



Napredni načini izrabe potenciala sirotke: prehod iz okoljskega problema v dragocen naravni vir



# Izkoriščanje kisle sirotke za gojenje mlečnokislinskih bakterij in pridobivanje koristnih metabolitov

Diana Paveljšek<sup>1</sup>, Jernej Oberčkal<sup>1</sup>, Timeja Planinšek Parfant<sup>2</sup>, Nika Osel<sup>2</sup>, Robert Roškar<sup>2</sup>, Bojana Bogovič Matijašić<sup>1</sup>

<sup>1</sup>Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za zootehniko, Inštitut za mlekarstvo in probiotike

<sup>2</sup>Univerza v Ljubljani, Fakulteta za farmacijo, Katedra za biofarmacijo in farmakokinetiko



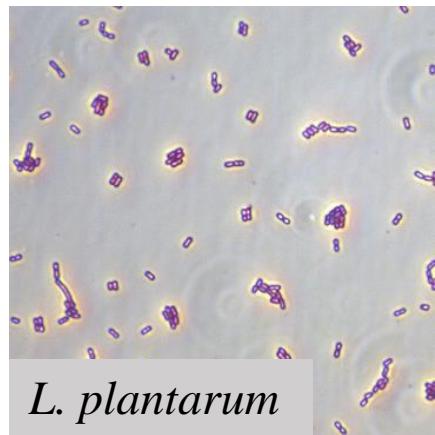
# Mlečnokislinske bakterije

## MKB in probiotiki

- 6 različnih sevov *Lactiplantibacillus (L.) plantarum* (IM1163, IM1164, IM943, IM930, IM527)
- *Lacticaseibacillus (L.) rhamnosus* GG

## Sev, ki proizvaja vitamin B12

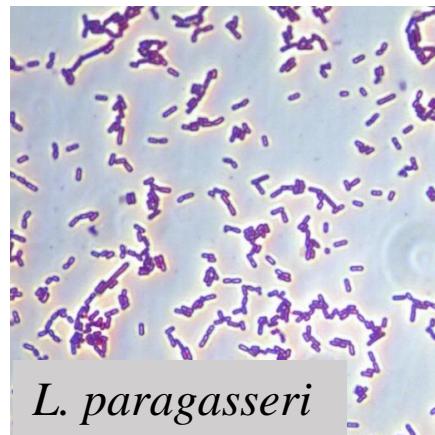
- *Propionibacterium (P.) freudenreichii* subsp. *freudenreichii* van Niel 1928



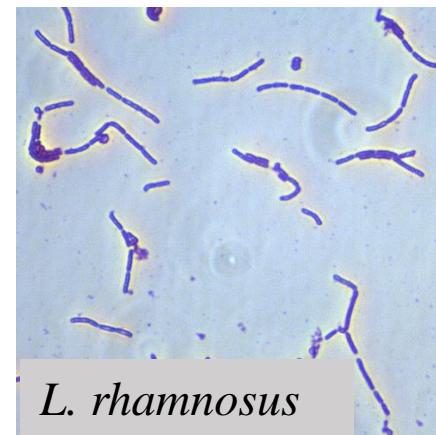
*L. plantarum*

## Sevi, ki proizvajajo bakteriocine

- *Lactobacillus (L.) paragasseri* K7 (**gasericini**)
- sevi vrste *Lactococcus lactis* IM407, IM143, IM145 (**nizin**)
- *L. johnsonii* IM123 (**laktacin F**)
- *Pediococcus (Pd.) acidilactici* IM1 (**pediocin AcH**)
- *Pd. acidilactici* IM2 (**pediocin PA-1**)
- *Enterococcus faecium* IM273 (**enterocini A, B, P**)

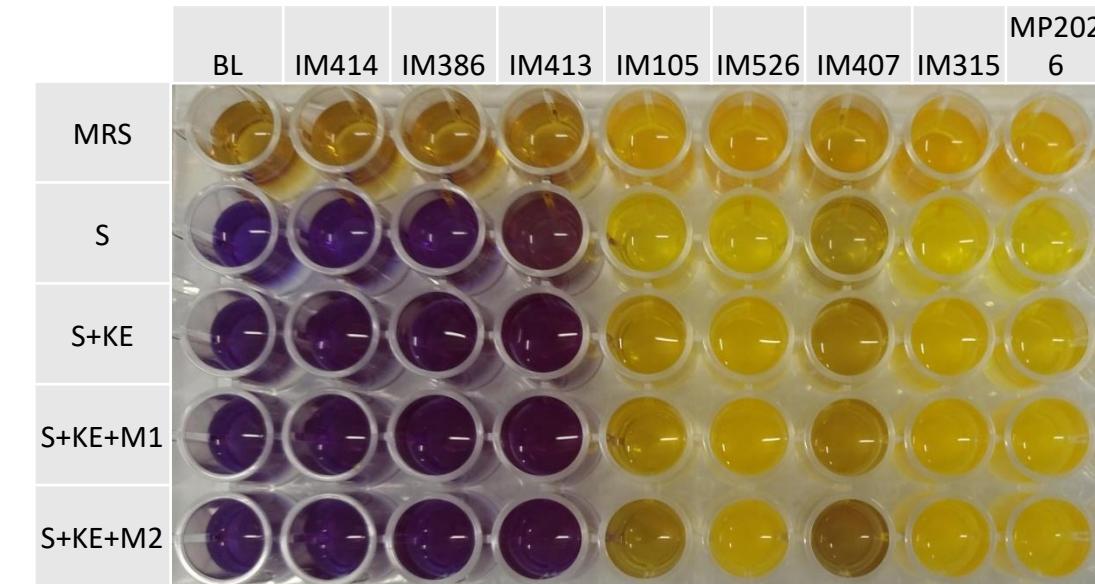


*L. paragasseri*



*L. rhamnosus*

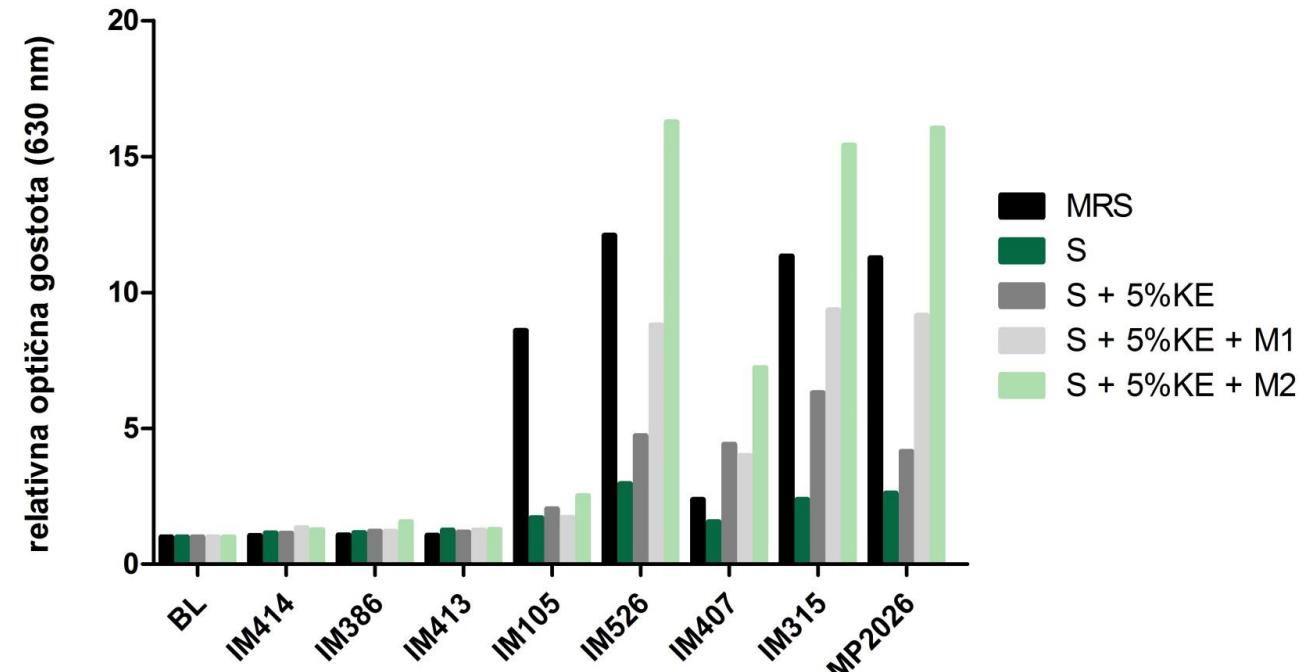
# Rast izbranih sefov v sirotki z različnimi dodatki



BROMKREZOL VIJOLIČNO



BROMKREZOL ZELENO



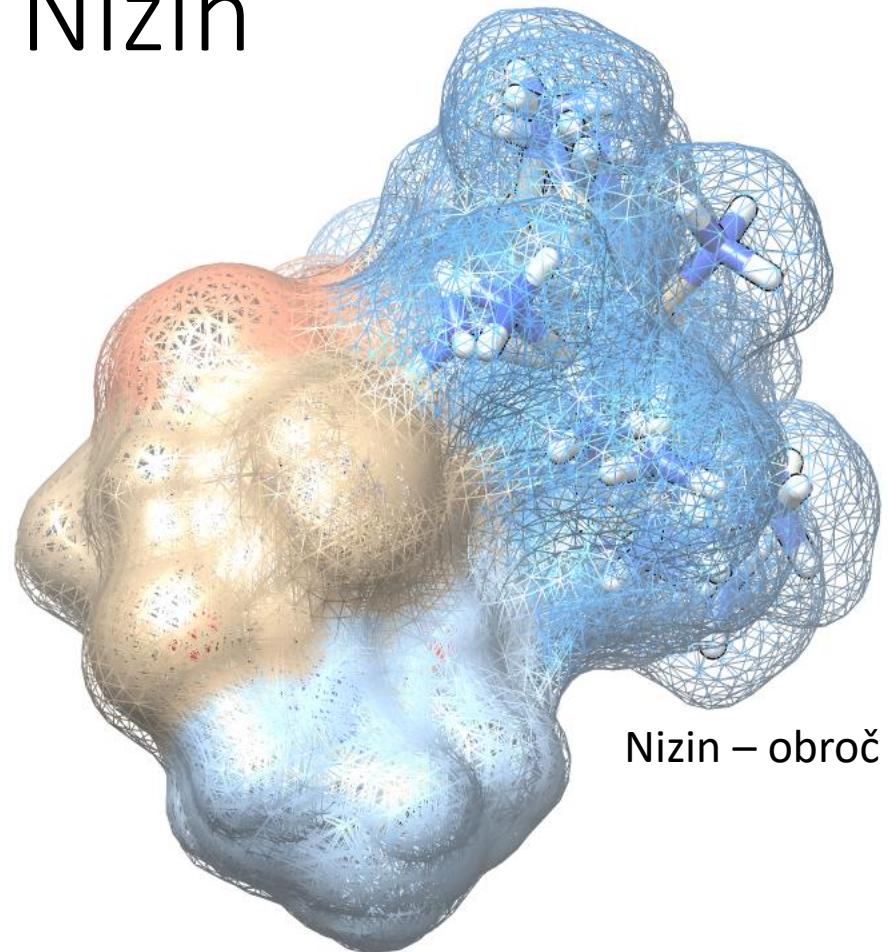
BL – kontrola

S – kisla sirotka

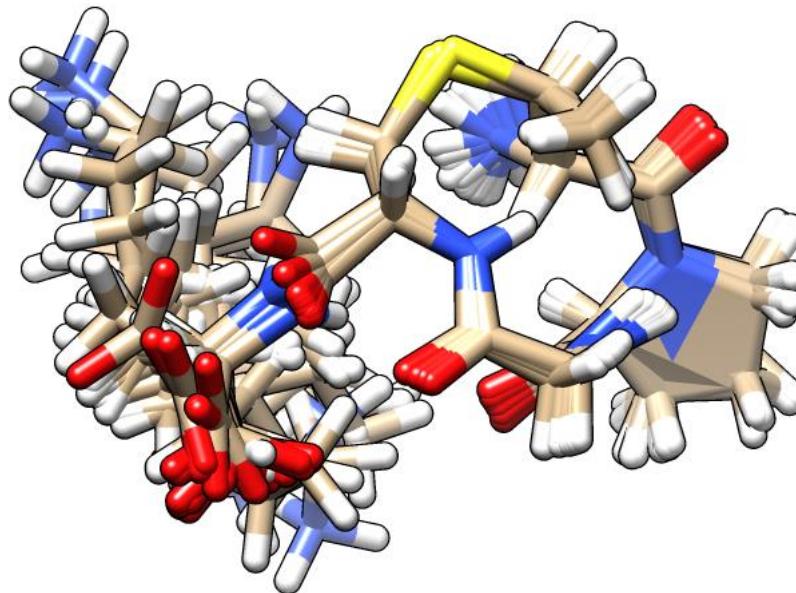
KE – kvasni ekstrakt

M1 in M2 – mineralne soli

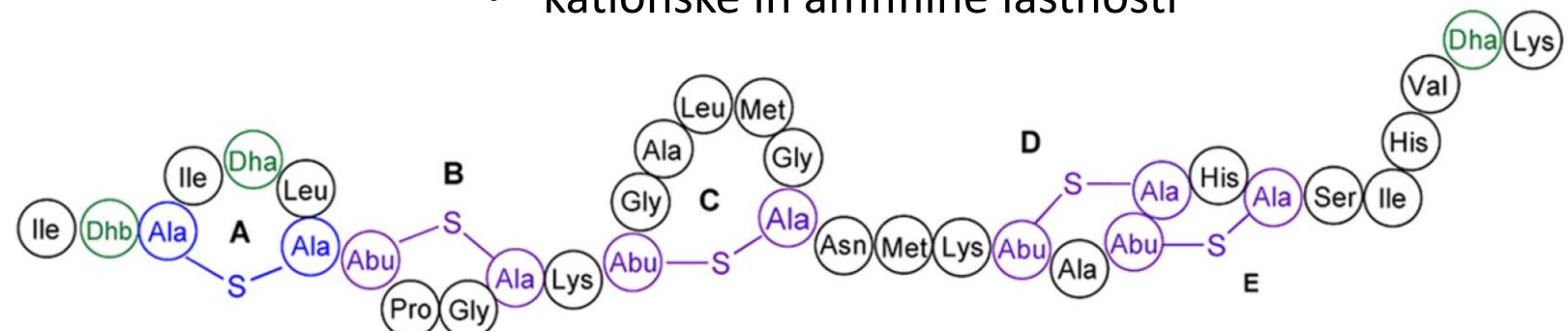
# Nizin



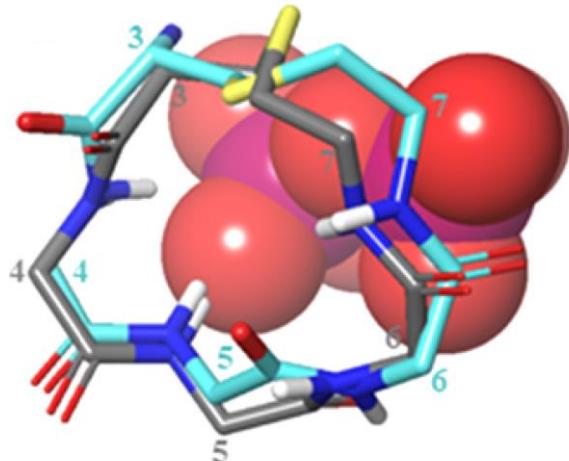
modra – zelo hidrofilno  
oranžna – zelo hidrofobno



