大葉大學九十一學年度　研究所博士班　招生考試試題紙					
系　所	組別	考試科目（中文名稱）	考試日期	節次	備　註
生物產業科技	甲	專業英文論文閱讀能力測驗	4月14日	第 1 節	總共四頁 P4-1

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）

1. 筆試題目共有十題，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. 人名、專有名詞以及無適當中文翻譯之名詞，如菌種、酵素名稱等，可依原文寫出。
4. 作答題數超過五題者，將按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。

題目：

1. A strain (designated DP-152), which produces an excellent flocculating substance, was isolated from soil samples and identified as *Bacillus* species. The major flocculating substance (bioflocculant DP-152) produced by *Bacillus* sp. DP-152 was purified by ethanol precipitation and cetylpyridinium chloride (CPC) precipitation and gel permeation chromatography. In kaolin suspension, the highest flocculating activity was obtained at the bioflocculant concentration of 1 mg/*l*. The bioflocculant DP-152, which has an estimated molecular weight of over 2×10^6 daltons, is a novel bioflocculant derived from sugar components consisting of glucose, mannose, galactose, and fucose in an approximate molar ratio of 8 : 4 : 2 : 1. Some of its physico-chemical properties were also determined.

2. The last decade has seen a great increase in innovative improvements of lactic acid bacteria used in industrial food fermentations. In order to allow the genetically modified lactic acid bacteria to reach the market place, their novel genetic combinations should be selected, stably maintained, and expressed using food-grade systems that are safe, stable, and sustainable. This paper aims to review the food-grade systems that have been constructed with specific attention for self-cloning approaches, and focuses on recent developments on food-grade selection markers, chromosome inactivation, stabilization, and amplification strategies, as well as approaches for controlled gene expression.

3. Calcium (Ca^{2+}) efflux from the cytosol modulates Ca^{2+} concentrations in the cytosol, loads Ca^{2+} into intracellular compartments, and supplies Ca^{2+} to organelles to support biochemical

functions. The Ca^{2+} /H^+ antiporter *CAX1* (for *CALCIUM EXCHANGER 1*) of Arabidopsis is thought to be a key mediator of these processes. To clarify the regulation of *CAX*1, we examined *CAX1* RNA expression in response to various stimuli. *CAX1* was highly expressed in response to exogenous Ca^{2+}. Transgenic tobacco plants expressing *CAX1* displayed symptoms of Ca^{2+} deficiencies, including hypersensitivity to ion imbalances, such as increased magnesium and potassium concentrations, and to cold shock, but increasing the Ca^{2+} in the media abrogated these sensitivities. Tobacco plants expressing *CAX1* also demonstrated increased Ca^{2+} accumulation and altered activity of the tonoplast-enriched Ca^{2+}/H^+ antiporter. These results emphasize that regulated expression of Ca^{2+}/H^+ antiport activity is critical for normal growth and adaptation to certain stresses.

4. A simple, yet robust and stable alternative to proportional, integral, derivative (PID) gain scheduling is developed using fuzzy logic. This fuzzy gain scheduling allows simple online duplication of PID control and the online improvement of PID control performance. The method is demonstrated with a physical model where PID control performance is improved to levels comparable to, model predictive control. The fuzzy formulation is uniquely characterized by; (i) one fuzzy input variable involving the PID manipulated variable, (ii) two parameters to be tuned, while previously tuned PID parameters are retained, and (iii) a gain scheduling differential equation which relates the fuzzy and conventional PID manipulated variables and enables fuzzy gain scheduling.

5. A potentiometric urea-sensitive biosensor using a NH_4^+-sensitive disposable electrode in double matrix membrane (DMM) technology as transducer is described. The ion-sensitive polymer matrix membrane was formed in the presence of an additional electrochemical inert filter paper matrix to improve the reproducibility in sensor production. The electrodes were prepared from one-side silver-coated filter paper, which is encapsulated for insulation by a heat-sealing film. A defined volume of the NH_4^+-sensitive polymer matrix membrane cocktail was deposited on this filter paper. To obtain the urea-biosensor a layer of urease was cast onto the ion-sensitive membrane. Poly (carbamoylsulfonate) hydrogel, produced from a hydrophilic polyurethane prepolymer blocked with bisulfite, served as immobilisation material. The disposable urea-sensitive electrode was combined with a disposable Ag/AgCl reference electrode to obtain the disposable urea biosensor. The sensor responded rapidly and in a stable manner to changes in urea concentrations between 7.2×10^{-5} and 2.1×10^{-2} mol/l. The detection limit was 2×10^{-5} mol/l urea and the slope in the linear range 52 mV/decade. By taking into consideration the influence of the interfering K^+- and Na^+- ions the sensor can be used for the determination of urea in human blood and serum samples (diluted or undiluted). A good correlation was found with the data obtained by the spectrophotometric routine method.

