

## РЕЗЮМЕТА НА ПУБЛИКАЦИИТЕ

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за участие в конкурса за професор по научната специалност “Процеси и апарати в химичната и биохимична технология”, шифър 02.10.09, обявен в Държавен вестник бр. 87/ 05.11.2010 г.

### I. Публикации

#### I.1. В списания с импакт фактор

1. **D.S.Yankov**, R.P. Stateva, J.P.M. Trusler, G. St. Cholakov  
“Liquid-liquid equilibria in aqueous two-phase systems of poly(ethylene glycol) and poly(ethyleneimine): experimental measurements and correlation”  
*J. Chem. Eng. Data*, 2006, **51**, 1056-1061

In this paper, which forms part of a study on aqueous two-phase systems (ATPS) for separation of low molar mass organic acids in the biotechnology industry, we present experimental and modeling results on the phase equilibria of an ATPS containing poly(ethylene glycol) (PEG) and poly(ethyleneimine) (PEI). The measurements were made at a temperature of 25 °C and a pressure of 1 bar, at pH ) 5.3, 7.5, and 9.2. The PEG had a mass-average molar mass of 4000 gâmol-1 and a polydispersity index of 1.05, while the PEI had a mass-average molar mass of 25 000 gâmol-1 and a polydispersity index of about 2.5. A UNIQUAC model, incorporating the polydispersity of the PEI, was found to give a satisfactory correlation of the experimental data. The experiment results demonstrated that the polymers are distributed unevenly in the two phases, and this suggests that organic acids (e.g., lactic acid) will partition preferentially to the PEI-rich phase through acid-base association.

2. K. Petrov, **D. Yankov**, V. Beschkov  
“Lactic acid fermentation by cells of *Lactobacillus rhamnosus* immobilized in polyacrylamide gel”  
*World J. Microb. Biotechn.*, 2006, **22**, 337-345

The process of lactic acid fermentation of lactose to lactic acid by *Lactobacillus rhamnosus* ATCC 7469 has been studied. The following processes have been explored: growth kinetics, as well as lactose utilization, production of lactic acid and further degradation of lactic acid. The immobilization experiments were conducted with microbial cells entrapped in polyacrylamide gels. Gels with different ratios of the monomer (acrylamide) and the cross-linking agent (N,N'-methylene-bis-acrylamide) have been tested. These were used in a repeat-batch process. The current processes inside and outside the gel particles were subjects of examination. The evolution of the activity of immobilized cells with repeated use showed that the particles served mainly as a donor of cells for the free culture. In all experiments a very high degree of conversion, 85–90% was observed. After several runs however, the particles were exhausted for microbial cells. A kinetic model of the process of lactic acid production was developed. This model allowed the evaluation of the effect of microbial growth and diffusion limitations inside the gel particles on the process rate and the separate contribution of the free and immobilized cells to the overall fermentation process upon multiple use.

3. M.S. Torz, **D.S. Yankov**, V.N. Beschkov  
“Biodegradation of mono- and dihaloacetic acids by *Moraxella* sp.B”  
*Compt. Rend. Acad. Bulg. Sci.*, 2006, **59**(3), 295-300.

The dehalogenation potential of the strain *Moraxella* sp. B growing on haloacetic acids used as a sole carbon source was studied. It was established that this strain could grow rapidly on monofluoacetic acid as well on its chloro- and bromo-analogues while the growth on dichloroacetate was much slower and it was accompanied by a very long lag-phase. No growth on difluoroacetic acid was observed. The kinetic parameters of microbial growth were evaluated for

monohalogenated acetates with the Andrew's model of inhibition. Stoichiometric halide release within a broad substrate concentration range was observed for all degraded acids. For concentrations exceeding 29 mM for monobromoacetate and 38 mm for monochloroacetate the degradation of substrate was no longer complete indicating possible product or substrate inhibition.

4. S. Stefanov, **D. Yankov** V. Beschkov

"Biotransformation of phytosterols to androstenedione in two phase water-oil systems"

*Chem Biochem. Eng. Q.*, 2006, **20**(4), 421-427

The microbial transformation of phytosterols to androstenedione (androst-4-ene- $\beta$ ,17-dione, AD) and androstadienedione (androsta-1,4-diene-3,17-dione, ADD) in two-phase water-oil systems by means of the strain *Mycobacterium* sp. *MB3683* has been studied. The effect of some process conditions, like the agitation speed, the age and amount of inoculum, the temperature and some additional carbon sources have been investigated. The highest conversion rates were attained with 10–15 % of inoculum of age  $t = 16\text{--}20$  h at  $T = 34\text{--}35$  °C and  $n = 400$  min $^{-1}$ . Media containing high concentrations of carbohydrates have a negative impact on the process, while the natural nitrogen sources influence beneficially the bioconversion. The use of silicon oil and polypropylene-glycol as solubilizing agents was found suitable for the above-mentioned biotransformation and permitted to increase considerably the amount of the substrate in the system. On the other hand, the biotransformation rate depended on the amount of the oils, because they inhibited the microbial cells growth.