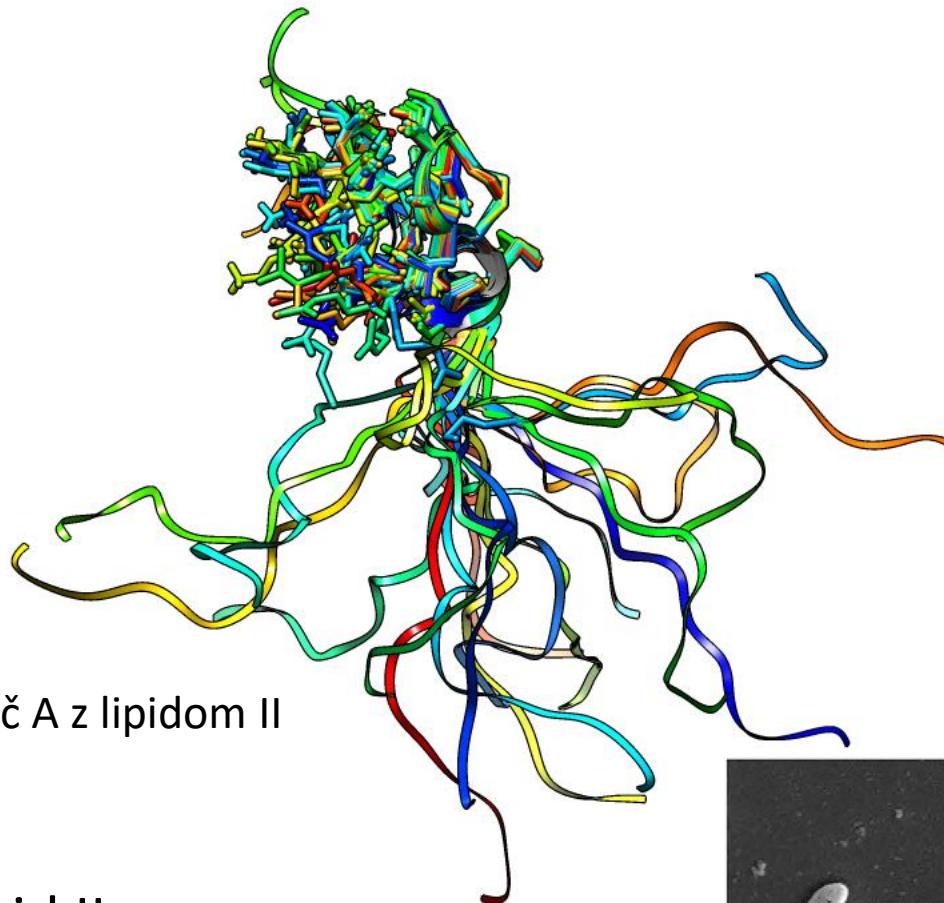
- več variant:
  - Nizin A, Z, F, Q → laktokoki
  - Nizin H, U, U2, P → streptokoki
- 3,4 kDa
- 34 aminokislinskih ostankov
- 5 obročev z disulfidnimi mostički
- spada k skupini lantibiotikov (razred I)
- posttranslacijske modifikacije: dehidroalanin, lantionin in  $\beta$ -metillantionin
- kationske in amfifilne lastnosti



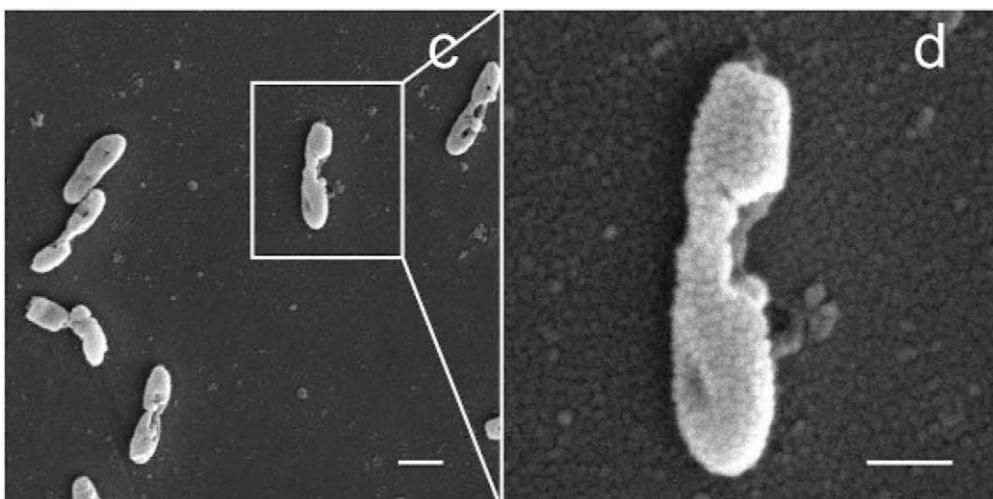
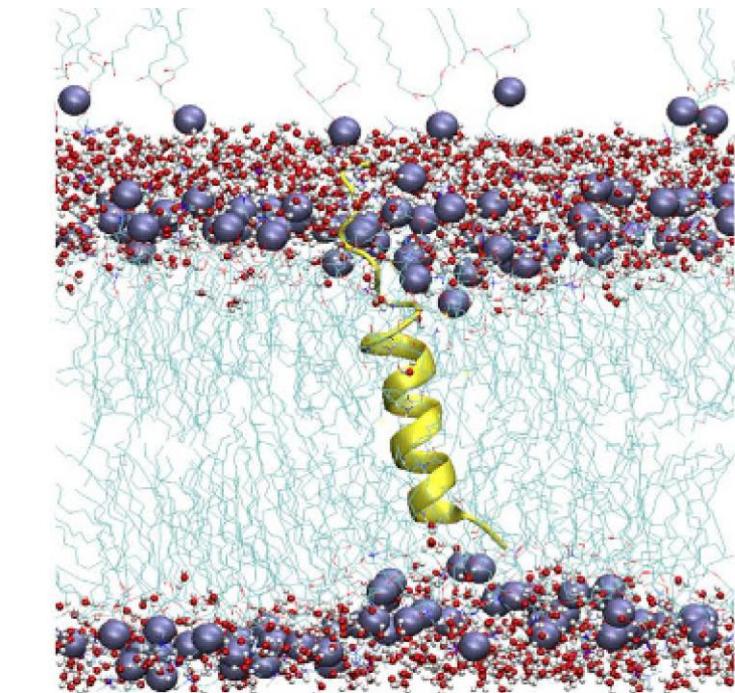
# Nizin



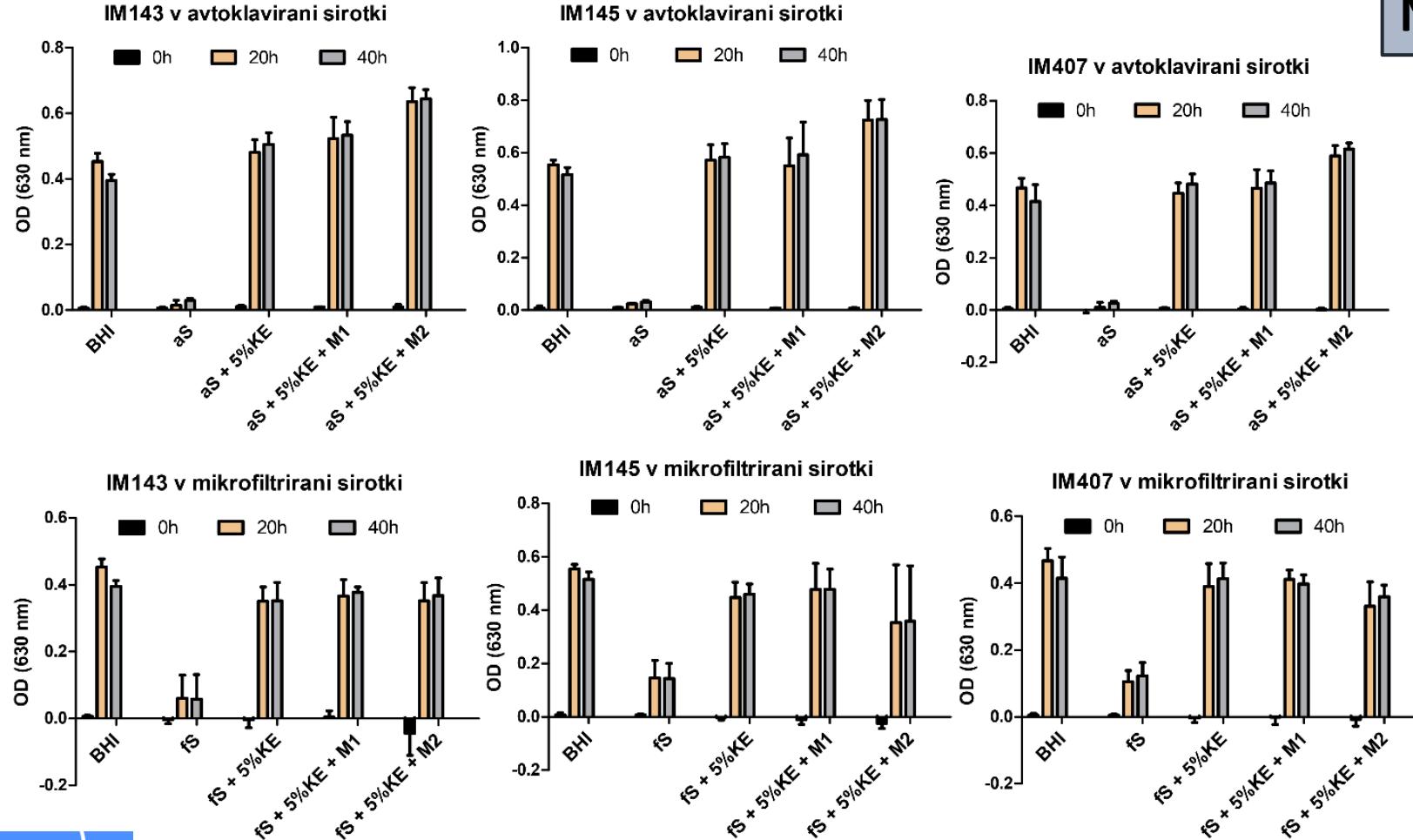
povezava nizin – obroč A z lipidom II



- selektivna vezava na lipid II
- dva mehanizma protimikrobnega delovanja:
  - motnje v sintezi celične stene
  - pore v membrani

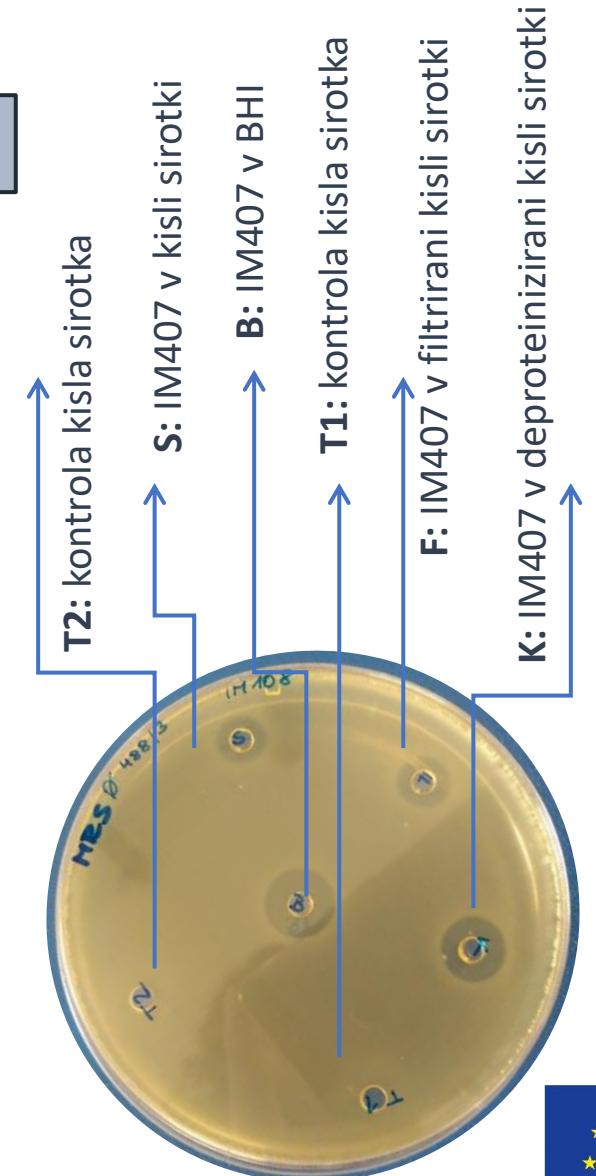


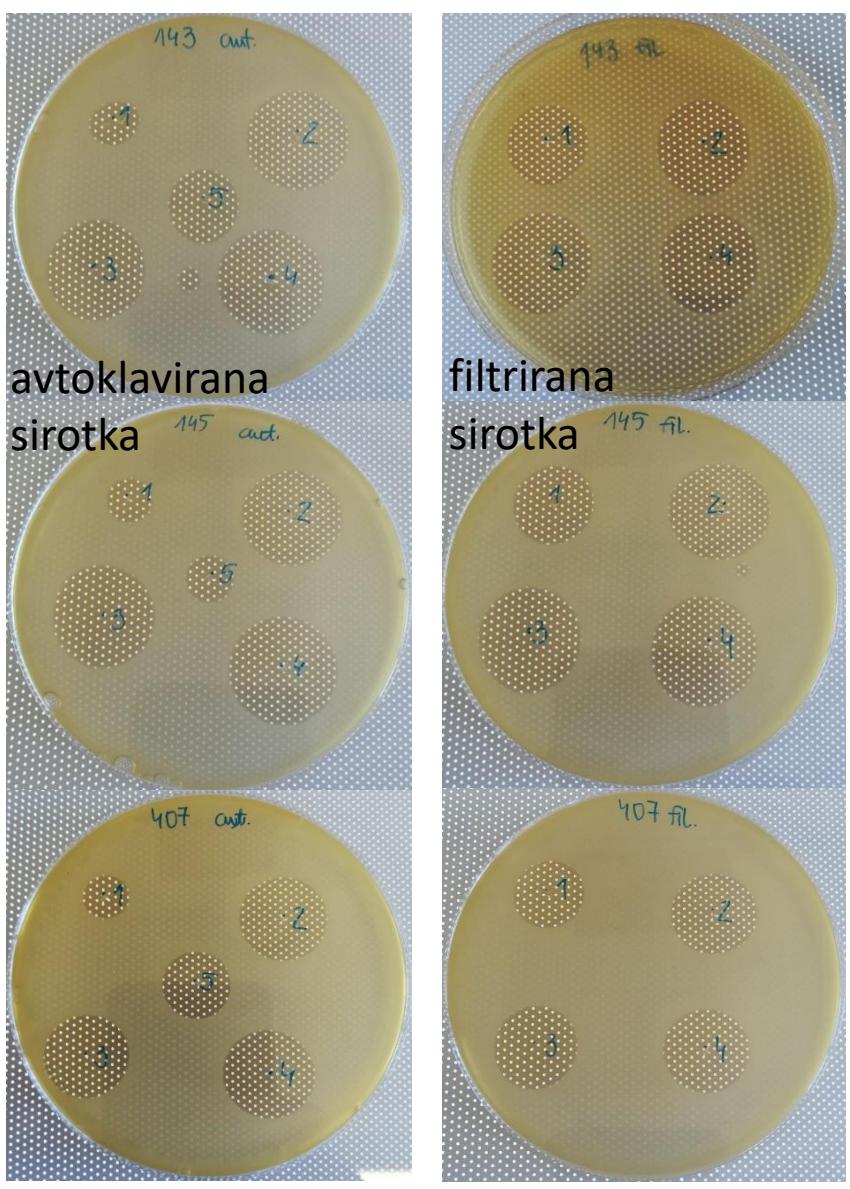
# Izraba kisle sirotke kot gojišča za laktokoke pri produkciji nizina – manjše merilo



