ABSTRACT

Fermentation and maturation are the most time consuming steps in the production of beer, the duration of which is typically between 5–7 and 7–30 days, respectively. The continuous fermentation process based on immobilised yeast cell technology allows producing an acceptable end product within as little as 2–3 days. In spite of the economic advantages that continuous beer fermentation offers, difficulties of technical and economic origin have retarded the implementation of the process at industrial scale so far. For example, the total investment costs depend significantly on carrier costs and applied technology. Thus the use of cheap carrier materials in a suitably designed bioreactor could favour the economics of the immobilised process, inspire researchers and encourage brewing engineers. Among the available yeast cell immobilisation techniques, flocculation of micro-organisms, due to its simplicity and low cost, is very attractive because there are no complex mechanical devices needed as well as any supporting material in this technique. This can be an advantage over other immobilisation techniques since it is well known that a support represents a major cost in immobilisation procedures.

Flocculation of yeast cells usually observed at the end of fermentation and is of great importance in beer brewing. It can occur naturally or it can be artificially induced by different agents. The focus of the study was to investigate the immobilisation of yeast cells onto Carbon Nanotubes (CNTs) using flocculation method. CNTs; long, thin cylinders of carbon; can be used as artificial agents to induce flocculation of yeast cells because they are increasingly being recognised as promising materials for catalysis, either as catalysts themselves, as catalyst additives or as a catalyst support. CNTs are inert and positively charged which enable them to attract negatively charged yeast cells to form flocs. The use of CNTs to improve yeast flocculation for fermentation processes has not been reported yet in literature.

CNTs were synthesised by Swirled Floating Catalyst Chemical Vapour Deposition (SFCCVD) method. The optimum conditions required to synthesise the best samples of CNTs were temperature of 800 ^{o}C, acetylene flowrate of 844 ml/min for a reaction time of 20 minutes. The synthesised CNTs were characterised by Transmission Electron Microscopy (TEM) and Raman Spectroscopy to obtain the type of nanotubes, morphology and their purity.

The yeast cells (*Saccharomyces cerevisiae* strain NRRL Y2084, a dry brewer's yeast obtained from National Food Products, Emmarentia, Johannesburg) were immobilised onto CNTs by flocculation method to produce a immobilised cells. The flocculation process was measured by two methods: a qualitative process of using the naked eye to rank the flocs as either -, +, ++ or +++ and a quantitative method of measuring the floc weight recovered using a centrifuge and dried in an oven at 40 ^{o}C for 24 hours. The flocculation of the immobilised cells was compared with a control experiment which had free cells. The immobilised cells and free cells were both recovered and dried using a freeze dryer for analysis and use in fermentation. Conditions required for the flocculation process were an agitation speed of 110 rpm, pH 5.60, a temperature of 30 ^{o}C and concentration of 53.57 μg/ml of CNTs. Addition of calcium ions at 5.49 mM resulted in good flocculation but the presence of glucose delayed onset of flocculation by 4 – 5 days. The flocculated cells were characterised by Scanning Electron Microscopy (SEM) and Optical Microscopy. The flocculation conditions used in the study were comparable with those in literature.

The immobilised cells and the free cells were introduced into malt extract for fermentation at 15 and 30 ^{o}C. The fermentation rates observed were compared with those found in literature by comparing the final ethanol concentration observed. Free cells produced more ethanol, 2.49 % (v/v) than the immobilised cells, 1.56 % (v/v) at 15 ^{o}C but these values were not comparable to literature with

5.50 % (v/v) for free cells and 5.20 % (v/v) using the immobilised cells. Free cells produced higher alcohol content (0.52 % v/v) than the biocatalyst (0.39% v/v) at 30 ^{o}C but these values were not comparable to the reported literature values of 6.20 % (v/v).