

Immobilized lipase B was used as a catalyst for butyl butyrate synthesis in [bmpy][TF2N].

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Protein refolding on ion-exchange chromatography: improving total mass recovery and investigating separation factors of folded and unfolded states

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Intracellular overexpression of recombinant proteins in prokaryotes such as *Escherichia coli* (*E. coli*) often forms inactive aggregates, referred to as “inclusion bodies.” These insoluble inclusion bodies must first be solubilized in denaturing solutions and then, refolded into an active state. For *in vitro* refolding of proteins, different methods have been tested; however, there are not enough studies on the protein refolding using various ion-exchange chromatographic columns. It is critical to identify the optimal refolding conditions in this process due to its considerable influence on the mass recovery. In the current study, we examined oxidative refolding of denatured lysozyme (DLys) in the strong cation-exchange chromatography with SP-3PW resin. For this purpose, two different chromatographic operation methods of gradient elution, via decreasing the concentration of urea, and isocratic elution, with high levels of urea, were tested. At the same time, we examined the impact of different concentrations of additives such as redox agents, reduced and oxidized glutathione, and L-arginine in washing and elution buffers on mass recovery. Under the optimal condition for mass recovery, more than 95% of lysozyme was recovered; however, the resolution peak of unfolded and folded proteins indicated a poor separation factor under the operating conditions.

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Bubble coalescence model effect on $k_L a$ prediction using CFD for non-Newtonian fluids in a stirred tank bioreactor

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There are several models to simulate bubble breakup and coalescence phenomenon in non-Newtonian applications. However accurate on gas–liquid mass transfer predictions depends on realistic modelling of implied phenomena. In this paper the effects of different bubble coalescence models on $k_L a$ calculations are analysed by using CFD simulations (computational fluid dynamics) in a 10L Bioreactor stirred by a Rushton turbine. The effects of turbulence, rotating flow, bubbles breakage and coalescence were simulated by using the $k-e$, MRF (multiple reference frame) and PBM (population balance model), respectively. Setting up simulations were based on typical fungi culturing conditions and values were compared to $k_L a$ an experimental data. Based on the latter mass transfer prediction was highly affected by coalescence model selection being the Coualologluo and Tavlarides coalescence

model the most accurate from those analysed in this work, with reached values of 14.0 h^{-1} that fit very well to experimental data (13.68 h^{-1}). The computational tools established here to estimate bubble coalescence could be used in non-Newtonian biochemical engineering applications for solving large scale and design optimization problems.

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Biosensors

Applications of whole bacterial cell imprinted biosensors

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Selective and sensitive detection of bacterial strains is of particular interest due to becoming increasingly important in clinical diagnostics, food and water analysis, product quality and process control, agricultural and industrial processing, bioprocess and environmental monitoring. Conventional methods applied for the detection of bacteria have some disadvantages and therefore, there is a growing need for the development of new techniques. Biosensor based technologies have great potential for replacing conventional methods due to their remarkable properties such as selectivity, sensitivity, multi-analyte testing capacity, speed and cost-effectiveness. They also provide high-throughput monitoring, label-free detection, real-time analysis, low detection limits. Molecular imprinting technology is an alternative approach for designing biosensor platforms working with increased selectivity. These biosensors having molecularly imprinted polymers defined as artificial recognition elements have unique features such as high bio-recognition capability, mechanical and chemical stability, easy preparation and low cost. Imprinted polymers have recognition cavities which are complementary in size, shape and/or chemical functionality to the template bacterial strain and they are highly selective against target bacterial cell selected to be used in the imprinting process. In our studies, it was shown that the quantifications of pathogenic and non-pathogenic bacterial strains were achieved using imprinting technology integrated with sensing systems.

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Comparison of two different methods for detecting *Escherichia coli* with a surface plasmon resonance-based sensor

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A surface plasmon resonance device was designed for detection of foodborne pathogens. The device is suitable for using two different injection port and allows to follow bacterial binding real time by pipetting bacteria solutions directly on to the chip surface or injecting to the system by using a peristaltic pump, continuously. In this work both incubation port and channel structure were used for detection of *E. coli* and the results were compared. The resonance angle was increased during the functionalization steps of EDC/NHS,

streptavidin and biotin conjugated antibodies. The immobilization of the anti-*E. coli* resulted in a remarkable change in resonance angle. The changes in the resonance angle obtained by bacterial binding with two methods were compared. Both incubation port and flow cell could be successfully used but the continuous system analysis gave higher signal increases during binding of analytes and bacterial solutions. Therefore, using a peristaltic pump can be a preferable way for detection and quantisation of food pathogens rapidly and this system can potentially be generalized to other pathogens.