*Lactococcus lactis* IM143, IM145, IM407

Nizin Z



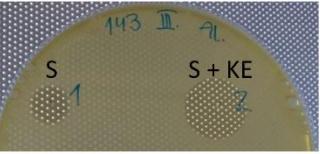


1 – sirotka; 2 – sirotka + 5 % KE; 3 – 5% KE + M1;  
4 – sirotka + 5% KE + M2; 5 – BHI

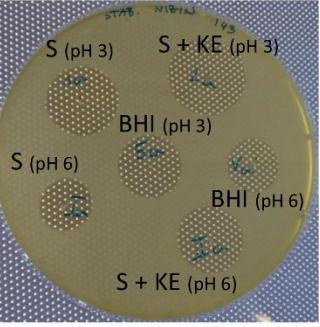
### STABILNOST

- sobna T
- 4 °C
- -20 °C

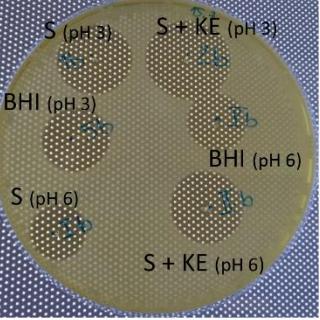
PRED  
LIOFILIZACIJO



PO  
LIOFILIZACIJI



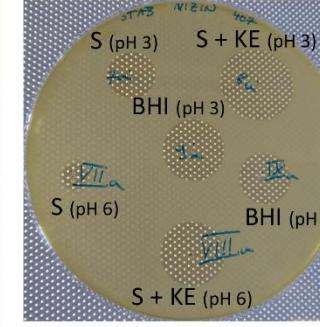
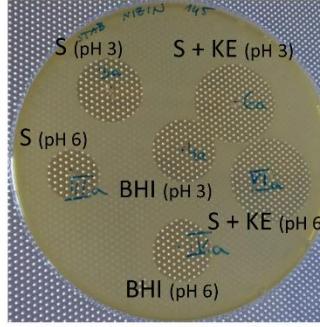
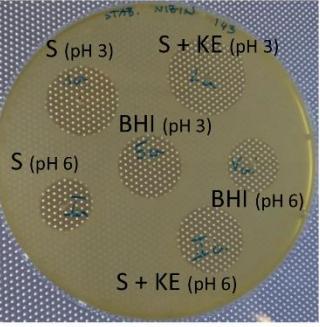
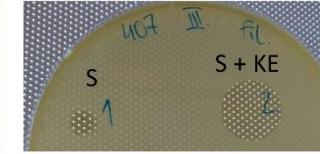
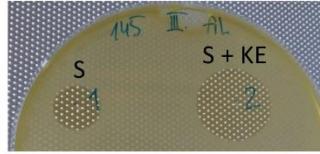
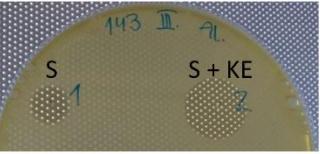
1 MESEC PO  
LIOFILIZACIJI  
sobna T



*L. lactis* IM143

*L. lactis* IM145

*L. lactis* IM407



ponovili tudi  
pol leta po  
liofilizaciji

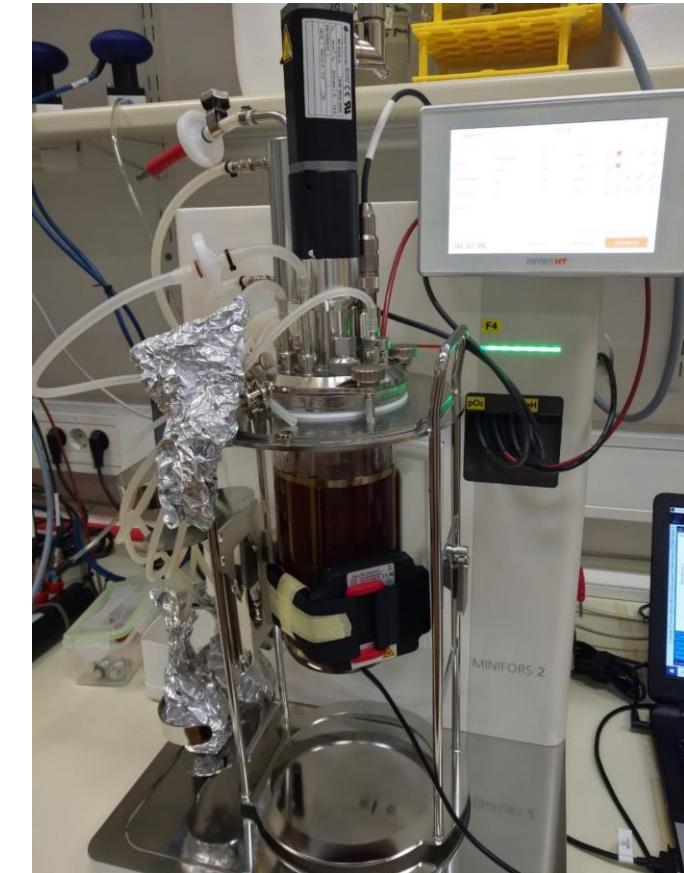
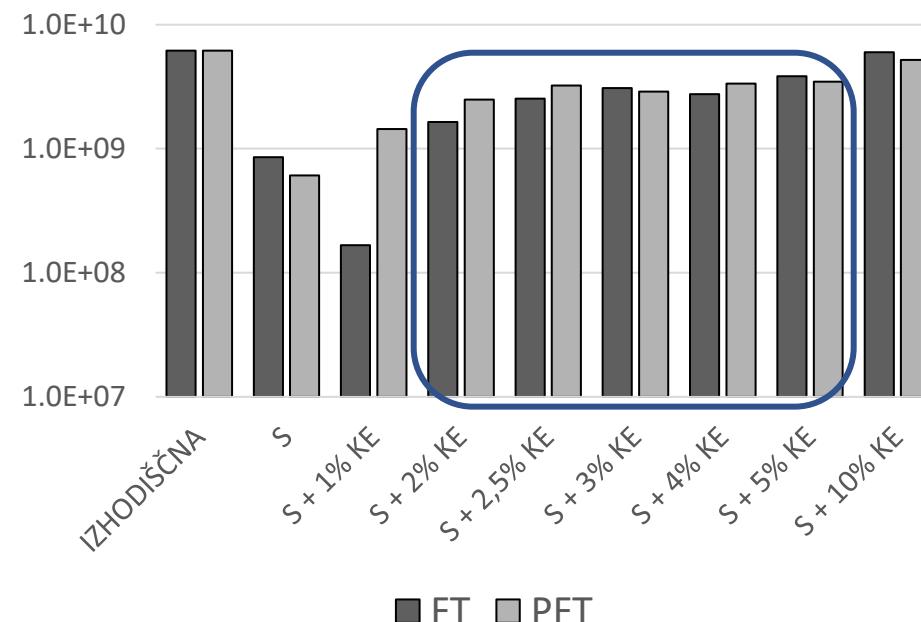
Protimikrobná aktivnosť sa najlepšie uchraňuje pri **pH 3** v syročine s **kvasným extraktom**. Med teplotami uchranenia nij sú veľké rozdiely, teda je aj na **sobnej teplote** nizin zelo odolný.

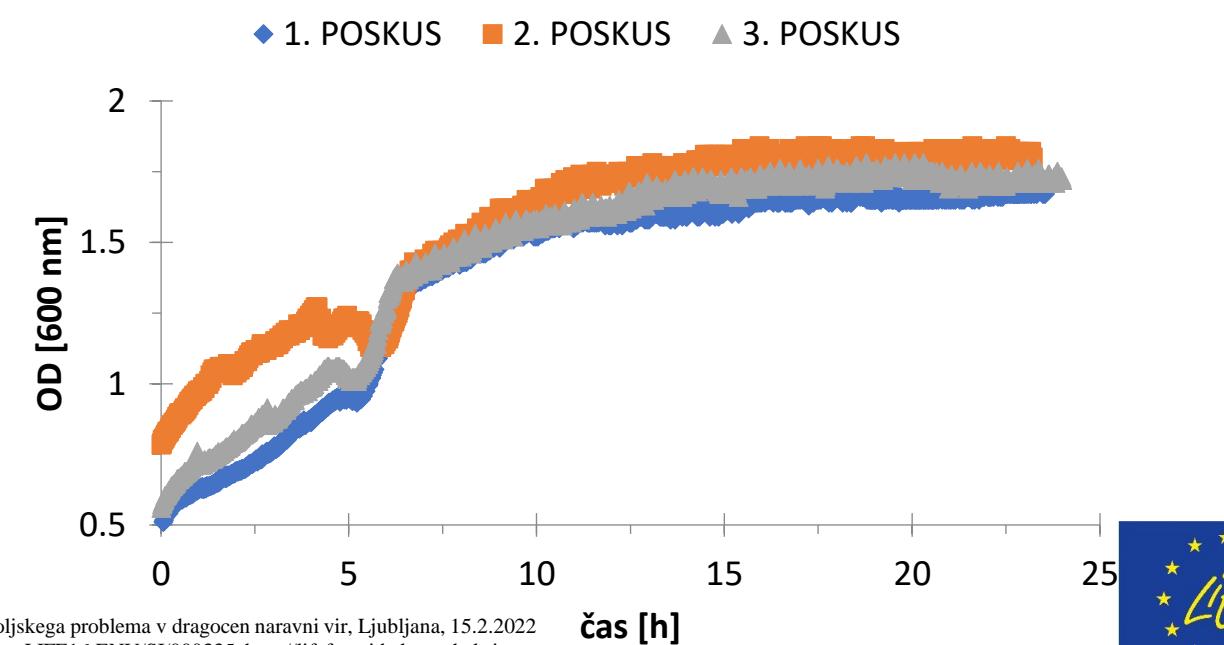
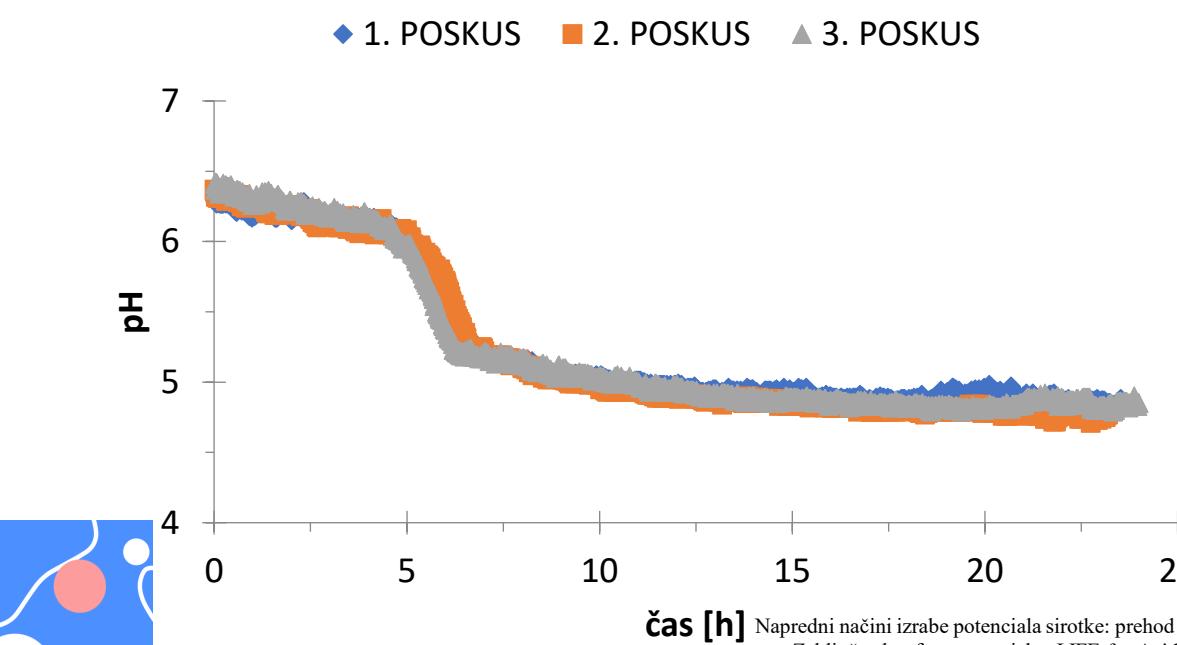
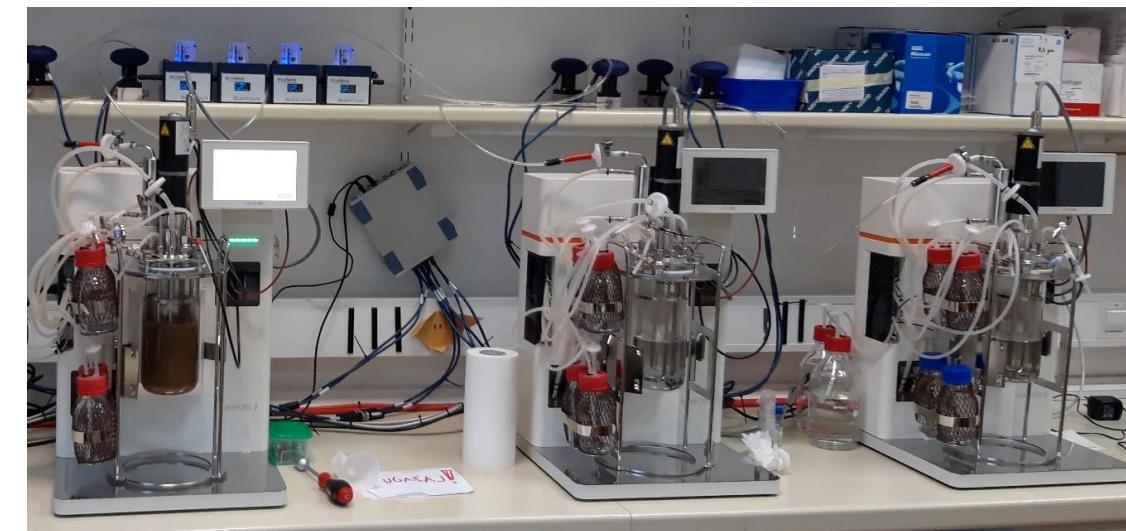
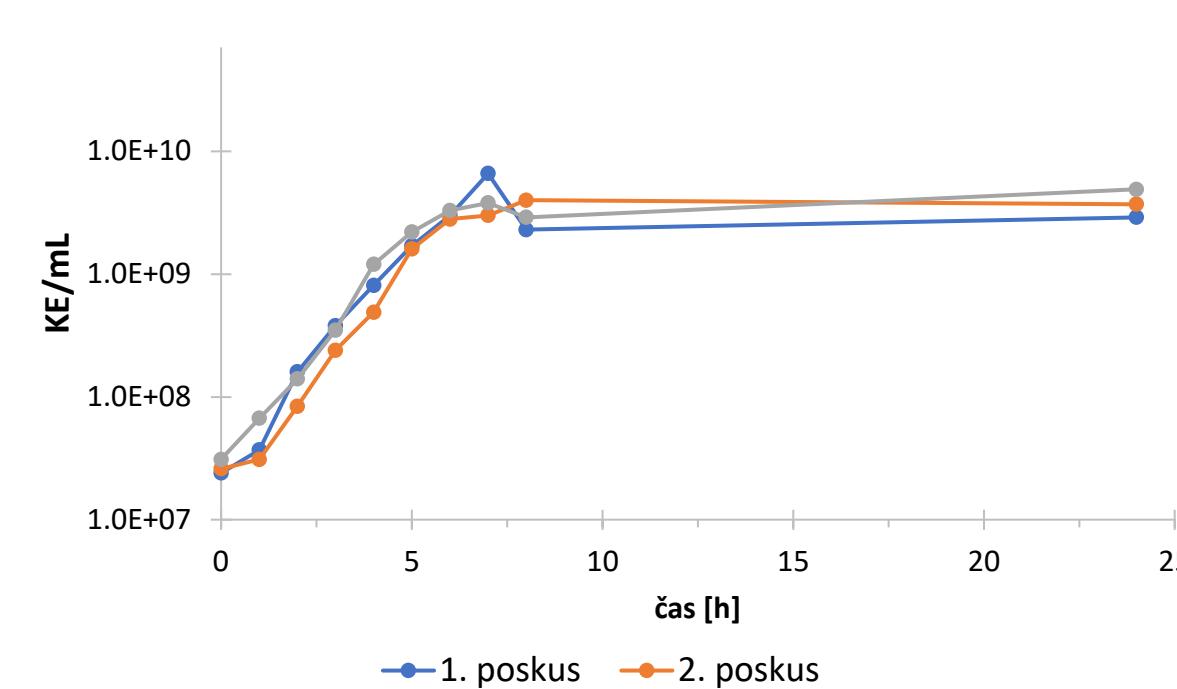
# Izraba kisle sirotke kot gojišča za laktokoke pri produkciji nizina – večje merilo



