

大葉大學 九十三 學年度 研究所博士班 招生考試試題紙

系 所 別	組 別	考 試 科 目 (中 文 名 稱)	考 試 日 期	節 次	備 註
生物產業科技學系		專業英文論文閱讀能力測驗	6月21日	第 1 節	共4頁,第1頁

註：考生可否攜帶計算機或其他資料作答，請在備註欄註明（如未註明，一律不准攜帶）

考生注意事項

1. 筆試題目共計十題，任選五題作答。每題 20 分，合計 100 分。
2. 作答方式：將英文摘要改寫成中文（不必抄題）。
3. 無適當中文翻譯之特殊專有名詞，如微生物學名、化合物、化學成分等，可依原文寫出。
4. 作答題數超過五題者，將依作答順序取前五題計分，其餘答案不予計分。
5. 一律橫式作答。
6. 作答時請註明「選答試題之題號」，以便閱卷老師之評分。

試題

第一題

During the stationary phase of a batch culture of the epipellic diatom *Cylindrotheca closterium*, accumulation of exopolysaccharides and intracellular carbohydrates was observed. When nitrogen was added to the culture in the stationary phase, growth was resumed and the accumulation of exopolysaccharides was delayed. This indicated that nitrogen depletion caused cessation of growth, and stimulated exopolysaccharide accumulation. Exopolysaccharide accumulation was also stimulated when cells were either resuspended in medium lacking N or P, or when they were inoculated in medium with low concentrations of N or P. Growth was not immediately affected by low N or P concentrations. S depletion only resulted in exopolysaccharide accumulation when growth was affected. Si or Fe depletion did not stimulate exopolysaccharide accumulation, even when growth rates were lowered. Apparently, stimulation of exopolysaccharide accumulation is dependent on the type of nutrient depletion. Intracellular storage carbohydrates did not accumulate when cells were incubated at low N or P concentrations. Cells grown with ammonium as nitrogen source produced more carbohydrates (both extracellular and intracellular) than cells grown with nitrate as nitrogen source, indicating that both exopolysaccharides and intracellular carbohydrates accumulated as a result of overflow metabolism.

第二題

Cathepsin-like proteinases in clam visceral extract was purified through the serial steps of acidification, cysteine-EDTA activation/stabilization, ultrafiltration concentration and ethanol fractionation. Overall, 77.8 % of the total proteolytic activity was recovered with 15.7-fold increase in enzyme purity. Effective separation of cathepsin B-like and D-like proteinases was achieved in different ethanol fractionations. Approximately 72.5 % of the total proteolytic activity of the 40-55 % ethanol fraction and 86.9 % of the total proteolytic activity of the 55-70 % ethanol fraction were contributed by cathepsin B-like and D-like enzymes, respectively. When rib muscles from a 6-year-old cow were dipped at 10 or 20 oC for 20 h with solutions of equivalent total proteolytic activity from different ethanol fractions, obvious and statistically significant differences ( $P < 0.05$ ) in Warner-Braztler shear forces and sensory tenderness scores were observed between control samples and samples treated with cathepsin B-rich ethanol fraction, but differences between control samples and samples treated with cathepsin D-rich ethanol fraction were less significant.

第三題

A trypsin inhibitor was purified from the egg of skipjack tuna *Katsuwonus pelamis* by ammonium sulfate precipitation, gel filtration on a Sephadex G-100 column, ion-exchange chromatography on diethylaminoethyl-Sepharose, rechromatography on a Sephadex G-100 column and reversed-phase high-performance liquid chromatography on a  $C_{18}$  column. The molecular mass of the purified trypsin inhibitor was approximately 78 kDa as estimated by gel filtration and 39 kDa by sodium dodecylsulfate polyacrylamide gel electrophoresis. The purified trypsin inhibitor was stable in the pH range from 4.0 to 10.0 and at temperatures below 40°C. The purified inhibitor was rich in Gly, Glu, Ser, Asp and Lys, but poor in Cys, Tyr, Phe, Ile and His. In addition, the activity of the purified trypsin inhibitor was increased in the presence of metal ions such as  $K^+$ ,  $Na^+$ ,  $Mg^{2+}$  and  $Ca^{2+}$ .

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第四題

Polysaccharides generally reduce flavor impact and should be added to the system before final flavoring is complete. There are many possible products to choose from. Propylene glycol alginates (PGA), made by esterifying alginates with propylene glycol, are a good hydrocolloid choice for fruit beverages. The ester groups make it harder for the polymer chains to self-associate in the presence of relatively high concentrations of hydrogen and calcium, so they are not as sensitive to calcium ions as sodium and potassium alginate. They are effective in a pH range of 3 to 6 and have emulsifying, stabilization and suspending capability. The degree of esterification (DE) determines each form of viscosity and emulsification levels. The hydrophobic ester groups allow the PGA molecules to associate slightly with the surface of oil droplets dispersed in water, so it sometimes acts as a "secondary" emulsifier. Blends of PGA and xanthan gum have been successfully used in fruit-juice beverages that require particle suspension, calcium fortification and viscosity control.

第五題

Two strains of *Bacillus subtilis* were isolated from the soil. Crude fungicides obtained from the culture broth of these strains grown aerobically in a medium containing chitin of marine waste displayed antifungal activity on pathogenic *Fusarium oxysporum*. *B. subtilis* W113 and *B. subtilis* W118 exhibited the maximal antifungal activity, when grown in a medium with the supplemented chitin being 1.75 and 0.75%, respectively. The inhibitory effects of the crude fungicides produced by these two strains were not significantly influenced by variation of pH. These crude fungicides were remarkably thermostable, and the inhibitory activities were retained to some extent even after the crude fungicides were heated at 100 .C for 30 min. These characteristics were unique in comparison with other known bio-fungicides. The utilization of chitin of marine waste to produce chitinolytic enzymes/fungicides by *B. subtilis* is seen for the first time.