5. N. Atanasova, P. Petrova, V. Ivanova, **D. Yankov**, A. Vassileva, A. Tonkova

"Isolation of Novel Alkaliphilic Bacillus Strains for Cyclodextrin Glucanotransferase Production"

*Appl Biochem Biotechnol*, 2008 **149**, 155–167

New alkaliphilic Bacillus producers of cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) were isolated from 17 Bulgarian alkaline and normal habitats (springs and soils) by three steps of a selection. None of the isolates obtained, producing CGTase, appeared to be thermophilic in character. One hundred and thirty-seven strains were estimated for CGTase activity by batch cultivation in a liquid alkaline medium. Twentyseven of them had a detectable CGTase activity in their culture supernatants under the enzyme assay conditions, despite of the significant growth of all isolates. The phenotypic properties of three selected strains (20RF, 8SB and 24WE) were determined. They were

aerobic endospore-forming Bacillus strains: two of them were obligated alkaliphiles (20RF and 8SB) and one, alkalitolerant (24WE). Both obligated alkaliphiles were further characterised by 16S rRNA analysis. According to the full 16S rRNA gene sequences obtained and deposited to the NCBI GenBank database, both isolated obligated alkaliphiles 20RF and 8SB were clustered into the group of alkaliphilic Bacillus species. The exhibited CGTase production by them (230–250 U ml $^{-1}$  for 20RF and 130–160 U ml $^{-1}$  for 8SB) defined these new isolates as promising producers of the enzyme, especially Bacillus sp. 8SB synthesising thermostable alkaline  $\beta$ -CGTase. Both new enzymes from 20RF and 8SB Bacillus strains formed only two types of cyclodextrins, beta and gamma, which could be of interest for their easy separation and industrial production.

6. **D.S.Yankov**, J.P.M. Trusler, B.Y. Yordanov, R.P. Stateva

"Influence of Lactic Acid on the Formation of Aqueous Two-Phase Systems Containing Poly(ethylene glycol) and Phosphates"

*J. Chem. Eng. Data*, 2008, **53**, 1309–1315

In this paper, which forms part of a study on aqueous two-phase systems (ATPS) for separation of lowmolar-mass organic acids in the biotechnology industry, we present experimental results on the phase equilibria of ATPS containing poly(ethylene glycol) (PEG) and either K<sub>2</sub>HPO<sub>4</sub> or KH<sub>2</sub>PO<sub>4</sub> or a fixed mixture of the two phosphates. The measurements were made at a

temperature of 298.15 K and a pressure of 1 bar. The PEG had a mass-average molar mass of either 10 000 g · mol<sup>-1</sup> or 20 000 g · mol<sup>-1</sup> and a polydispersity index of 1.05. The influence of 2-hydroxypropanoic acid (lactic acid) on the formation of the ATPS was studied, and the partition coefficient for this compound between the coexisting phases was found to be close to unity.

7. R. Georgieva, P. Koleva, D. Nikolova, **D. Yankov**, Sv. Danova

“Growth parameters of probiotic strain *Lactobacillus plantarum*, isolated from traditional white cheese”

*Biotechnol. & Biotechnol. Eq.*, 2009, **23** (2), special edition, 861-865

Different aspects, including safety, functional and technological characteristics, have to be taken into consideration in the selection of each probiotic microorganism. The aim of the present work was to determine the kinetic growth parameters of *L. plantarum* strain after cultivation in media with different carbon sources. The strain was isolated from traditional Bulgarian white cheese and previously characterized as putative probiotic, based on commonly accepted in vitro criteria. For further biotechnological implementation was necessary to select a suitable and economically relevant growth media. Thus, reconstituted permeate (6% w/v) and the following modification of de Man, Rogose Sharpe media (MRS): (i) MRS-glucose; (ii) MRS-lactose; (iii) MRS-galactooligosaccharide; (iv) MRS-fructooligosaccharide were used. The strain growth, lactic acid production and carbon source utilization were monitored by pH and cell number determination, and HPLC analysis at different time points of the cultivation process. The highest cell growth and carbohydrate conversion were detected in the presence of glucose and lactose. The main product of the fermentation was lactate with detectable level of acetate. The permeate and MRS-galactooligosaccharide also support good growth and lactic acid production, which indicate a great potential for industrial applications of studied *L. plantarum* strain into the food system.