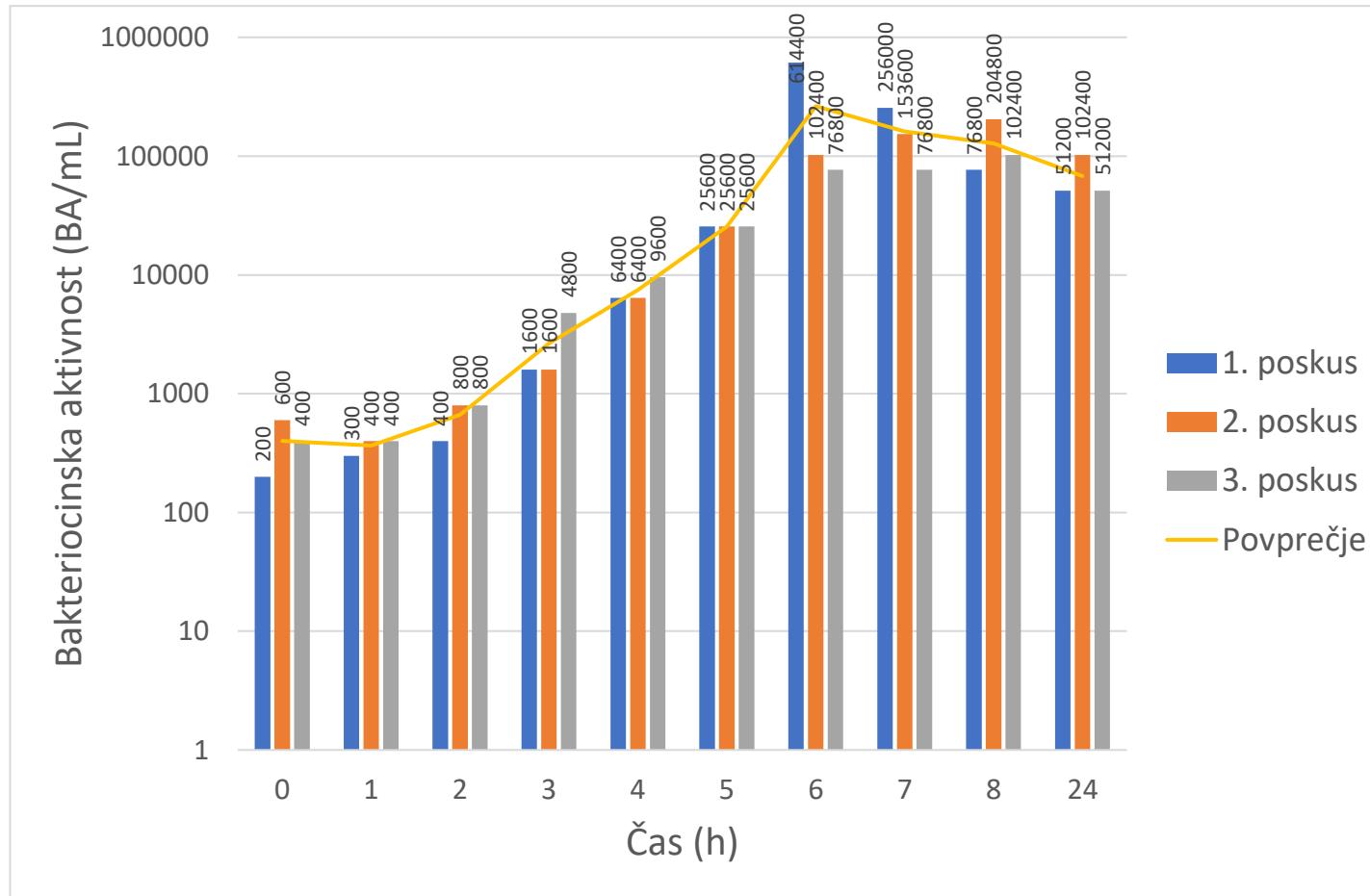
**IM145**

Primerjava FT in PFT





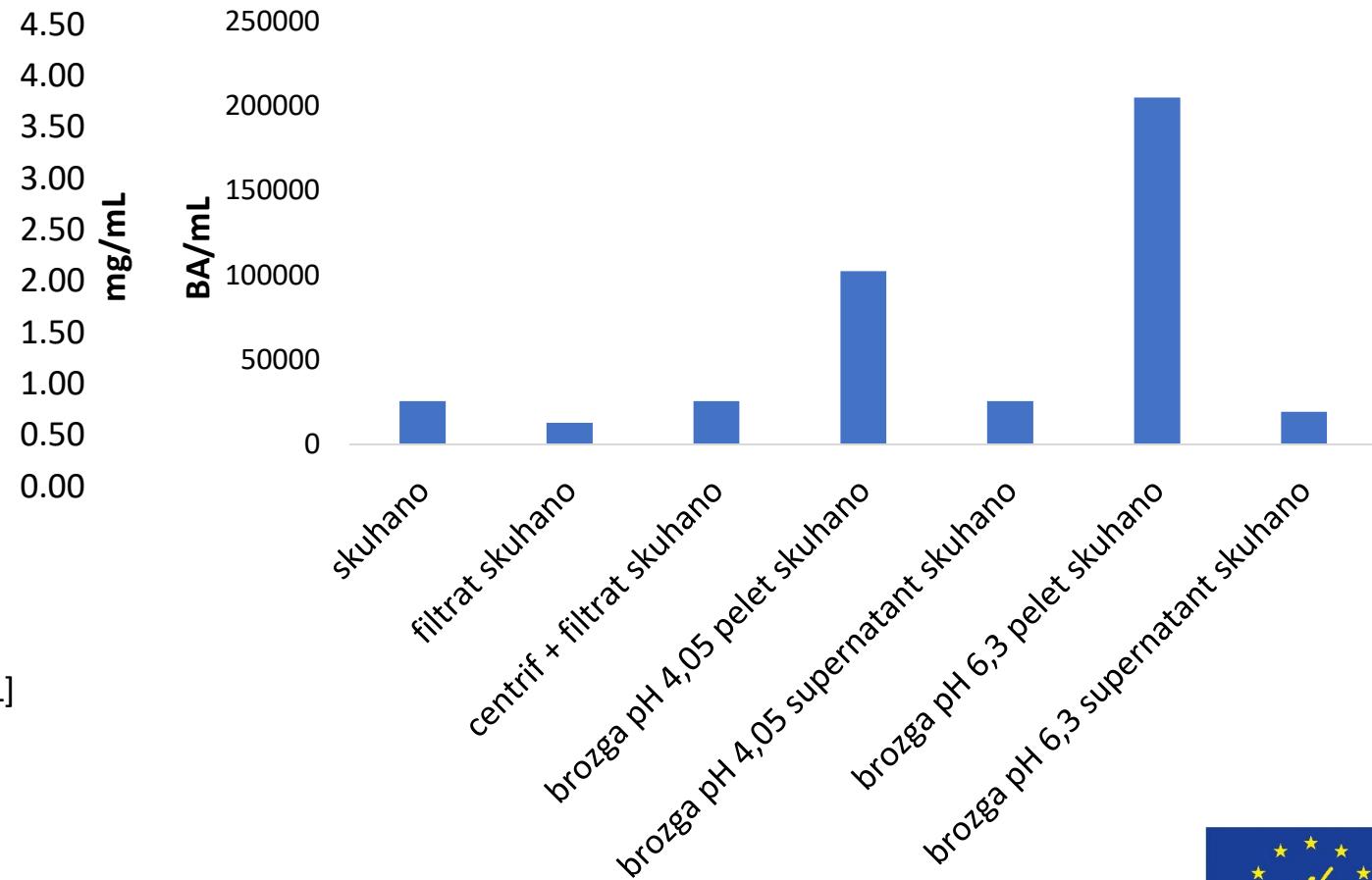
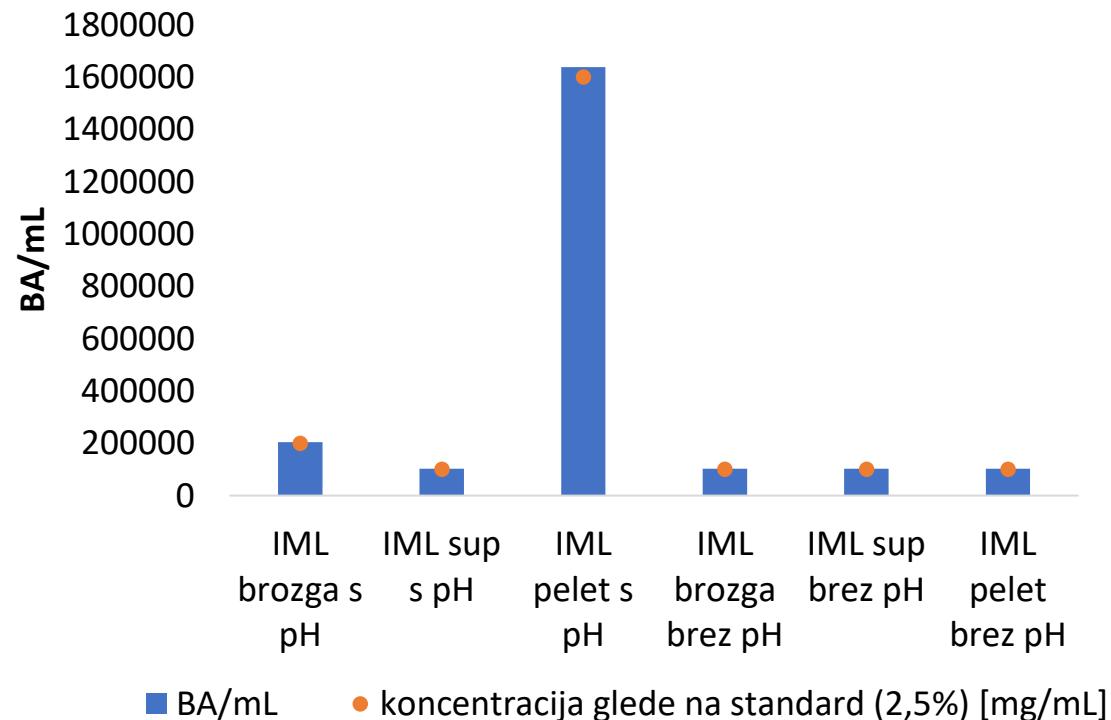
# Protimikrobska aktivnost nizina proti indikatorskemu sevu *Lactobacillus sakei* IM108 (ATCC15521)