6. Since lipid auto-oxidation during wort boiling is a determining for the appearance of staling flavor in aged beers, we have investigated the reducing power of hops added in the boiling kettle. An assay based on the inhibition of linoleic acid oxidation in the presence of an initiator [2,2'-azobis(2-amidino-propane) dihydrochloride = AAPH] enabled us to distinguish hop varieties and conditionings. Large differences in hop flavanoid contents explained the higher antioxidant activity of low-α-acid samples versus bitter varieties and CO_2 hop extracts. As expected, adding hop pellets to the kettle effectively increased the overall reducing activity of wort. Supercritical CO_2 hop extracts had no significant effect due to their extremely low level of polyphenols. The concentration of the very well-known marker of beer ageing, *trans*-2nonenal, was lower in boiled wort exhibiting a better reducing power. The AAPH reducing power test applied to hops or worts was thus efficient to predict the nonenal synthesis during boiling. Hop varieties and conditionings emerged from this work as key-parameters for improving the reducing power wort and the flavor stability of the final product.

7. A simple and improved method of preparing highly soluble chitosan (half N-acetylated chitosan) was developed using a series of chitosan samples of low molecular weights, and the solubility of the half N-acetylated chitosan in water and organic solvents was investigated in detail. To reduce the molecular weight, chitosan was treated with $NaBO_3$ under the condition that chitosan was homogeneously dissolved in aqueous acetic acid. Weight-average molecular weights of the obtained chitosan samples were determined using a size-exclusion chromatography system equipped with a low-angle laser light-scattering photometer. Each chitosan sample was then N-acetylated with acetic anhydride under the condition that chitosan was homogeneously dissolved in aqueous acetic acid again. The water solubility of the half N-acetylated chitosan thus prepared increased with decreasing molecular weight. From ^{1}HNMR spectroscopy, it was suggested that the sequence of N-acetylglucosamine residues was random. The solubility of the half N-acetylated chitosan of low molecular weight was rather high even in aqueous dimethylacetamide and dimethylsulfoxide.

8. The above five strains of *Monascus* species were maintained on potato dextrose agar plates at 25℃. In the investigation of the culture condition, growth was carried out in a basal medium containing 0.1% yeast extract, 0.1% polypepton, 0.1% K_2HPO_4, and 0.05% $MgSO_4 \cdot 7H_2O$, 0.01% $FeSO_4 \cdot 7H_2O$, 0.3% $NaNO_3$, 0.05% KCl (pH7), and gradually supplemented with the various carbon sources to be investigated. The major ingredients being investigated included sucrose, SCSP, and chitin. They were added and investigated in one kind at a time fashion. 100 ml of the resultant medium in a 250-ml Erlenmeyer flask was aerobically cultured at 25℃ for 48hrs on a rotary shaker (180 rpm). After centrifugation (12,000 x *g*, 4℃, for 20 min, Beckman J2-21 M/E), the supernatant was collected for measurement of chitinase activity and antifungal activity against *F. oxysporum*. Usually an effective experiment prior condition was used as the

basis for the later experiment until the optimal culture composition was obtained. With the use of the optimal culture composition, the effects of the initial pH, temperature, culture volume, and cultivation time on the production of antimicrobial chitinase were investigated in the same fashion until the optimal condition was found. *M. purpureus* CCRC 31499 showed the highest capability in producing antifungal chitinase.

9. For the production of the chitinase, *M. purpureus* CCRC31499 was grown in 100ml of liquid medium in an Erlenmeyer flask (250 ml) containing 1% shrimp and crab shell powder, pH 7. Two milliliters of the seed culture was transferred into 100 ml of the same medium and grown in an shaking incubator for 4 days at 25℃ and pH 7. The culture broth was centrifuged (4℃ and 12,000 x *g* for 20 min.), and the supernatant was used for further purification by chromatography. To the cell-free culture broth (1600 ml), ammonium sulfate was added (608 g/L). The resultant mixture was kept at 4℃ overnight and the precipitate formed was collected by centrifugation at 4℃ for 20 min at 12,000 x *g*. The precipitate was dissolved in a small amount of 50 mM sodium phosphate buffer (pH 7), and dialyzed against the buffer. The resultant dialysate (90 ml) was loaded onto a DEAE-Sepharose CL-6B column (5 by 30 cm) pre-equilibrated with 50 mM sodium phosphate eluting buffer (pH 7). The unadsorbed materials were washed from the column with the same eluting buffer, and the enzymes were fractionated with a linear gradient of 0 to 1 M NaCl in 50 mM phosphate buffer. The flow rate was 75 ml/h. The eluted fractions were dialyzed against 50 mM sodium phosphate buffer (pH 7, 4℃) for 24 h to remove NaCl, and assayed for antifungal and chitinase activities. The resultant dialysate (25 ml) was loaded onto a Sephacryl S-200 gel filtration column (2.5 by 120 cm) which had been equilibrated with 50 mM phosphate buffer (pH 7), then eluted with the same buffer at a flow rate of 20 ml/h. Fractions (5 ml each) were automatically collected, and assayed for antifungal activity and chitinase activity.

10. The percentages of adsorption of Rhodamine 6G (a kind of dye) from water using both chitosan and activated carbon adsorbents at three particle sizes (0.5, 0.75, and 1.0 mm), temperatures (30, 45, and 60℃), and initial pH values (7, 8, and 9) were compared. The Box-Behnken design was applied in a second-degree quadratic polynomial regression model to test the effects and interactions of the variables using three-factorial experimental designs. The statistical analysis revealed that the second-degree quadratic model gave a good fit with an R^2-value of 0.9704 and 0.9989, and an F-value of 25.5 and 719.9 with chitosan and activated carbon, respectively. The significance for each variable on the adsorption of dye Rhodamine 6G was identified, and the optimal conditions for dye removal were obtained.