8. N. Atanasova, Ts. Kitayska, **D. Yankov**, M. Safarikova A. Tonkova

“Cyclodextrin glucanotransferase production by cell biocatalysts of alkaliphilic bacilli”, *Biochemical Engineering Journal*, 2009, **46** (3), 278-285

Cells of obligated alkaliphiles *Bacillus pseudocaliphilus* 20RF and *Bacillus pseudocaliphilus* 8SB isolated from Bulgarian habitats, producers of cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19), were immobilized by three different techniques: on two types of polysulphone membranes; entrapped in agar-gel beads containing magnetite and by nanoparticles of silanized magnetite covalently bound on the cell surface. The biocatalysts obtained demonstrated the opportunity for a significantly enhanced CGTase production compared to free cells for a long period of time (10 days semicontinuous cultivation) without impact on their mechanical stability. The cell membrane-biocatalysts exhibited the highest enzyme activity after 240 h repeated batch cultivation and retained 1.3–2.3-fold increase of the CGTase yield compared to free cells at the end of the process. Membrane biocatalysts were applied for a direct cyclodextrin (CD) production. The results obtained demonstrated the possibility of starch conversion into cyclodextrins by immobilized cells without using of crude or purified enzyme. The membrane biocatalysts of both obligated alkaliphiles formed mainly β- and γ -CDs after 6 h enzyme reaction at pH 9.0 of the reaction mixture. Under these conditions, the quantity of γ-CDs was a relative high, to 35–37% of the total CD amount.

9. N. Marinova, **D. Yankov**

"Toxicity of some solvents and extractants towards *Lactobacillus Casei* cells"

*Bulgarian Chemical Communications*, 2009, **41**(4), 368-373

Various organic compounds used as diluents, modifiers and extractants in lactic acid extraction have been tested to determine their toxicity towards *Lactobacillus casei* cells. The toxicity on molecular and phase level has been investigated. In general, the tested hydrocarbons

were non-toxic on molecular level and showed variable toxicity on phase level. Among the tested alcohols octanol and oleyl alcohol were non-toxic, whereas decanol and dodecanol were toxic on the molecular level. All the alcohols showed high phase toxicity. The tested extractants were very toxic both on molecular (except for tridodecylamine) and on phase level. Two extraction systems composed of trioctylamine/oleyl alcohol and tridodecylamine/oleyl alcohol have also been studied. Both systems were non-toxic on molecular level and showed medium toxicity on phase level. They can be used successfully for *in situ* extractive fermentation of lactic acid.

10. **D. S. Yankov**, J.P. M. Trusler, R. P. Stateva, G. St. Cholakov  
"Influence of pH and acid solutes on the phase behaviour of aqueous solutions containing poly(ethylene glycol) and poly(ethyleneimine)"  
*Biochemical Engineering Journal*, 2009, **48**, 104-110

In this paper, which forms part of a study on aqueous two-phase systems (ATPS) for separation of lowmolar-mass organic acids in the biotechnology industry, we examine the phase behaviour of ATPSs containing poly(ethylene glycol) (PEG) and poly(ethyleneimine) (PEI) as a function of pH, the nature of the acid used for titrating PEI, and the addition of lactic acid. We show that increasing the pH leads to contraction of the two-phase regions and that titrating PEI with a higher polyvalent acid results in a larger two-phase region. We propose a mechanism explaining the experimentally observed phase behaviour. Finally, we demonstrate that the lactic acid partition coefficient is very favourable (the acid partitions preferentially to the PEI-rich phase), which confirms that the (PEG + PEI) ATPSs could be used to advantage as a medium for lactic acid bio-transformation.

11. G. Kyuchoukov, **D. Yankov**  
"Theoretical and experimental study of lactic acid stripping from loaded organic phase"  
*Ind. & Eng. Chem. Res.* 2010, **49**, 8238–8243

The process of stripping lactic acid with different stripping agents was investigated for two organic-phase systems containing trioctylamine (TOA). On the basis of theoretical considerations for extraction mechanism, equations for stripping were developed. The theoretical values of the extent of stripping and the concentrations of lactic acid in organic and aqueous phases were compared with experimental values in the cases of water, NaOH, NaCl, HCl, and carbonates as the stripping agents. The possibilities of repeated use of the extractants in extraction/stripping runs were investigated. The best results were obtained with a system containing 20% TOA, 30% dodecane, and 50% decanol with  $(\text{NH}_4)\text{HCO}_3$  as the stripping agent.

