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Optimization of L-(+)-Lactic Acid Production by Ring and Disc Plastic Composite Supports through Repeated-Batch Biofilm Fermentation†

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Four customized bioreactors, three with plastic composite supports (PCS) and one with suspended cells (control), were operated as repeated-batch fermentors for 66 days at pH 5 and 37°C. The working volume of each customized reactor was 600 ml, and each reactor's medium was changed every 2 to 5 days for 17 batches. The performance of PCS bioreactors in long-term biofilm repeated-batch fermentation was compared with that of suspended-cell bioreactors in this research. PCS could stimulate biofilm formation, supply nutrients to attached and free suspended cells, and reduce medium channelling for lactic acid production. Compared with conventional repeated-batch fermentation, PCS bioreactors shortened the lag time by threefold (control, 11 h; PCS, 3.5 h) and sixfold (control, 9 h; PCS, 1.5 h) at yeast extract concentrations of 0.4 and 0.8% (wt/vol), respectively. They also increased the lactic acid productivity of *Lactobacillus casei* subsp. *rhamnosus* (ATCC 11443) by 40 to 70% and shortened the total fermentation time by 28 to 61% at all yeast extract concentrations. The fastest productivity of the PCS bioreactors (4.26 g/liter/h) was at a starting glucose concentration of 10% (wt/vol), whereas that of the control (2.78 g/liter/h) was at 8% (wt/vol). PCS biofilm lactic acid fermentation can drastically improve the fermentation rate with reduced complex-nutrient addition.

A lactic acid molecule has two optical active isomers, the D-(−) and L-(+) forms (12). Optically pure lactic acid is important for the production of polylactide because the physical properties of the polylactide are dependent on the stereochemistry of the individual lactic acid molecule (10). Microbial fermentation is the only source for producing optically pure lactic acid isomers. *Lactobacillus casei* subsp. *rhamnosus* is a homofermenter that produces solely L-(+)-lactic acid (7). Cell immobilization is a common way to increase cell density in fermentation. However, immobilization of cells through the use of calcium alginate beads and polyacrylamide gels is not widely employed in industry because of the high cost of immobilization, mass transfer limitations, lack of stability of the biocatalysts, and changes in product patterns of reactions catalyzed by certain immobilized cells (3).

Biofilms are a natural form of cell immobilization (1). Demirci and Pometto (4) demonstrated that lactic acid fermentation was enhanced when biofilm fermentation was carried out with chips of plastic composite supports (PCS) containing 75% (wt/wt) polypropylene (PP) and 25% (wt/wt) agricultural material. Ho et al. (8, 9) evaluated 24 PCS disc blends containing 50% (wt/wt) PP and 50% (wt/wt) agricultural materials for L-(+)-lactic acid biofilm fermentation in minimal medium with no pH control. Each PCS blend was evaluated for biofilm development, slow release of nutrients, surface contact angle, hydrophobic compatibility with *L. casei*, porosity, and lactic acid absorption. The PCS disc that consistently demonstrated the highest performance contained 50% (wt/wt) PP, 35% (wt/wt) soybean hulls, 5% (wt/wt) soybean flour, 5% (wt/wt) yeast extract, 5% (wt/wt) dried bovine albumin, and mineral salts (SFYB+). Hence, the goal of this study was to compare the performances of bioreactors with and without SFYB+ in long-term biofilm repeated-batch fermentation at a controlled pH (pH 5) and temperature (37°C).

The effects of PCS nutrient leaching on long-term lactic acid fermentation in an environment with a low concentration of complex nutrients were studied. We optimized lactic acid fermentation by lowering the yeast extract concentration in the medium, increasing the starting glucose concentration in the fermentor, and reducing the medium flow rate in the bioreactor's recycling loop. The reduction of nutrients benefits industrial lactic acid fermentation by lowering production costs and enhancing the downstream lactic acid recovery process.

This study demonstrated that PCS bioreactors significantly shortened the lag phase and total fermentation time. Maximum productivity was also improved through biofilm formation and leaching of complex nutrients from PCS.

