1.0 ABSTRACT

Lactic acid can be produced chemically using petrochemical feed stock or biotechnologically by fermenting carbohydrate feed stock. As growing interest emerges in renewable feed stocks, microbial fermentation using *Lactobacillus casei* subsp. *rhamnosus* has been studied in this work. Preliminary studies were done batchwise with the free cells in basal synthetic medium. For cultivation 42°C was selected and NH_4OH was used as the neutralizing agent. The organism preferred lactose to glucose. When the bacteria was cultivated in basal synthetic medium containing $50gl^{-1}$ lactose, the productivity obtained was $2.02gl^{-1}h^{-1}$. Under optimized conditions the cells were grown in whey permeate medium. The productivity ($2.83gl^{-1}h^{-1}$) and substrate utilization were improved by supplementing whey permeate with yeast extract ($10gl^{-1}$) along with $MnSO_4.H_2O$ ($0.03gl^{-1}$). As yeast extract supplementation constitutes 33% of the production cost, it was decided to hydrolyse the whey proteins and to utilize the peptides for cell multiplication and lactic acid production. The whey protein was hydrolysed with an endoprotease, Neutrase gave the highest productivity of $2.1gl^{-1}h^{-1}$. Further supplementation of whey hydrolysate medium with yeast extract ($2.5gl^{-1}$) and $MnSO_4.H_2O$ ($0.03gl^{-1}$) increased the productivity to $2.8gl^{-1}h^{-1}$ (effective whey medium). To further increase the lactic acid production either $70gl^{-1}$ lactose or glucose (whey production medium) was added to the effective whey medium. Glucose supplementation was incompletely utilized while lactose ($70gl^{-1}$), was completely utilized, and $88.5gl^{-1}$ lactic acid and $3.1gl^{-1}h^{-1}$ productivity were obtained. As lactic acid inhibits the fermentation, *L. casei* subsp. *rhamnosus* cells immobilized to foam glass beads (Pora-bact A, commercial name) were used for continuous lactic acid production. For 1 and 2% PEI coated beads, the time taken to complete the initial cell adsorption batch were 44 and 74h respectively. The cells immobilized to beads coated with 1% PEI gave the average reactor productivity of $4.6gl^{-1}h^{-1}$ during 8 repeated recycle fermentation. When the cells immobilized to the beads coated with 2% PEI were used, the suspended free cells present in the medium decreased and increased the number of possible repeated recycle batches to 12 with the average reactor productivity of $4.3gl^{-1}h^{-1}$. The decrease in yeast extract supplementation to 1 gl^{-1} did not affect the productivity, but the D-lactic acid content was decreased while maintaining the L-lactic acid level at or above 95% of the total lactic acid. When the medium volume was scaled up, sugar consumption and lactate production efficiency were unaltered until the medium volume was increased to 10 times that of the immobilized boicatalyst (with the productivity of $3.43gl^{-1}h^{-1}$). As the immobilized cells preferred lactose to glucose both in synthetic

(containing yeast extract and salts in addition to sugar) and whey production medium, the effect of different ratios of lactose to glucose in the synthetic medium was studied. At lactose to glucose ratios of 3 : 7 and 1 : 19 in the synthetic medium, the productivities were 3.9 and 2.8$gl^{-1}h^{-1}$ respectively and suspended free cells densities of 8.6 and 3.9 (OD 620nm) respectively were obtained. The effect of age of the cells on their ability to get adsorbed to PEI coated beads indicated that, allowing the cells to grow for 8h (log phase) shortened the time required for the initial cell adsorption batch by 52h compared to immediate commencement of recirculation after inoculation. To avoid the channelling effect, 12-16mm mean diameter beads were used instead of 2-4 mm beads and the productivity decreased to 2.2$gl^{-1}h^{-1}$. Highest lactic acid productivity (3.9$gl^{-1}h^{-1}$) was obtained when 50gl^{-1} total sugar (at lactose to glucose ratio of 1 : 19) was used while at 125gl^{-1} total sugar, immobilized cells did not use the substrate completely. The inhibitory effect of lactic acid on immobilized and free cells were studied using the synthetic medium containing 50gl^{-1} total sugar (lactose : glucose = 1 : 19). When 0 and 40gl^{-1} lactic acid were added to the medium the productivities obtained were 3.9 and 1.34$gl^{-1}h^{-1}$ for immobilized cells and for free cells 2.4 and 1.2$gl^{-1}h^{-1}$ respectively. When 50gl^{-1} lactic acid was added to the medium, the sugar was not utilized at all by both the immobilized and free cells. In the range of pH 6.0 to 6.2, lactic acid was the only product and the productivities obtained were almost same (2.8$gl^{-1}h^{-1}$) for the immobilized cells while for free cells in the pH range of 5.5 to 6.0 only lactic acid was produced (productivity 2.3$gl^{-1}h^{-1}$). Lactic acid was purified by precipitating as calcium lactate or by using ion-exchange resin. When lactic acid was precipitated as calcium lactate, 72% was recovered and contained 1.6gl^{-1} total sugar. Amberlite IRA-400 had higher adsorption capacity (186.3g lactic acid / 1000g resin), than the Amberlite IRA-401 at pH for 6.0. However the adsorption capacity decreased to 126g lactic acid /kg resin, when decolourised fermented broth was purified. The elution of lactic acid with 2N HCl gave 1.6 fold increase in concentration and the final recovery of lactic acid was 82% with no sugars and by products like formic and acetic acids.