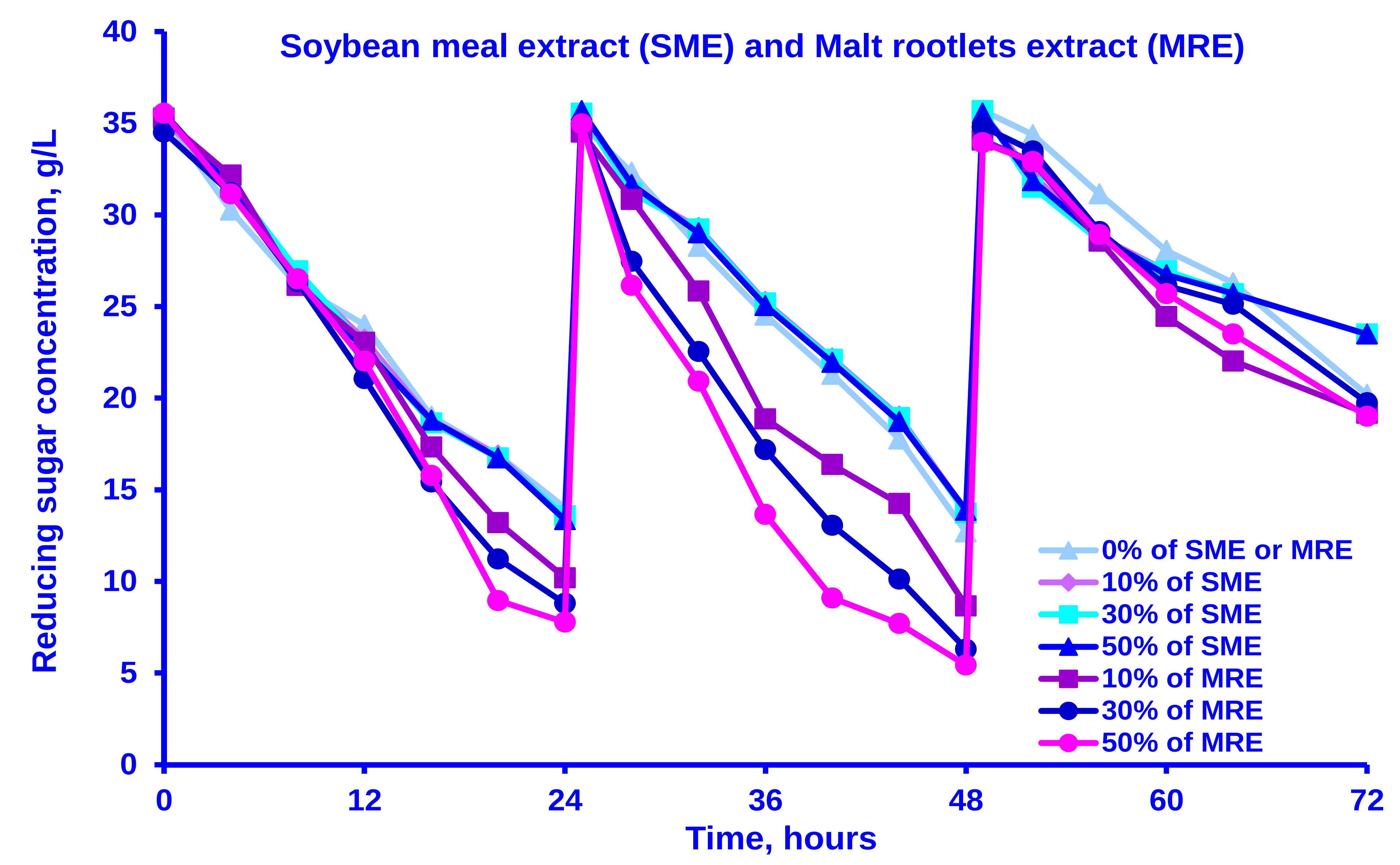

***L. rhamnosus*** **ATCC 7469 cell viability in batch fermentations with soybean meal extract (SME) or malt rootlets extract (MRE) addition**