## Uravnavanje pH tekom procesa

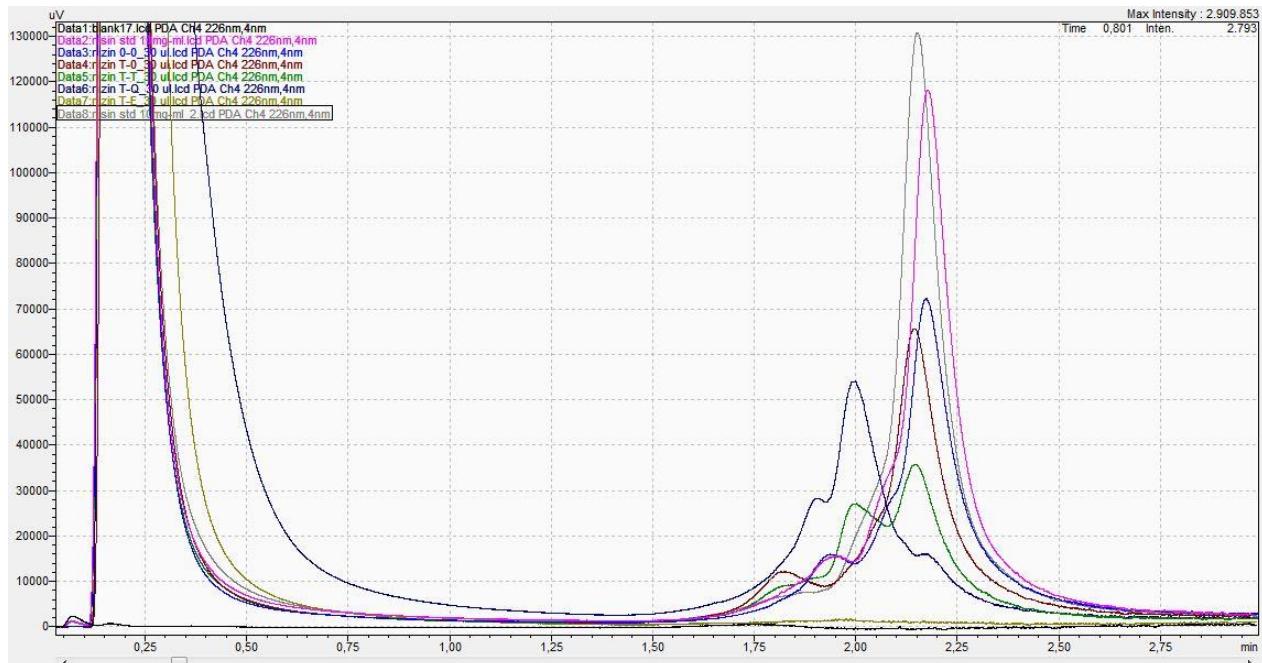
→ večja produkcija nizina

→ višji pH – več nizina povezanega z bakterijskimi celicami

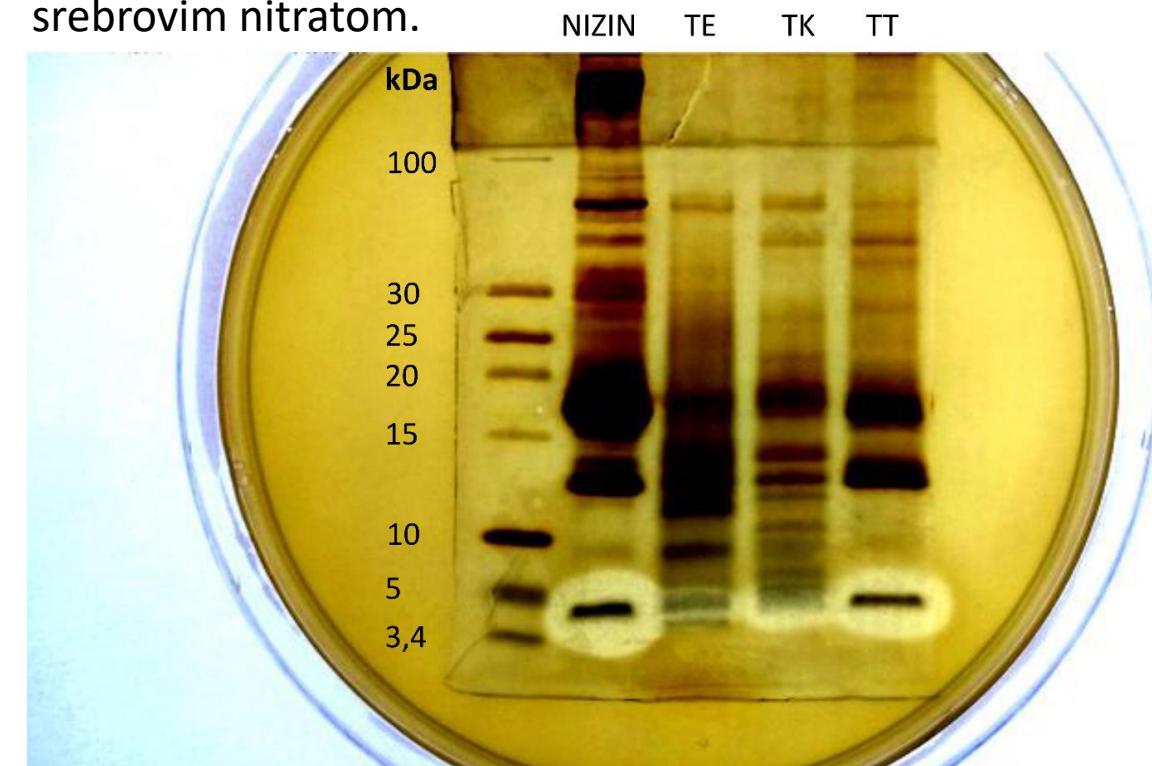


# Čiščenje in koncentriranje nizina: Kationsko-izmenjevalna kromatografija standard (Sigma)

kolona CIMac-SO3

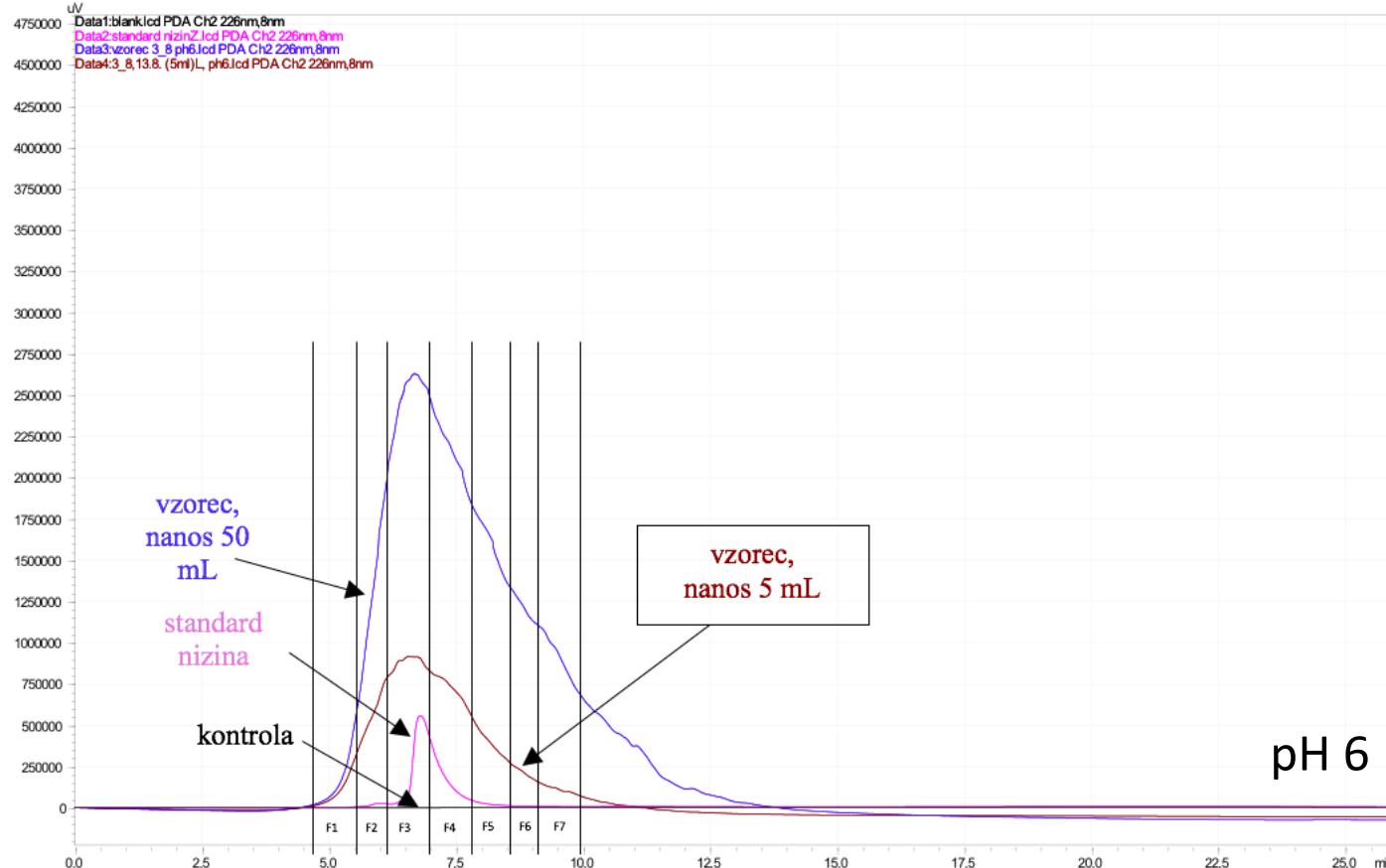


Protimikrobnna aktivnost nizina na gelu (NaDS-PAGE), ki je bil predhodno obarvan s Coomassie Brilliant Blue in srebrovim nitratom.

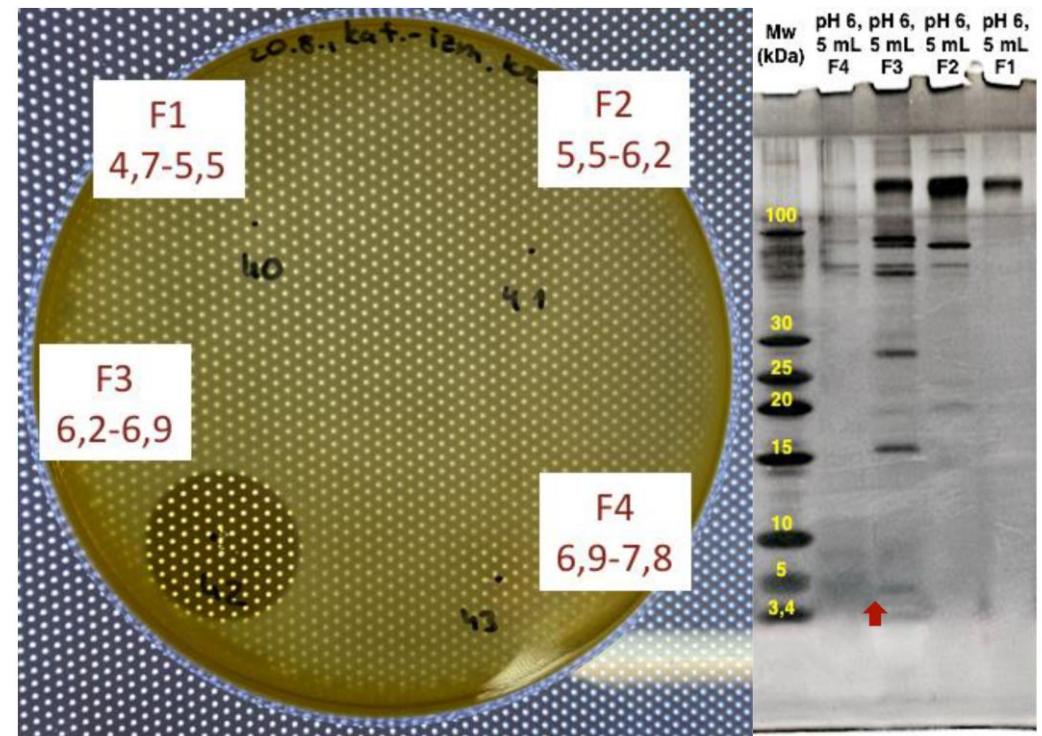


TE – nizin, obdelan s pronazo E; TK – nizin, obdelan z  $\alpha$ -kimotripsinom; TT – nizin, obdelan s tripsinom.

# Kationsko izmenjevalna kromatografija brozga po koncu bioprocesa - preparativa

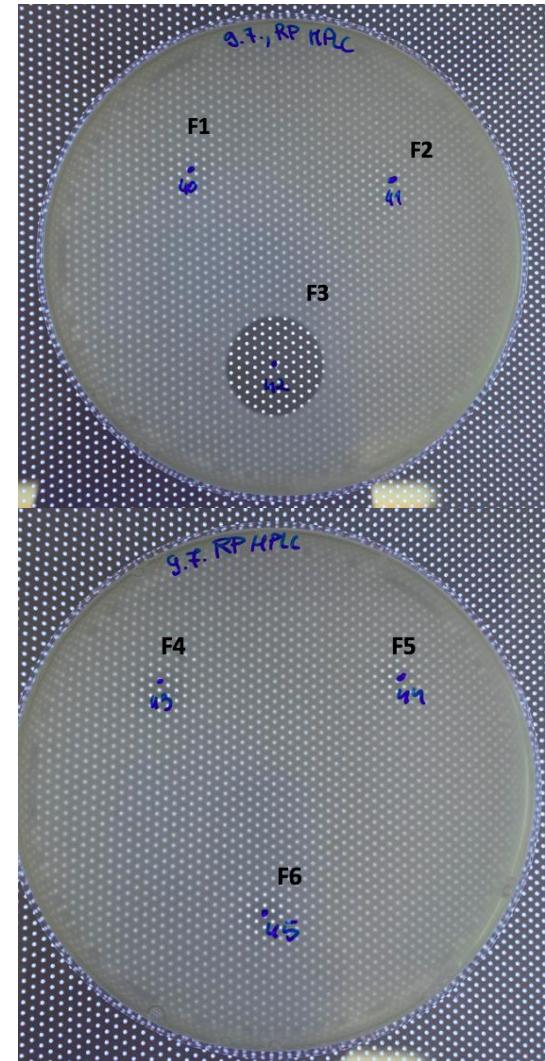
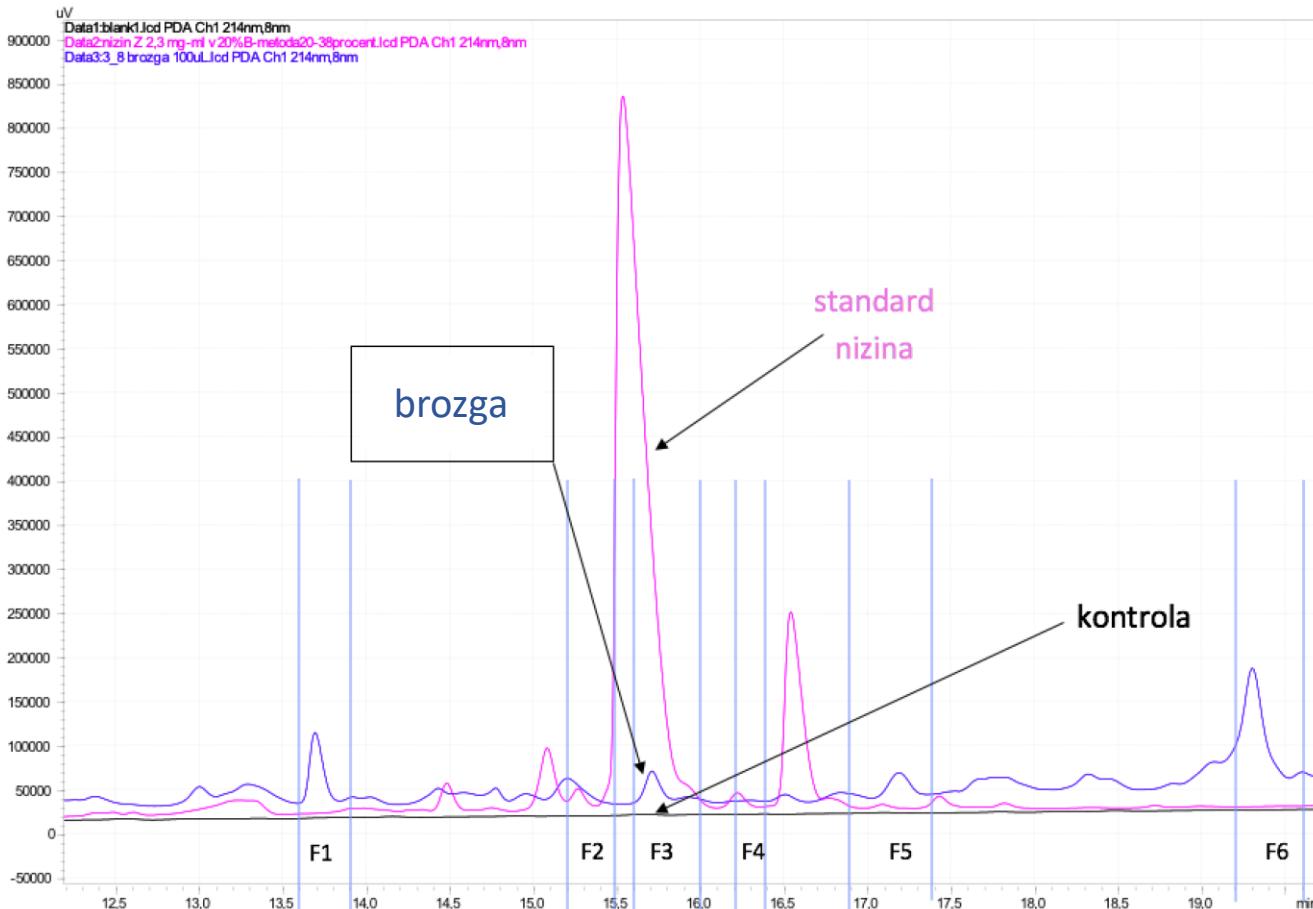