第六題

Nanoparticles formulated from biodegradable polymers such as poly(lactic acid) (PLA) and poly(lactide-co-glycolide) (PLGA) are being extensively investigated as non-viral gene delivery systems due to their controlled release characteristics and biocompatibility. PLGA nanoparticles for DNA delivery are mainly formulated by an emulsion-solvent evaporation technique using PVA as a stabilizer generating negatively charged particles and heterogeneous size distribution. The objective of the present study was to formulate cationically modified PLGA nanoparticles with defined size and shape that can efficiently bind DNA. An Emulsion-diffusion-evaporation technique to make cationic nanospheres composed of biodegradable and biocompatible co-polyester PLGA has been developed. PVA-chitosan blend was used to stabilize the PLGA nanospheres. The nanospheres were characterized by atomic force microscopy (AFM), photon-correlation spectroscopy (PCS), and Fourier transform infrared spectroscopy (FTIR). Zeta potential and gel electrophoresis studies were also performed to understand the surface properties of nanospheres and their ability to condense negatively charged DNA. The designed nanospheres have a zeta potential of 10mV at pH 7.4 and size under 200nm. From the gel electrophoresis studies we found that the charge on the nanospheres is sufficient to efficiently bind the negatively charged DNA electrostatically. These cationic PLGA nanospheres could serve as potential alternatives of the existing negatively charged nanoparticles.

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第七題

The yeast transcription factor Yap I activates expression of antioxidant genes in response to oxidative stress. YapI regulation involves nuclear accumulation, but the mechanism sensing the oxidative stress signal remains unknown. We provide biochemical and genetic evidence that upon  $H_2O_2$  treatment, YapI is activated by oxidation and deactivated by enzymatic reduction with Yap I-controlled thioredoxins, thus providing a mechanism for autoregulation. Two cysteines essential for YapI oxidation are also essential for its activation by  $H_2O_2$ . The data are consistent with a model in which oxidation of Yap I leads to disulfide bond formation with the resulting change of conformation masking recognition of the nuclear export signal by Crml/Xpol, thereby promoting nuclear accumulation of the protein. In sharp contrast to  $H_2O_2$ , diamide does not lead to the same YapI oxidized form and still activates mutants lacking cysteines essential for  $H_2O_2$  activation, providing a molecular basis for differential activation of YapI by these oxidants. This is the first example of an  $H_2O_2$ -sensing mechanism in a eukaryote that exploits the oxidation of cysteines in order to respond rapidly to stress conditions.

第八題

In this study, evidence for two novel metabolic processes catalyzed by a filamentous fungus, *Graphium* sp. strain ATCC 58400, is presented. First, our results indicate that this *Graphium* sp. can utilize the widely used solvent diethyl ether (DEE) as the sole source of carbon and energy for growth. The kinetics of biomass accumulation and DEE consumption closely followed each other, and the molar growth yield on DEE was indistinguishable from that with *n*-butane. *n*-Butane-grown mycelia also immediately oxidized DEE without the extracellular accumulation of organic oxidation products. This suggests a common pathway for the oxidation of both compounds. Acetylene, ethylene, and other unsaturated gaseous hydrocarbons completely inhibited the growth of this *Graphium* sp. on DEE and DEE oxidation by *n*-butane-grown mycelia. Second, our results indicate that gaseous *n*-alkane-grown *Graphium* mycelia can cometabolically degrade the gasoline oxygenate methyl *tert*-butyl ether (MTBE). The degradation of MTBE was also completely inhibited by acetylene, ethylene, and other unsaturated hydrocarbons and was strongly influenced by *n*-butane. Two products of MTBE degradation, *tert*-butyl formate (TBF) and *tert*-butyl alcohol (TBA), were detected. The kinetics of product formation suggest that TBF production temporally precedes TBA accumulation and that TBF is hydrolyzed both biotically and abiotically to yield TBA. Extracellular accumulation of TBA accounted for only a maximum of 25% of the total MTBE consumed. Our results suggest that both DEE oxidation and MTBE oxidation are initiated by cytochrome P-450-catalyzed reactions which lead to scission of the ether bonds in these compounds. Our findings also suggest a potential role for gaseous *n*-alkane-oxidizing fungi in the remediation of MTBE contamination.

第九題

The chemical composition of the largely used tea from lemon verbena, *Aloysia triphylla*, was investigated. The qualitative and quantitative composition of the main aromatic and polyphenolic constituents of tea made by infusion from leaves were examined. The results showed that the relative proportions of the active constituents differ from those of the original leaves. The tea contained a large amount of polyphenolic compounds (mean value 675 mg/l; extraction yield 65%) including verbascoside (400 mg/l) and luteolin 7-diglucuronide (100 mg/l). It contained also 42 mg/l of essential oil (extraction yield 51%) with much more citral (77% of the essential oil) than the original leaves (41%).

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第十題

This article presents a novel drug release model that combines drug dissolution, diffusion, and immobilization caused by adsorption of the drug to the tablet constituents. Drug dissolution is described by the well-known Noyes-Whitney equation and drug adsorption by a Langmuir-Freundlich adsorption isotherm, and these two processes are included as source and sink terms in the diffusion equation. The model is applicable to tablets that disintegrate into a number of approximately spherical fragments. In order to simplify the analysis it is assumed that liquid absorption, matrix swelling, and tablet disintegration are much faster than drug dissolution and subsequent drug release. The resulting model is shown to yield release characteristics in good agreement with those observed experimentally.