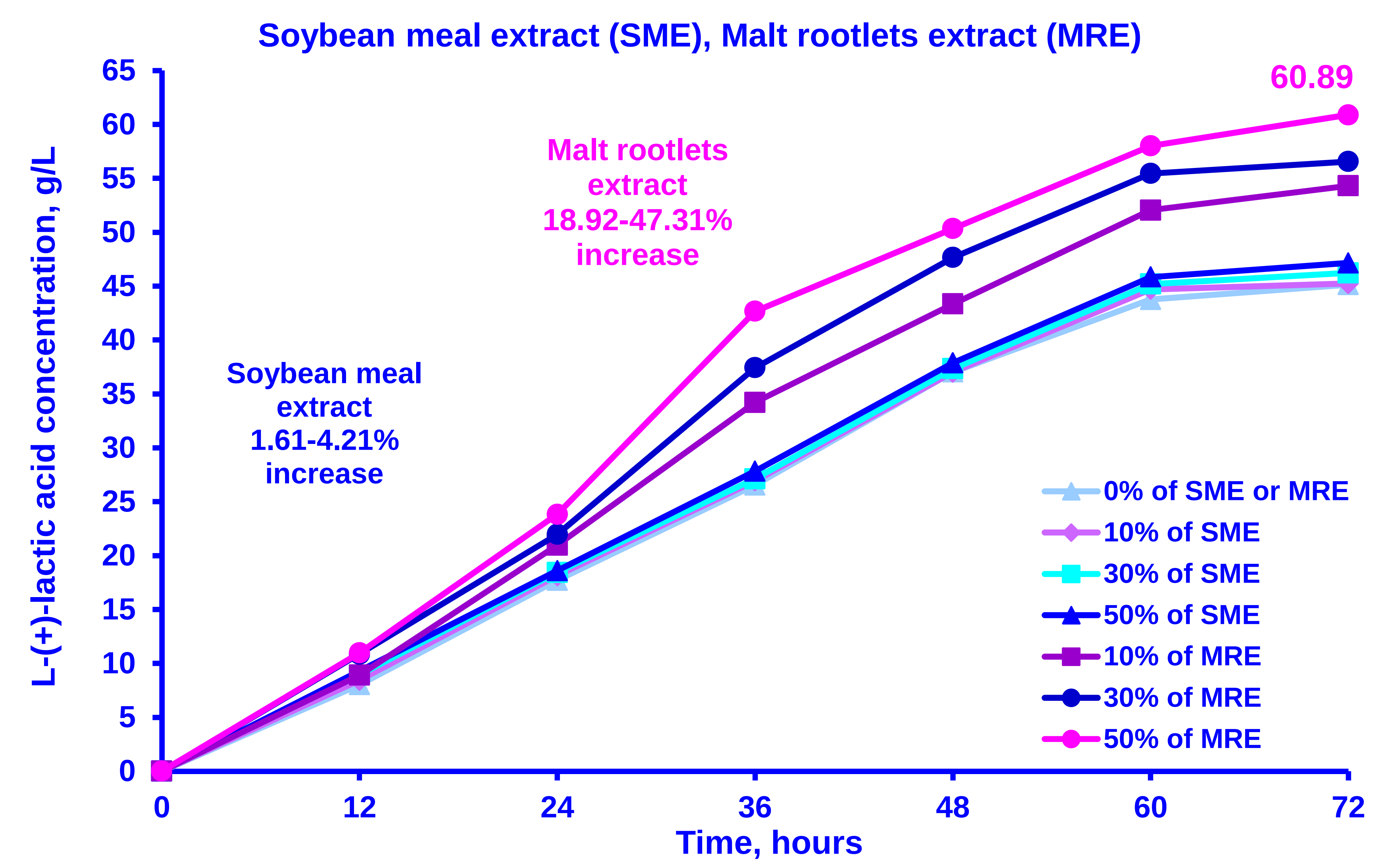

**L-(+)-lactic acid yield in batch fermentations with soybean meal (SME) or malt rootlets extract (MRE) addition**

# L-(+)-lactic acid volumetric productivity in batch fermentations with malt rootlet or soybean meal extract addition

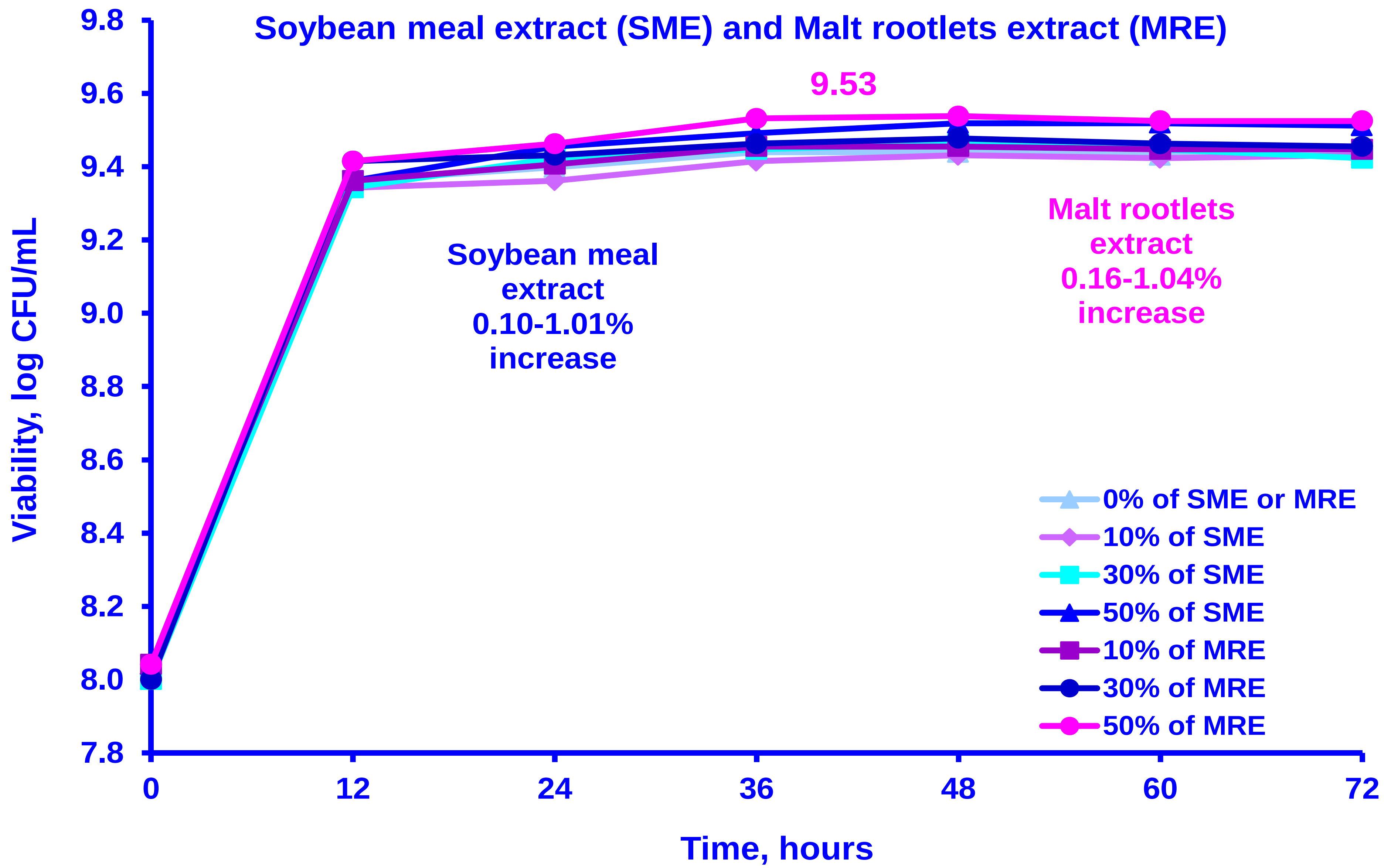
| Time, hours | Volumetric productivity ($g/L \cdot h^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Without addition | 10% soybean meal extract | 30% soybean meal extract | 50% soybean meal extract | 10% malt rootlets extract | 30% malt rootlets extract | 50% malt rootlets extract |
| 12 | 0.64 | 0.72 | 0.75 | 0.76 | 0.75 | 0.88 | 0.91 |
| 24 | 0.61 | 0.70 | 0.72 | 0.74 | 0.83 | 0.91 | 0.95 |
| 36 | - | 0.62 | 0.66 | 0.68 | 0.63 | 0.68 | 0.71 |