preparativna kolona CIMmultus™ S03-1



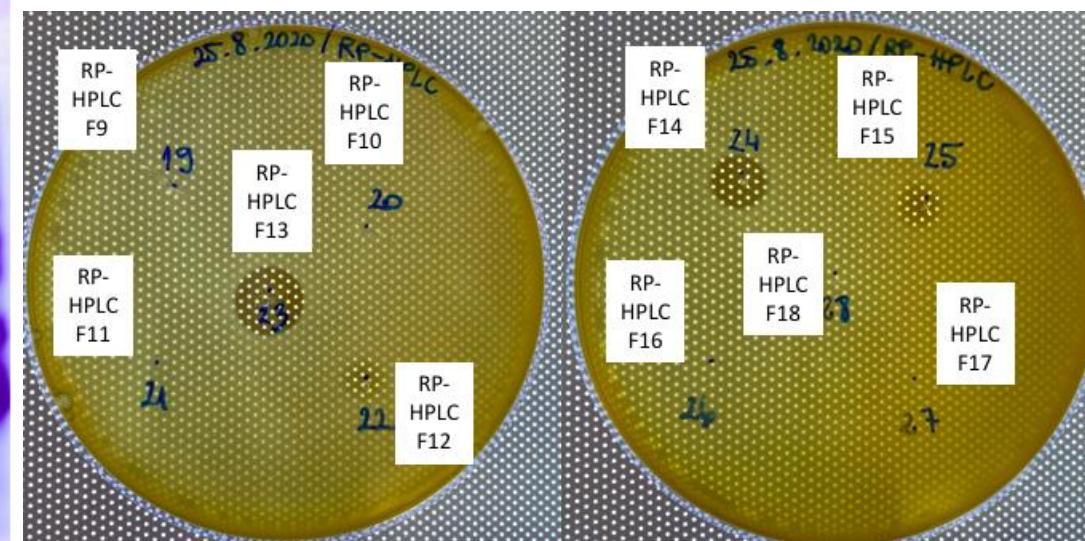
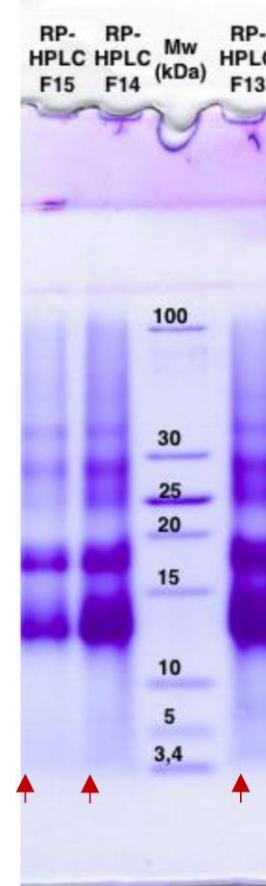
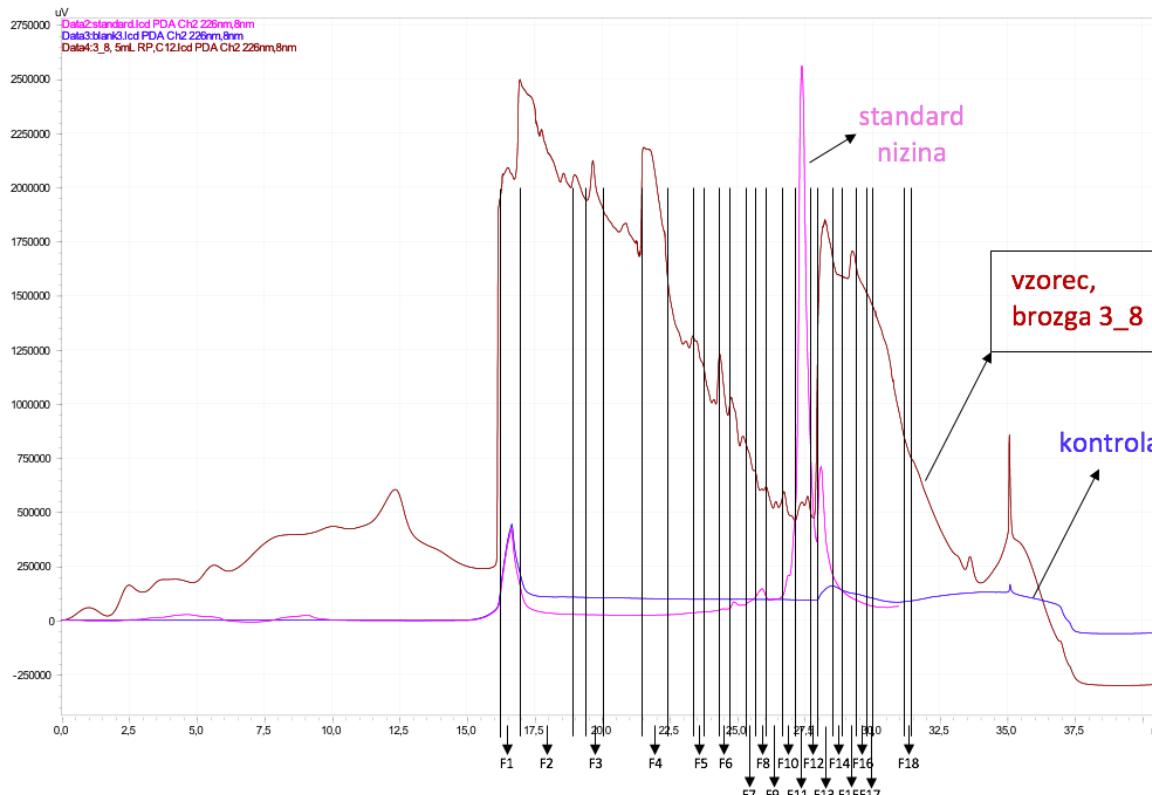
# Kromatografija z obrnjeno fazo (RP-HPLC) brozga po koncu bioprocesa - analitika

analitska kolona C8, Widespore Aeris



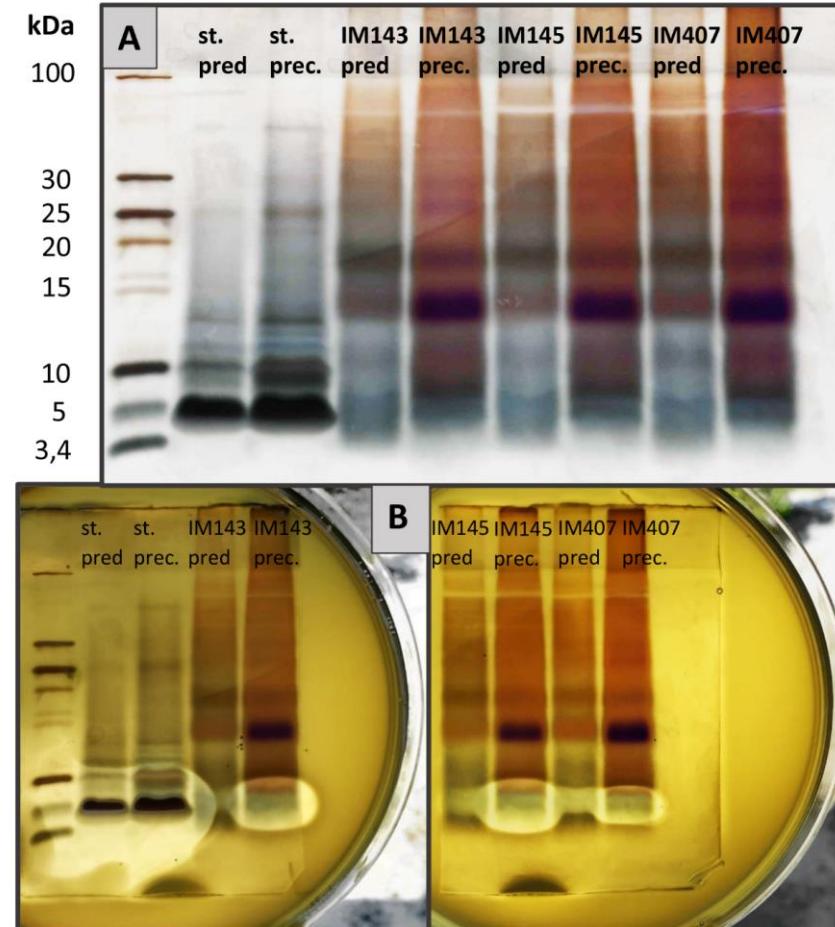
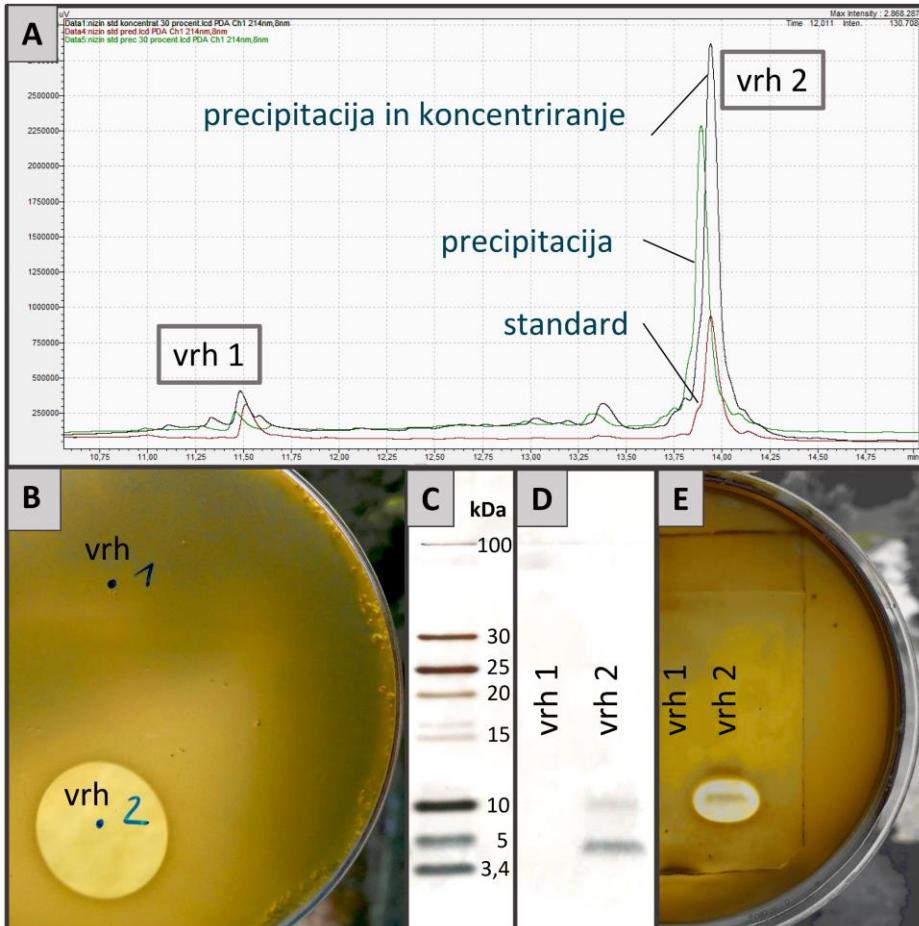
# Kromatografija z obrnjeno fazo (RP-HPLC) brozga po koncu bioprocesa - preparativa

preparativna kolona C12 Jupiter



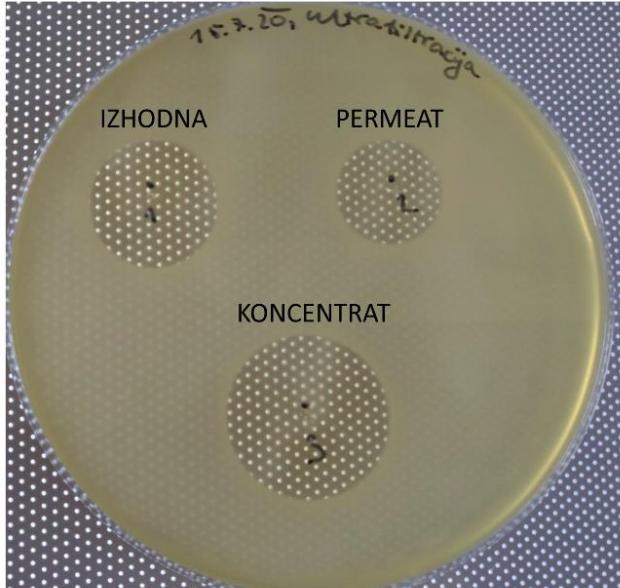
# Precipitacija nizina z amonijevim sulfatom