12. **D. S. Yankov**, V. N. Beschkov, R. P. Stateva  
Insights on the Influence of Acid Solutes on the Phase Behaviour of Aqueous Two-Phase Systems, Containing Poly(ethylene glycol) and Poly(ethyleneimine)  
*Bulgarian Chemical Communications*, 2010, 42(4), 327-334

This paper is part of a study on the possibility of using aqueous two-phase systems (ATPSs) for separation of low-molar-mass organic acids in the biotechnology industry. We present for the first time experimental data on the influence of titrating organic acids on the phase behaviour of the ATPSs, containing poly(ethyleneglycol) (PEG) and poly(ethyleneimine). The underlying hypothesis of the mechanism advanced is discussed from the point of view of the acid structure, and is validated by the observed ATPSs phase behaviour. Finally, we demonstrate the opposing influence of the lactic acid and titrating acids in these systems.

13. Atanasova N, Kitayska Ts, Bojadjieva I, **Yankov D**, Tonkova A.  
"A novel cyclodextrin glucanotransferase from *Bacillus pseudocaliphilus* 20RF: purification and properties."

*Process biochemistry*, 2011, **46**( 1), 116-122

A new cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) from the obligated alkaliphile *Bacillus pseudocaliphilus* 20RF, isolated from Bulgarian soils, was purified up to 18-fold by ultrafiltration and starch adsorption with a recovery of 63% activity. The enzyme was a monomer with a molecular weight 70 kDa estimated by SDS-PAGE. The CGTase exhibited two pH optima at pH 6.0 and 9.0 and was optimally active at 60 °C. The enzyme could be effectively used for conversion of raw starch into cyclodextrins (CDs) in a wide pH range, from 5.0 to 10.0 and temperatures 60–70 °C. The enzyme was more heat resistant after its pretreatment in alkaline pH 9.0 at high temperatures 65–70 °C, than at pH 6.0 under the same reaction conditions. The CGTase showed a significant stability in the presence of 15mM various metal ions and reagents after 30 min incubation at 25 °C. The purified CGTase could be used for an efficient cyclodextrin production without any additives which is of an industrial interest. The achieved high conversion of an insoluble raw commercial corn starch into cyclodextrins (47%) with production of only two types of cyclodextrins,  $\beta$ - and  $\gamma$ -CD (80%:20%) in alkaline pH 9.0, makes *B. pseudocaliphilus* 20RF CGTase industrially desired for cyclodextrin manufacture.

## II. Доклади в пълен текст

14. **D. S. Yankov**, J.P. Martin Trusler, R. P. Stateva, G. St. Cholakov

“Effects of Temperature, pH and Lactic Acid Concentration on the Phase Behaviour of Aqueous Solutions of Poly(ethylene glycol) and Poly(ethyleneimine)”

Proceedings of the 23rd European Symposium on Applied Thermodynamics, ESAT 2008, May 29<sup>th</sup> – June 1<sup>st</sup>, Cannes, France, p. 119-126

The present work is part of a broader study devoted to the application of aqueous two-phase systems (ATPS) for *in situ* separation of low molar mass organic acids in the biotechnology industry. The ATPS studied comprise poly(ethyleneglycol) (PEG) and poly(ethyleneimine) (PEI). The PEG average molar mass was 4000 g·mol<sup>-1</sup> with a polydispersity index of 1.05, and the PEI molar mass was 25000 g·mol<sup>-1</sup> with a polydispersity index of about 2.5. Measurements of the binodal curves of the ATPS were performed at  $T = (25 \text{ and } 36)^\circ\text{C}$ ,  $p = 1 \text{ bar}$ ,  $5 \leq \text{pH} \leq 9$  and at two different concentrations of lactic acid. The partition coefficient of the lactic acid was also measured at  $T = 36^\circ\text{C}$  and  $\text{pH} = 7.5$ . The experimental results which we present demonstrate that, while the influence of the temperature on the phase formation of the ATPSs is almost negligible, that of the pH value is clearly pronounced, with increasing pH leads to shrinkage of the two-phase region. The addition of lactic acid acts as an ATPS phase breaker as demonstrated at  $\text{pH} = 7.5$  for two lactic acid concentrations. Furthermore, lactic acid distributes preferentially to the PEI rich phase of the ATPSs with partition coefficients  $K = (2-4)$ . Finally, the equilibrium compositions (tie-lines) of the (PEG + PEI) ATPS with two different lactic acid concentrations were obtained from experimental measurements at  $T = 36^\circ\text{C}$  and  $\text{pH} = 7.5$  by means of a gravimetric method.