MATERIALS AND METHODS

PCS. SFYB+ discs and rings containing 35% (wt/wt) soybean hulls, 5% (wt/wt) yeast extract, 5% (wt/wt) soybean flour, 5% (wt/wt) dried bovine albumin, and 50% (wt/wt) PP were produced by high-temperature extrusion by the procedures of Ho et al. (8, 9). The tubes for the SFYB+ rings and discs were extruded through a 12.7-mm-outside-diameter (o.d.) large pipe die with 9.5- and 3.2-mm inside diameters (i.d.), respectively. The SFYB+ tubes were then cut into rings (1-cm i.d., 1.5-cm o.d.) and discs (0.3-cm i.d., 1.1-cm o.d.) with a utility knife.

Bacterial culture preparation. *L. casei* subsp. *rhamnosus* (ATCC 11443), a homofermenter of L-(+)-lactic acid, was obtained from the American Type Culture Collection (Rockville, Md.) and maintained according to the methods described by Ho et al. (8). All fermentation inocula were prepared by transferring 10 ml of an active *L. casei* culture (18 h in MRS broth at 37°C) into 100 ml of lactic acid fermentation medium (20 g of glucose per liter, 4 g of yeast extract [Ardamine Z; Champlain Industries Inc., Clifton, N.J.] per liter, and mineral salts solution [0.5 g of KH_2PO_4 per liter, 0.5 g of K_2HPO_4 per liter, 1 g of sodium acetate per liter, 0.6 g of $MgSO_4 \cdot 7H_2O$ per liter, and 0.03 g of $MnSO_4 \cdot 7H_2O$ per liter]) (5), followed by an 18-h incubation at 37°C.

Medium preparation. All of the dry ingredients of the mineral salts stock solution were mixed with 80 liters of deionized water in a B-Braun 100-D

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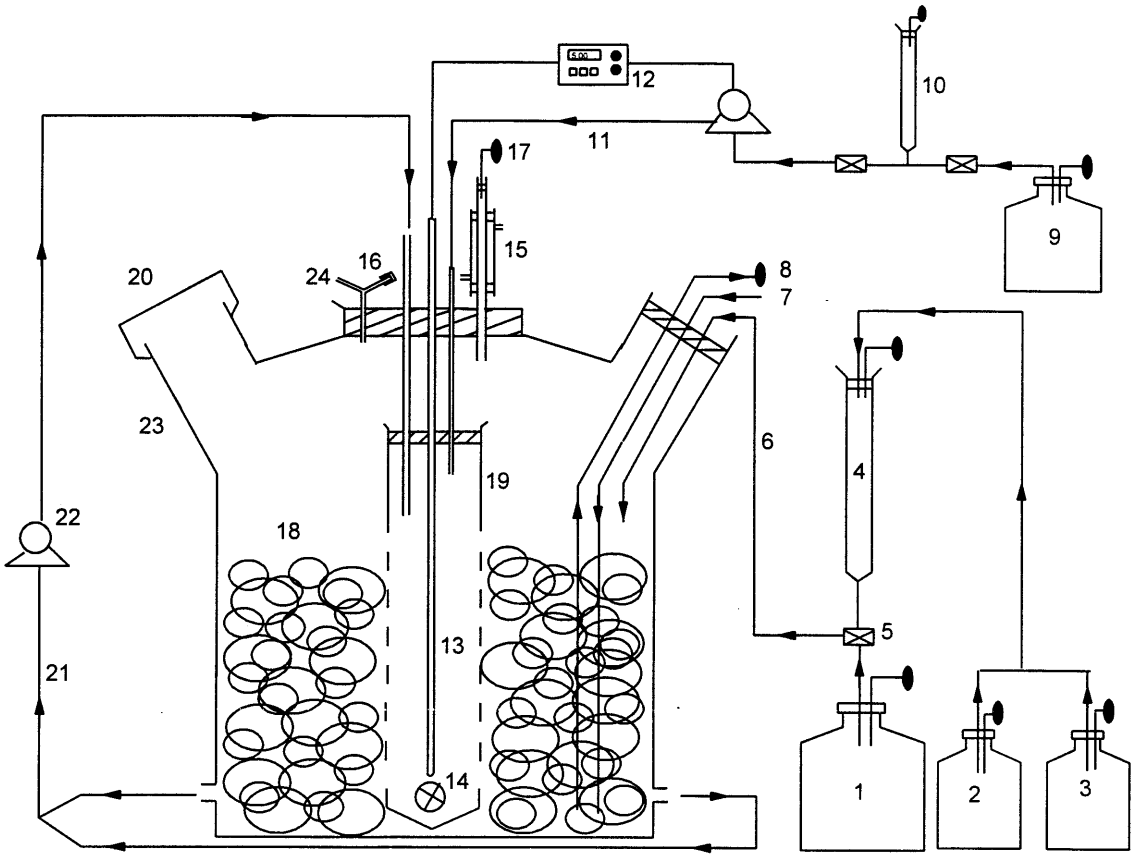

