ABSTRACT

The actinomycete *Amycolatopsis* sp. RSP-3 is used for the production of rifamycin B by fermentation. Various fermentation strategies such as submerged fermentation, solid state fermentation and whole cell immobilization were exploited to maximize the production of rifamycin B by the selected actinomycete. A two step methodology was employed for optimization of rifamycin B. In the first stage, the conventional one variable at a time methodology was employed to select the suitable fermentation parameters. In the second stage, taguchi methodology was employed to fine tune the levels of the critical parameters those noticed to influence the production of rifamycin B drastically during optimization studies carried out via conventional one variable at a time methodology

The production of rifamycin B under submerged fermentation was maximized by optimizing the physical process parameters such as pH, temperature, speed of agitation, medium volume, incubation time, age and size of inoculum level, concentration of calcium carbonate and nutritional parameters such as carbon and nitrogen sources and investigated the effect of barbital a very well known inducer of rifamycin B. Under submerged fermentation, 4.5 g/L of rifamycin B production was observed when fermentation was carried out with 25 ml medium volume, containing glucose as carbon source and soya bean meal and potassium nitrate as organic and inorganic nitrogen sources adjusted to pH 8.0 when inoculated with 72 hours grown 10% inoculum and incubated at 28 ^{o}C at an agitation speed of 250 rpm, for a period of 9 days. Addition of barbital after 24 hours of inoculation elicited a 10% improvement in rifamycin B

production. During optimization studies it was observed that the optimum concentration of barbital depends on the concentration of inorganic nitrogen source selected.

Further enhancement of rifamycin B under submerged fermentation was carried out using taguchi methodology with L18 orthogonal array. Six critical fermentation controlling factors such as inoculum level, soyabean meal, glucose, calcium carbonate, barbital concentrations were selected for this study. The design of experimental methodology by taguchi approach aided in identification of most significant parameters that effected rifamycin B production at interactive and individual levels. The data suggested that production of rifamycin B was mostly influenced by the variation in concentration of soyabean meal. It is interesting to note that under both methodologies (conventional i.e., one variable at a time as well as Taguchi) this nutrient was observed to be significant for production of rifamycin B. Approximately a 13.19% improvement was observed in rifamycin B yields on optimizing significant parameters using this methodology.

Whole cell immobilization of *Amycolatopsis* sp. RSP-3 was attempted using various supports such as alginate, agar and coconut fiber. Of all the supports that were, screened coconut fiber was found to be suitable support for the whole cell immobilization of *Amycolatopsis* sp. RSP-3. Comparative evaluation of different supports, suggested that growth of the microorganism should not be restricted and free diffusion of gases and nutrients should be facilitated. Repeated batch production of rifamycin B suggested that yield (4.5±0.1g/L) was observed to be consistent up to 5 cycles and then decreased further. Though the production of rifamycin B was observed to be consistent upto 5 cycles, a 10% decrease in production was observed when compared to free cells.

Under immobilized conditions process parameters such as substrate weight, medium volume, level of aeration, size of inoculum, number of cycles, concentrations of glucose and soyabean meal were investigated. Four grams of coconut fiber was found to support rifamycin production. All other factors remained the same as that for free cells.

The production of rifamycin B under solid state fermentation conditions was investigated. Various agricultural wastes which act as a source of carbon were selected for screening. Rice bran, corn husk, wheat bran, and corn cobs were screened. Among all these agriwastes, corn husk supported more rifamycin B production by selected microorganism. Hence it was selected as the solid substrate and further studies were carried out to maximize the rifamycin B production under solid state fermentation.

The process parameters such as initial pH, moisture level, particle size, age and size of inoculum, length of incubation time, effect of barbital, glucose, soyabean meal, potassium nitrate were optimized under solid state fermentation conditions. Here too taguchi methodology was employed to optimize the maximum requirements of critical factors such as inoculum level, length of incubation time, concentration of nutritional factors such as soyabean meal, potassium nitrate, glucose and barbital. An L-18 orthogonal array with the above mentioned parameters as controlling factors was used for fine tuning the antibiotic production. The data revealed an overall yield of 18.75 g/Kgds of rifamycin B which accounted for nearly 100% improvement in rifamycin B production.

Extraction of rifamycin B from solid substrate was carried out using phosphate buffer at pH -7.3, under agitation at 200 rpm and temperature of 28 ^{o}C for 4 hours.

This is the first report on production of rifamycin B using corn husks and coconut fiber discs as solid substrate and immobilization support respectively. The investigations suggest that production of rifamycin B by solid state fermentation is more beneficial compared to other fermentation strategies(submerged(5.12 g/L) and whole cell immobilization(4.5±0.1 g/L)) as it supported high titers of rifamycin B production(18.75 g/Kgds).