surface methodology was used to optimize the concentration of nitrogen sources. A strong stimulation on avermectin B1a production in *S. avermitilis* was observed by 3% glucose feeding at the time of residual glucose being 1%. The avermectin B1a productivity could be further improved by another glucose feeding at 206 hour of cultivation. Avermectin B1a titer was increased by 86.3% and the proportion of avermectin B1a in the total avermectins was increased from 38% to 45% through medium optimization and glucose feeding process. These results would be very useful for enhancing productivity of avermectin B1a in up-scaled processes.

H311

Development of a Simplified Purification Process of Avermectin B1a from Fermentation Broth of *Streptomyces avermitils* YA99-40

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Avermectin B1a is the most potent anthelmintic and insecticidal antibiotic among eight avermectin derivatives produced by *Streptomyces avermitilis*. To circumvent difficulty in the purification of avermectin B1a from the other avermectins, especially avermectin B1b, a new *S. avermitilis* mutant YA99-40 was developed by mutagenesis through ultraviolet light irradiation and protoplast fusion. Avermectins were extrated with 5:1 mixture of ethylacetate and acetone, then applied to a column packed with XAD-2000 resin. The avermectins were eluted with 95% methanol. The pooled avermectins B1a was concentrated and further purified by crystallization. Analysis result of the purified avermectin B1a showed that the proportion of avermectin B1 was more than 95%. This process was simple and applicable to up-scaled purification processes

H312

Synthesis of 7a-hydroxycephalosporin C by Immobilized Enzyme

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In the chemical synthesis of 7a-methoxylated cephalosporins from cephalosporins substrate, difficulties have been experienced such as low conversion yield and generation of toxic wastes. Enzymatic 7a-methoxylation has been studied to alleviate these problems. The methoxylation of cephalosporin C by enzymes was reported to proceed by two step reactions which involves the formation of 7a-hydroxy cephalosporin C with subsequent methylation to yield 7a-methoxycephalosporin C. We have attempted to synthesize 7a-hydroxycephalosporin C from cephalosporin C as the first step with immobilized cephalosporin 7a-hydroxylase. First of all, the conversion of cephalosporin C to 7a-hydroxycephalosporin C was examined with the cell-free extract of several cephamycin producing strains for the selection of converting strain. *Streptomyces clavuligerus* ATCC 27064 was the most potent strain for the activity of cephalosporin 7a-hydroxylase. The cephalosporin 7a-hydroxylase was purified to near homogeneity through DEAE-sephacel chromatography, ammonium sulfate

fractionation, Sephadex G-75 gel filtration, and Affi-gel blue chromatography. Partially purified and immobilized cephalosporin 7a-hydroxylase with resins were used to synthesize 7a-hydroxy cephalosporin C from the substrate, cephalosporin C. The molecular weights of the product isolated from the reaction mixture were determined by ESI-Mass to be 431. ^{1}H NMR also support the conversion of cephalosporin C to 7a-hydroxycephalosporin C by immobilized enzyme.

H313

Neural Networks and Molecular Analyses of Recycling Piggery Slurry Treatment System

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A recycling reactor system operated under sequential anoxic and oxic conditions for the swine wastewater has been developed, in which piggery slurry is fermentatively and aerobically treated and then part of the effluent is recycled to the pigsty. The most dominant heterotrophs were *Alcaligenes faecalis* (TSA-3), *Brevundimonas diminuta* (TSA-1) and *Abiotrophia defectiva* (TSA-2) in order whereas lactic acid bacteria (MRS-1, *etc.*) were dominantly observed in the anoxic tank. One of the most dominant aerobes was *Alcaligenes faecalis* (TSA-3). The most dominant species of LAB was strain MRS-1 that was yet to be characterized. In this study, we tried to model the treatment process for each tank in the system based on population densities of heterotrophic and lactic acid bacteria. Principal component analysis (PCA) was first applied to identify a relation between input (microbial densities and parameters for the treatment such as population densities of heterotrophic and lactic acid bacteria, suspended solids (SS), COD, NH_4^+-N, *ortho*-P, and total-P) and output. Multi-layer neural networks using error back propagation learning algorithm were then employed to model the treatment process for each tank. PCA filtration of input data as microbial densities was found to facilitate the modeling procedure for the system monitoring even with a relatively lower number of input. Neural networks independently trained for each treatment tank and their subsequent combined data analysis allowed a successful prediction of the treatment system for at least two days. The isolated heterotrophs were found to uptake ammonium as a sole nitrogen source for their growth. The molecular analysis data (PCR and Southern hybridization) showed that glutamine synthetase (GS) could be involved in ammonium utilization by the heterotrophic bacteria isolated from the treatment system.

H801

Overexpression and Purification of *Listeria grayi* p60 Protein in *Escherichia coli.*

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The *iap*(invasion associated protein) gene from *Listeria grayi* encoding listerial major extracellular protein p60 was cloned and expressed with expression vector