Keys:

1.	Mineral salt solution	13.	pH probe
2.	70% glucose solution	14.	Magnetic stirrer
3.	10% yeast extract solution	15.	Condenser
4.	Media mixing reservoir	16.	Innoculation port
5.	Stopcock	17.	0.45-um sterilized filter
6.	Medium feed line	18.	Plastic composite-supports
7.	CO_2 feed line	19.	pH-controlled basket
8.	Sampling and medium draining port	20.	PCS sampling port
9.	5 N NH_4OH stock solution	21.	Medium recycling line
10.	Alkaline reservoir	22.	Peristaltic pump
11.	Alkaline feed line	23.	Nalgene magnetic culture vessel
12.	pH meter	24.	Extra port

FIG. 1. Schematic diagram of the repeated-batch fermentation system.

fermentor (New Brunswick Scientific, Allentown, Pa.) and sterilized with continuous agitation for 20 min at 121°C and 15 lb/in^2. The pH of the mineral salts stock solution was adjusted to 5 by using 4 N NaOH. The 80 liters of sterilized mineral salts stock solution was then aseptically distributed into four separate sterilized (2 h at 121°C) 20-liter carboys. The 70% (wt/vol) glucose stock solution (Cerelose dextrose 2001 CPC Brand; International Ingredient Corporation, St. Louis, Mo.) and the 10% (wt/vol) yeast extract stock solution for each bioreactor were autoclaved (30 min at 121°C) separately in a 3-liter carboy. Thirty-two liters of 5 N NH_4OH was filter sterilized with a 142-mm cellulosic Triton-free 0.45-μm-pore-size filter (MSI, Westboro, Mass.) and aseptically distributed into four sterilized (1 h at 121°C) 10-liter carboys. All carboys containing stock solutions were equipped with an air vent capped with a 0.45-μm air filter and a medium delivery line with a liquid break to prevent reservoir contamination.

Repeated-batch fermentation system. Four sets of customized bioreactors (A, B, C, and D) were built for long-term repeated-batch lactic acid biofilm fermentation. Each set of customized bioreactors (Fig. 1) was composed of a modified Nalgene magnetic culture vessel (MCV), an alkaline reservoir, and a stock solution mixing reservoir. The right arm of the MCV was equipped with a

medium feed line, a filter-sterilized (0.45 μm) carbon dioxide feed line, and a medium draining and sampling port. The left arm of the MCV was covered by a screw cap, which acted as the SFYB+ sampling port.

The central opening of the MCV contained an inoculation port, a medium recycling line, an alkaline feed line, a condenser, and a pH probe. The alkaline feed line, the medium recycling line, and the pH probe were further connected to a PCS-free pH-controlled basket inside the MCV. The pH-controlled customized basket was made from a 60-ml syringe with 100 bored holes (5-mm i.d.). A magnetic stirrer was placed at the bottom of the pH-controlled basket to ensure that the recycling medium was well mixed with the added alkali for pH control. This allowed immediate detection of alkali addition by the pH probe and prevented overshooting of the alkali into the bioreactor. The system pH was constantly controlled at 5 ± 0.05.

A hole was drilled on each side of the MCV (2 mm above the base) for connecting a 1/4-in. barbed bulkhead fitting. The side of the 1/4-in. barbed bulkhead fitting facing the interior of the MCV was fitted with a section of the silicone tubing that stretched across the bottom of the MCV. The other side of the 1/4-in. barbed bulkhead fitting was linked to a pump, which was connected to the medium recycling line. The medium in the reactor was continuously recirculated from the bottom to the top by the circulation pump at 60 ml/min (unless otherwise stated). The design of the medium recycling loop was done to ensure the homogeneous mixing of the medium throughout the fermentation process.