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Biotechnology & Ethics

The insulin based alternative glucose uptake activity assay with eukaryotic unicellular *Tetrahymena thermophila*

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Insulin is a peptide hormone that helps lower the blood glucose levels. To determine the function of recombinant insulin derivatives, rabbit and mouse are used in the blood glucose drop assay instead of an in-vitro assay. According to the principles of humane experimental techniques, replacement rule of the 3Rs implies the use of alternative invertebrate organisms or cell culture instead of vertebrate animals. In this study, we report an alternative insulin activity assay based on unicellular invertebrate *Tetrahymena thermophila*. Experimentally, 0.5 mg/ml glucose was added to 24-hours starved *T. thermophila* cells. Then, different doses of bovine and human insulin were tested for glucose dropping function. Samples from control and experimental groups were taken with time intervals. Glucose concentration was determined by a repeated test group. As a result, while insulin free control group's glucose concentration was only dropped 2–4%, bovine and human insulin dropped glucose level to 12–16% in 10 min depending on the insulin doses, consistent in all repeat experiments. In conclusion, the data presented here suggest that *T. thermophila* as a unicellular invertebrate could be used as an alternative experimental organism in place of rabbit or mouse of insulin based on blood glucose drop assay.

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Environmental biotechnology

Dynamic observation of changes in myocardial angiogenesis of mice chronically exposed to chromium

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The health consequences of environmental pollution have significant importance in public health. The burden of disease caused by pesticides and other toxic substances is extremely increasing. We aimed to study the influence of sodium dichromate to myocardial angiogenesis of mice. After obtaining ethical committee approval, 80 outbred mouse stocks were enrolled to this study. The experimental models were exposed to sodium dichromate *per os* with the dose of 7.5 mg/kg. The animals were sacrificed in the first (20 mice) and the second (20 mice) months after exposure. 2 months after exposure, animals were paired and offspring (20 mice) were exposed to environmental chemicals after lactation. The last group were observed after 1 month of exposure to pollutants. Morphological, histological studies and statistical analyses were conducted using SAS software. In the first month of exposure, the myocardial capillary walls (CWs) on posterior side of ventricles (PV) were 1.3 times thicker than in control group. In the second month of exposure, on the PV CWs were 1.5 times higher and on the other parts of myocardium 1.2 times thicker. No changes were observed on the CWs of the offspring. We conclude that exposure to chromium has significant influence on myocardial angiogenesis.

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Study of some responses of biotechnological and ecotoxicological importance of marine yeasts

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Marine microbial bioprospecting searches for extremophile microorganisms with biodegradative capabilities towards highly polluting compounds (insecticides Chlorpyrifos-CP/TCP metabolite). Yeasts are eukaryotic unicellular microorganisms that are models for environmental biotechnology and microbial ecotoxicology. Isolation of yeasts from sediments and their biochemical characterization may be of benefit for applications in the control of environmental chemicals. Bay of Cartagena sediments were characterized physicochemically with multiparameter analyser (conductivity/pH/temperature/NO₃/dissolved oxygen), TOC (colorimetric test) and Chlorpyrifos (GC-MS-TSQ); yeasts were isolated in YPD medium, characterized biochemically (API20C), generating a profile of carbohydrate assimilation; symbiosis/antibiosis test was done in agar by sowing pure yeasts in contact with each other (inhibition) and esterase profile (Tween 80/red methyl indicator); yeast were subjected to growth 4 °C/45 °C and NaCl (4%/10%) and minimum inhibitory concentration-MIC was tested by placing a volume on agar at different concentrations (CP/TCP) to see inhibition. The results show decrease (DO/conductivity/NO₃) towards the south of the bay and chlorpyrifos (0.189–1.429 ng/g); 10 yeasts charac-