analitska kolona C8, Widespore Aeris

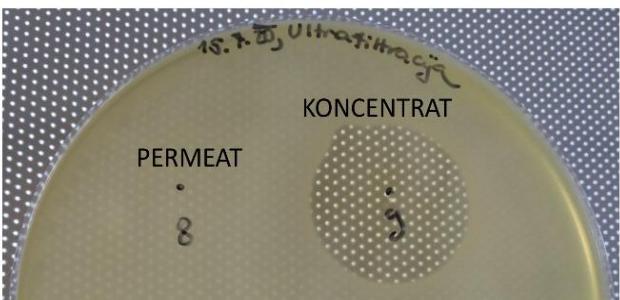


# Ultrafiltracija in penjenje z detergentom

Prepustnost 30 kDa



Prepustnost 10 kDa



1: kontrola

2: izhodni nizin pred prepihovanjem

3: pena po pepihavanju pH 4,8

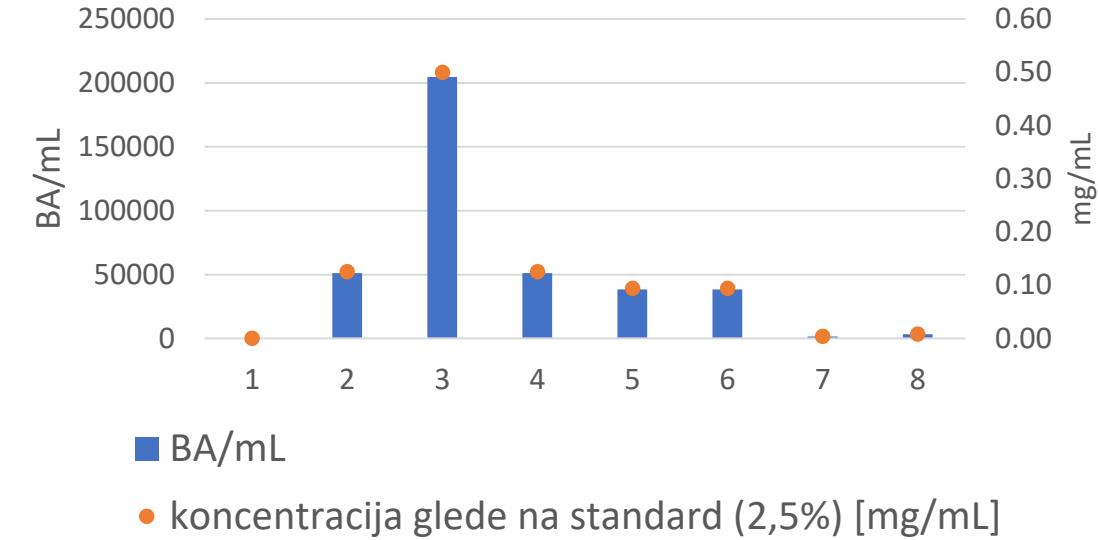
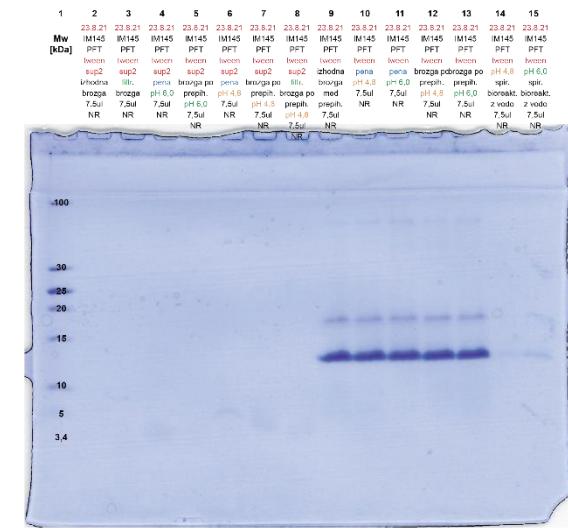
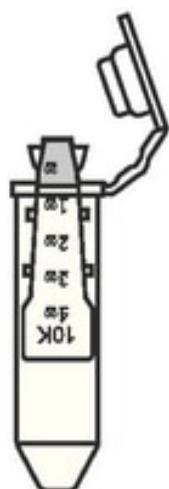
4: pena po pepihavanju pH 6,0

5: brozga po koncu prepihovanja pH 4,8

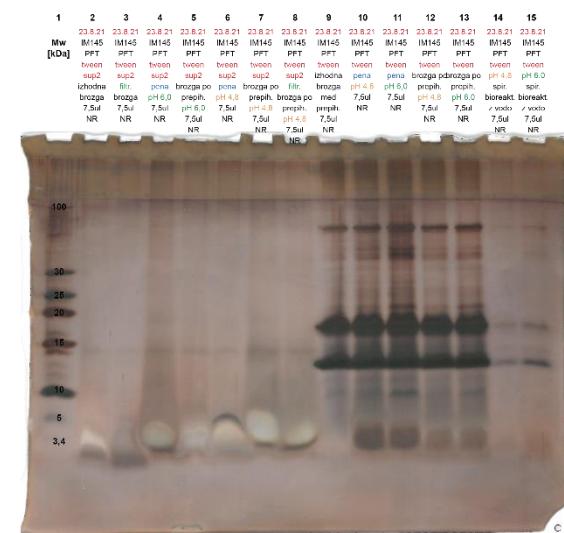
6: brozga po koncu prepihovanja pH 6,0

7: spirek posode s 50 mL vode pH 4,8

8: spirek posode s 50 mL vode pH 6,0

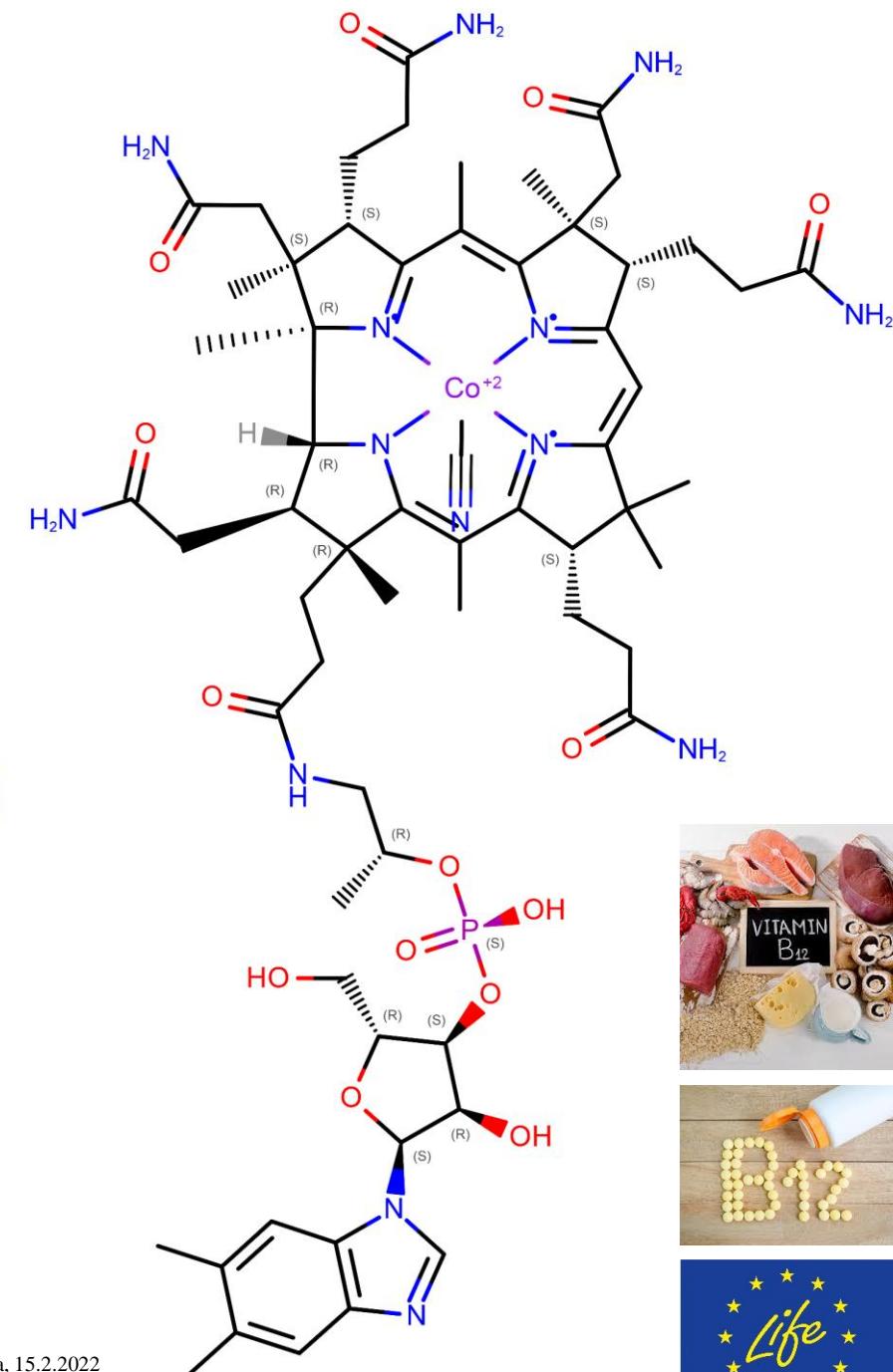
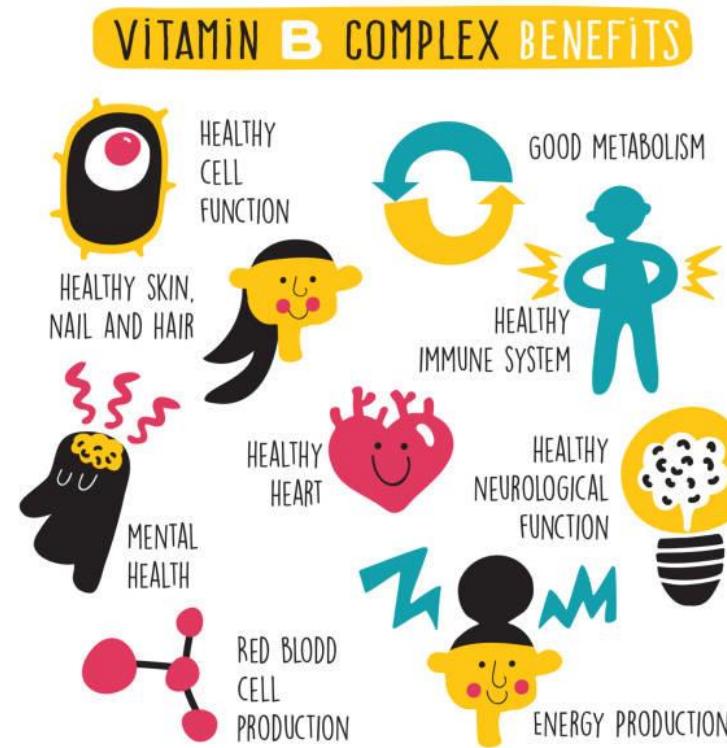


● koncentracija glede na standard (2,5%) [mg/mL]



# Vitamin B12

- **tetrapirolni obroč s centralnim kobaltovim atomom**
- stabilen pri pH 4-7
- vzdrži temperaturo 120 °C
- občutljiv na **svetlobo**
- glede na vrsto molekule, ki je vezana na **centralni kobaltov atom** imamo 4 različne oblike
  - adenozilkobalamin
  - metilkobalamin
  - cianokobalamin
  - hidroksikobalamin

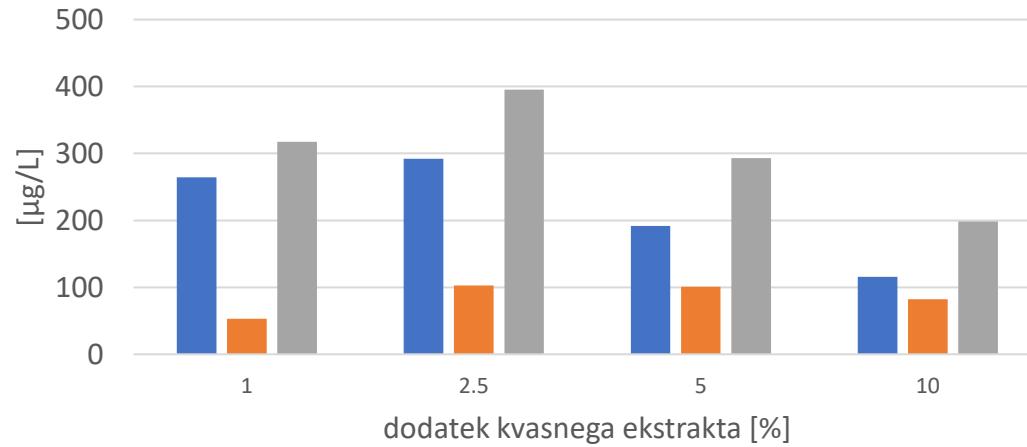


doi: [10.3390/nu2030299](https://doi.org/10.3390/nu2030299)