The refillable alkaline reservoir (a 50-ml burette) was connected to the 5 N NH_4OH stock solution and to a pump for controlling alkali addition. The stock solution mixing reservoir (a 500-ml burette) was connected to various stock solutions (mineral salts, 10% [wt/vol] yeast extract, and 70% [wt/vol] glucose). The medium formulation flexibility was achieved by the stock solution mixing reservoir, which permitted aseptic blending of different amounts of glucose, yeast extract, and mineral salts stock solutions.

The space between the pH-controlled basket and the MCV of bioreactors A, B, and C was filled with 145.0 g of SFYB+ (406 discs [72.7 g] and 377 rings [72.3 g]). The SFYB+ was sterilized dry (45 min at 121°C) in a 2-liter fleaker beaker before transfer into the MCV. After the addition of the SFYB+, 300 ml of deionized water was added to each bioreactor, and the whole system was autoclaved (20 min at 121°C). The sterilized customized bioreactor was then aseptically connected to the 5 N NH_4OH, glucose, yeast extract, and mineral salts stock solution carboys. The soaking water in the bioreactor was subsequently drained through the medium draining port, and the fermentation system was ready to be filled with the specific medium blend.

Repeated-batch fermentation. Four customized bioreactors, three with SFYB+ and one with suspended cells (control), were operated as repeated-batch fermentors for 66 days at controlled pH 5 and 37°C. The ratio of the culture medium working volume (600 ml) to the SFYB+ bulk volume (560 ml) was kept at 1.07 throughout the study. Every 2 to 5 days (depending on the rate of the bioreactors) the media from the bioreactors were drained. Each bioreactor was then rinsed with 500 ml of mineral salts stock solution and refilled with 600 ml of fresh medium with a specific formulation. Seventeen repeated-batch fermentations were performed consecutively within the 66 days.

The entire 66 days of repeated-batch fermentation was divided into three phases. The first phase (first to sixth repeated-batch fermentations) aimed to evaluate the effect of the yeast extract concentration in the medium (0.2, 0.4, and 0.8% [wt/vol]) on the performance of *L. casei*. During this phase, the recycling flow rate and the starting glucose concentration in each bioreactor were 60 ml/min (6 working volumes/h) and 8% (wt/vol), respectively. The yeast extract concentrations in the three SFYB+ bioreactors, A, B, and C, were kept constant at 0.2, 0.4, and 0.8% (wt/vol), respectively, throughout the 66 days. The first to fourth repeated-batch fermentations of bioreactors A, B, and C were used for building up biofilms on the supports, and only the fifth and sixth repeated-batch fermentation results were used for data interpretation. The yeast extract concentration in reactor D (control) in the first to sixth repeated-batch fermentations varied randomly from batch to batch, and a total of two batch fermentations with 0.2, 0.4, and 0.8% (wt/vol) yeast extract medium were run.

The second phase (7th to 10th repeated-batch fermentations) aimed to determine the effect of the medium recycling flow rate on the maximum productivity of each bioreactor with different medium formulations. In this phase, a starting glucose concentration of 8% (wt/vol) was also used. The maximum productivity for each repeated-batch fermentation was determined by measuring the slope on the steepest region of the lactic acid production curve (at least three points were used). The medium yeast extract concentration in bioreactors A, B, C, and D was kept at 0.2, 0.4, 0.8, and 0.8% (wt/vol), respectively. The medium recycling flow rates evaluated were 60, 30, 15, and 7.5 ml/min, which were equivalent to 6, 3, 1.5, and 0.75 working volumes/h, respectively.

The third phase (12th to 17th repeated-batch fermentations) aimed at analyzing the effects of the starting glucose concentration on bacterial performance. The medium yeast extract concentration in each bioreactor was identical to that in the second phase. The medium recycling flow rate was kept at 60 ml/min. The six starting glucose concentrations evaluated were 4, 6, 8, 10, 12, and 16% (wt/vol). In addition, bioreactor D's performance was compared with that of a standard bench-top continuous-stir tank fermentor (2-L B-Braun Biostat M B-Braun; New Brunswick Scientific) which was operating with starting concentrations of 8% (wt/vol) glucose and 1% (wt/vol) yeast extract in the medium at 37°C and controlled pH 5.