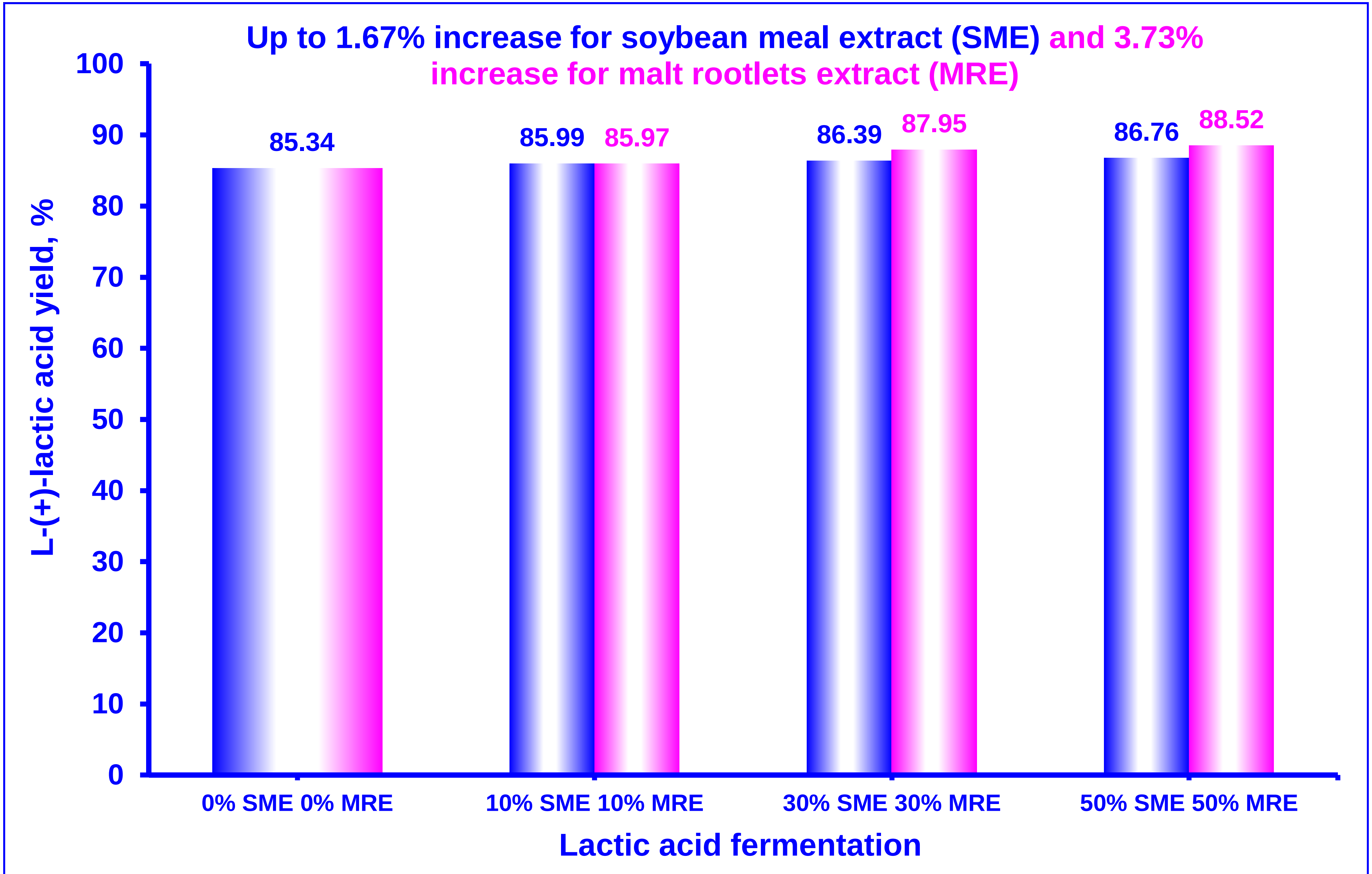

**Reducing sugar concentration in fed-batch fermentations with soybean meal extract (SME) or malt rootlets extract (MRE) addition**




**L-(+)-lactic acid concentration in fed-batch fermentations with soybean meal extract (SME) or malt rootlets extract (MRE) addition**




***L. rhamnosus* ATCC 7469 cell viability in fed-batch fermentations with soybean meal extract (SME) or malt rootlets extract (MRE) addition**




**L-(+)-lactic acid yield in fed-batch fermentations with soybean meal (SME) or malt rootlet extract (MRE) addition**

## L-(+)-lactic acid volumetric productivity in fed-batch fermentations with malt rootlets or soybean meal extract addition

| Time, hours | Volumetric productivity ($g/L \cdot h^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Without addition | 10% soybean meal extract | 30% soybean meal extract | 50% soybean meal extract | 10% malt rootlet extract | 30% malt rootlet extract | 50% malt rootlet extract |
| 12 | 0.66 | 0.70 | 0.74 | 0.77 | 0.74 | 0.91 | 0.91 |
| 24 | 0.73 | 0.75 | 0.75 | 0.78 | 0.87 | 0.92 | 0.99 |
| 36 | 0.73 | 0.75 | 0.77 | 0.78 | 0.95 | 1.04 | 1.19 |
| 48 | 0.76 | 0.77 | 0.78 | 0.79 | 0.90 | 0.99 | 1.05 |
| 60 | 0.73 | 0.74 | 0.75 | 0.76 | 0.87 | 0.92 | 0.97 |
| 72 | 0.62 | 0.63 | 0.64 | 0.65 | 0.75 | 0.79 | 0.85 |

**Colleagues:**

**Ljiljana Mojović**
**Sunčica Kocić-Tanackov**
**Miloš Radosavljević**
**Milana Pribić**
**Aleksandra Djukić-Vuković**
**Dragana Mladenović**

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**Carlsberg Serbia**

**Novozymes, Denmark**


THANK YOU
KINDLY!