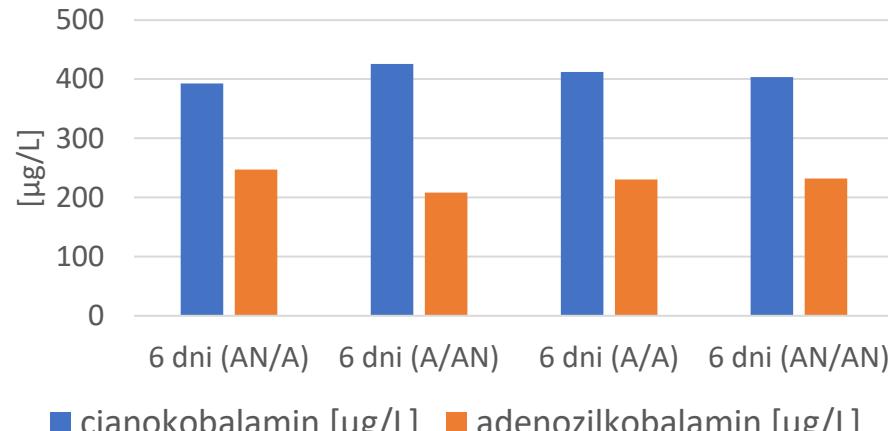
<https://www.istockphoto.com/vector/>

# Optimizacija pogojev gojenja

*Propionibacterium (P.) freudenreichii* subsp.  
*freudenreichii* van Niel 1928



■ cianokobalamin ■ adenozilkobalamin ■ celokupna vsebnost



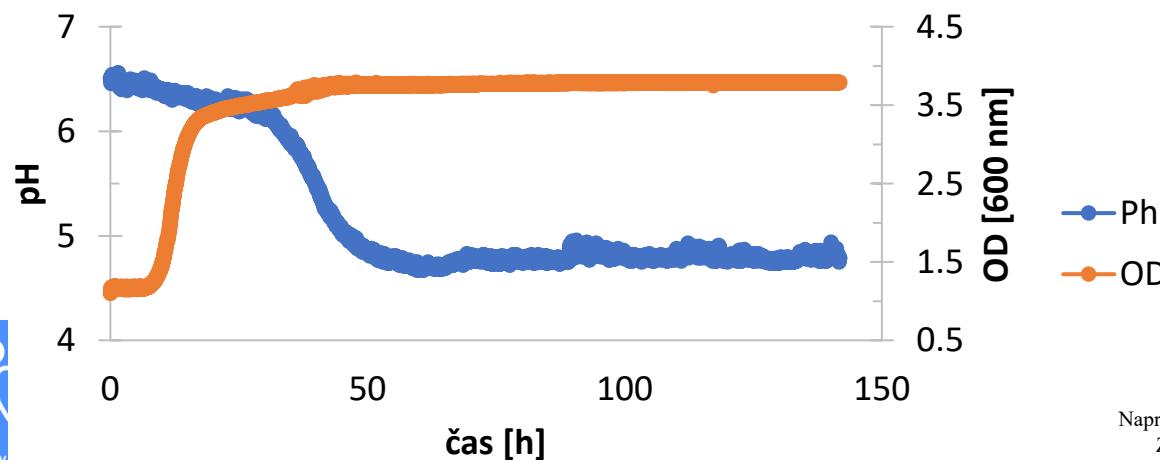
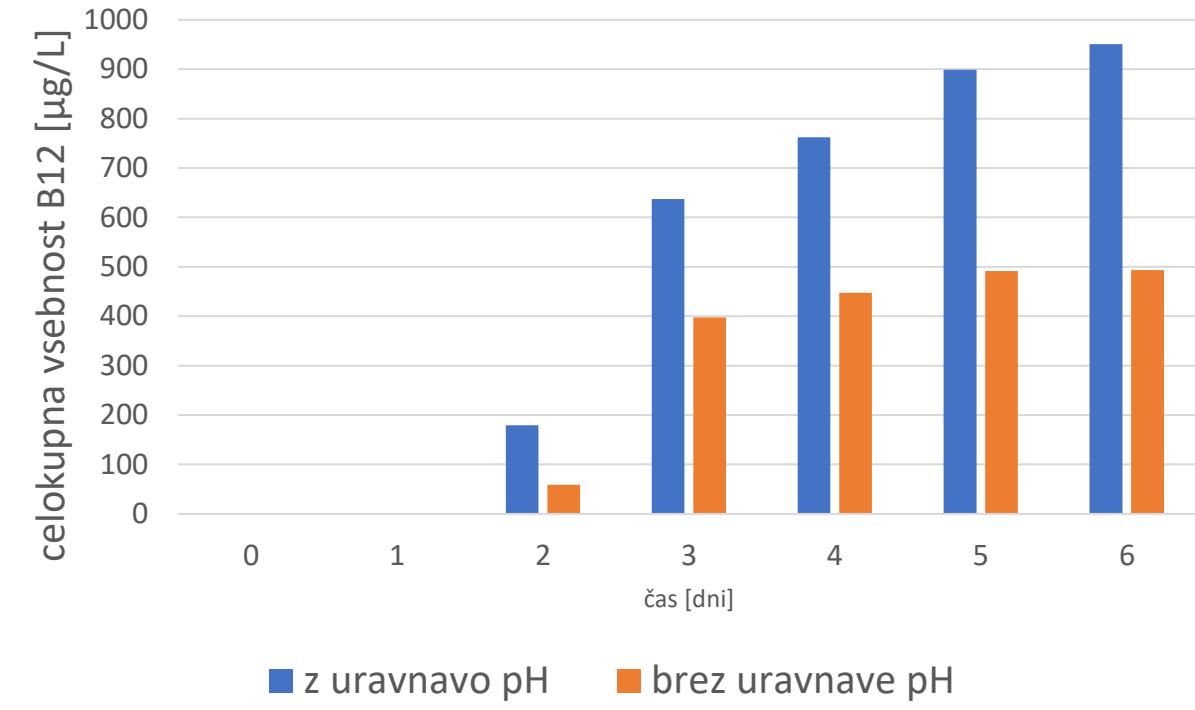
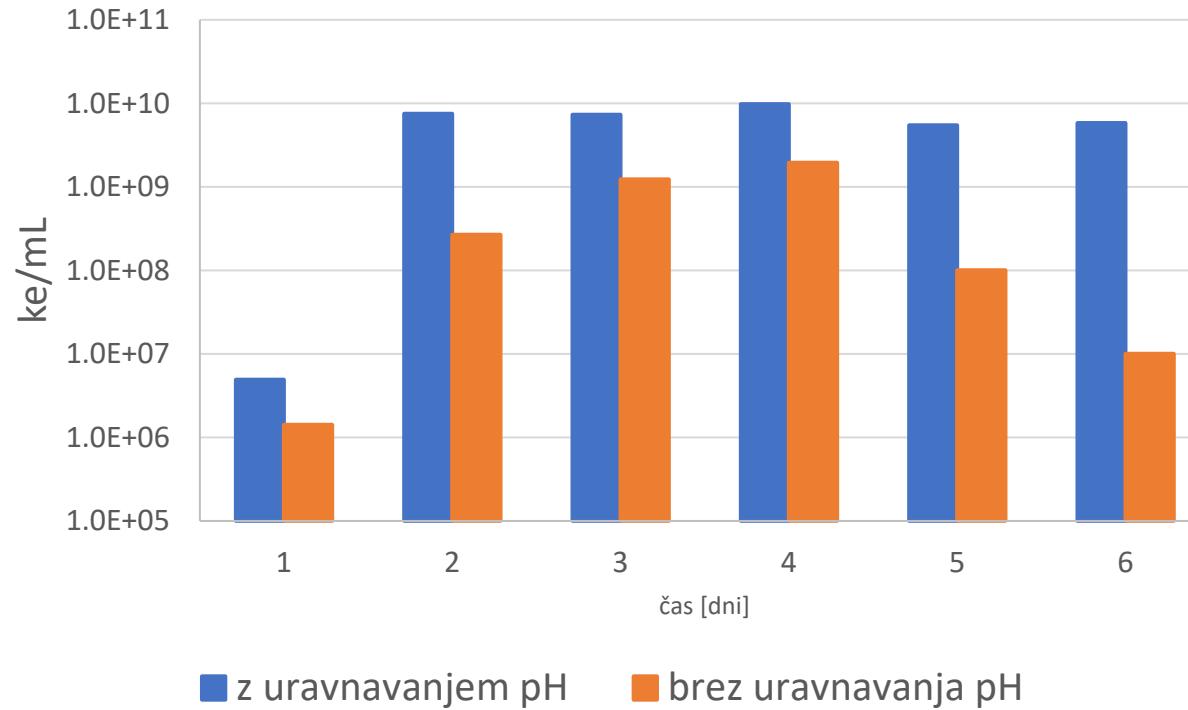
■ cianokobalamin [ $\mu\text{g/L}$ ] ■ adenozilkobalamin [ $\mu\text{g/L}$ ]



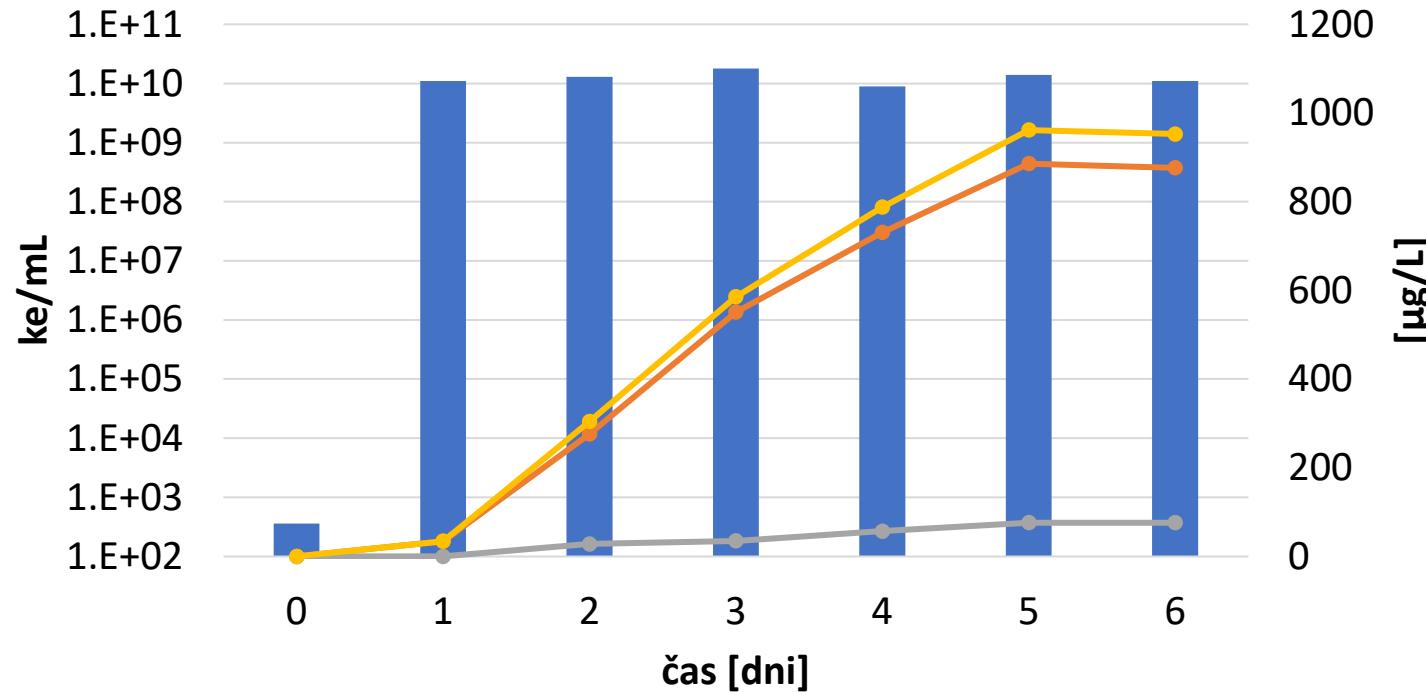
■ cianokobalamin [ $\mu\text{g/L}$ ]

■ adenozilkobalamin [ $\mu\text{g/L}$ ]

# Producija vitamina B12 v bioreaktorju

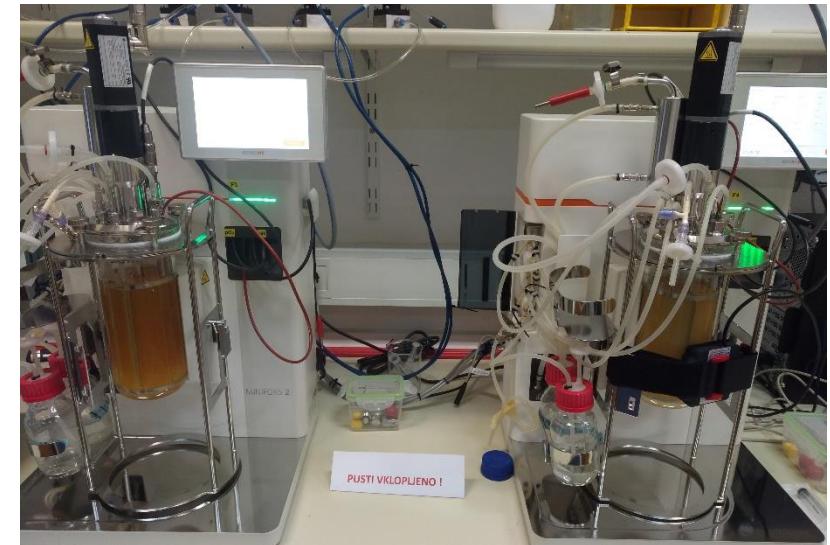


# Produkcija vitamina B12 v bioreaktorju

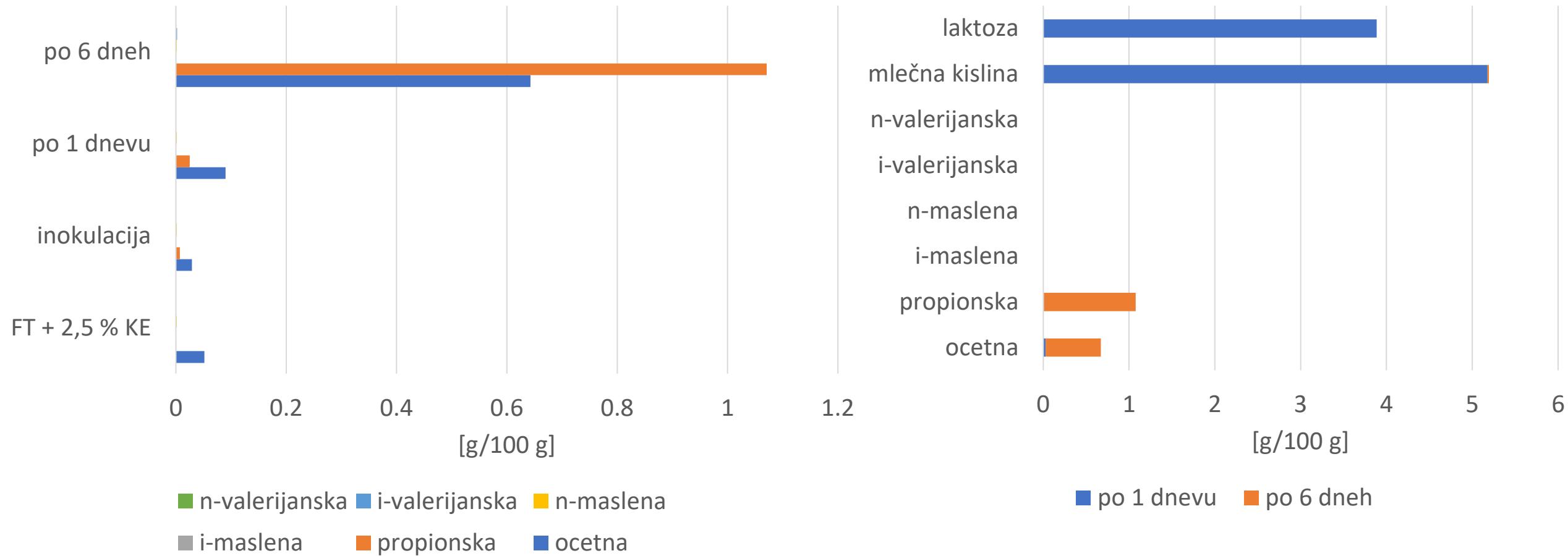


$\mu_{\max}$ (1/h)	$t_g$ (h)
0,071	9,80

- Vsebnost cianokobalamin
- Vsebnost adenozilkobalamin
- Celokupna vsebnost



# Odpadna brozga po koncu bioprocesa



# Hvala za pozornost