Biofilm formation analysis. Five SFYB+ discs and rings (approximately 1 g) were aseptically retrieved from bioreactors A, B, and C, and their biofilm populations were determined by the stripping-sand method (8). The sample from each bioreactor was serially diluted, and CFU of the 10^7 to 10^9 dilutions were determined by using *Lactobacillus* MRS agar spread plates in duplicate. Finally, the five sand-stripped discs and rings from each bioreactor were rinsed with water, convection oven dried (70°C, 24 h), and reweighed.

The biofilm population on the SFYB+ was also evaluated by scanning electron microscopy (SEM). Broken SFYB+ discs and rings were prepared by the procedures described by Ho et al. (8). SEM micrographs of gold-coated critical-point-dried supports were taken with a JSM-35 scanning electron microscope (JEOL, Tokyo, Japan) at 25 kV.

Biofilm formation was further analyzed indirectly by measuring the amount of exopolysaccharides (EPS) (total complex carbohydrates) present in the fermentation media of the four bioreactors by comparing the reducing sugar concentration as determined by Somogyi-Nelson and phenol-sulfuric acid assays (13). If a polysaccharide was present, then the phenol-sulfuric acid assay would hydrolyze the polymer to monosaccharides, which would translate into a high concentration of reducing sugars, whereas the Somogyi-Nelson assay measured only the free reducing sugars in freeze-dried medium. The total weight loss of SFYB+ in bioreactors A, B, and C was determined by calculating the difference between the initial weight of SFYB+ and its convection-dried (60°C overnight) weight after the 66 days of repeated-batch fermentation.

Fermented-medium analysis. L-(+)-Lactic acid and D-glucose concentrations were analyzed by high-performance liquid chromatography by procedures described by Ho et al. (8). Growth of suspended-cells in the medium was monitored by measuring the absorbance at 620 nm with a Spectronic 20 spectrophotometer (Milton Roy Co., Rochester, N.Y.).

Statistical analysis. Statistical analysis was carried out by using the two-way analysis of variance in the Statistical Analysis System package (version 6.03) (SAS Institute Inc., 1985).

RESULTS AND DISCUSSION

Effects of SFYB+ and yeast extract concentrations on repeated-batch fermentation. The fermentation of reactor D (control) with 0.8% (wt/vol) yeast extract medium demonstrated a pattern between type I (growth associated) and type II (non-growth associated) fermentation (2) (Fig. 2, top). *L. casei* in reactor D reached its stationary phase after 12 h of fermentation, while the concentration of lactic acid continued to increase. The non-growth-associated fermentation (as indicated by the continued decrease of the glucose consumption curve) was probably a result of the need of *L. casei* to produced energy (ATP) for maintaining its internal pH by pumping out protons as the lactic acid concentrations inside and outside the cell increased (7, 11).

In contrast, reactor C (PCS bioreactor in 0.8% [wt/vol] yeast extract medium) under the same fermentation conditions as used for reactor D demonstrated a type I fermentation pattern (Fig. 2, bottom). Most likely this was the result of the biofilm population in reactor D constantly producing lactic acid (including during the lag phase) under the type II fermentation pattern. Hence, when the suspended cells reached stationary phase, all of the glucose in reactor D was consumed, as indicated by the glucose consumption curve. This also would explain the higher lactic acid production rates by the *L. casei* in the PCS bioreactors.

The final suspended-cell densities of *L. casei* in reactors A, B, and C were 7.2, 10, and 13.5 absorbance units, respectively (Fig. 3). Similarly, the suspended-cell densities in reactor D (control) were 5, 12.5, and 16 absorbance units when the medium yeast extract concentration was 0.2, 0.4, and 0.8%, respectively (Fig. 3). This indicated that the medium yeast extract concentration had a great impact on *L. casei* growth.

Although the glucose consumption and L-(+)-lactic acid production rates in reactor C (maximum lactic acid productivity, 3.6 g/liter/h) were much higher than those in reactor D (maximum lactic acid productivity, 2.5 g/liter/h) in 0.8% yeast extract medium, the medium cell density in reactor C (final cell density, 13.5 absorbance units) was significantly lower than that in reactor D (final cell density, 16 absorbance units) (Fig. 3,

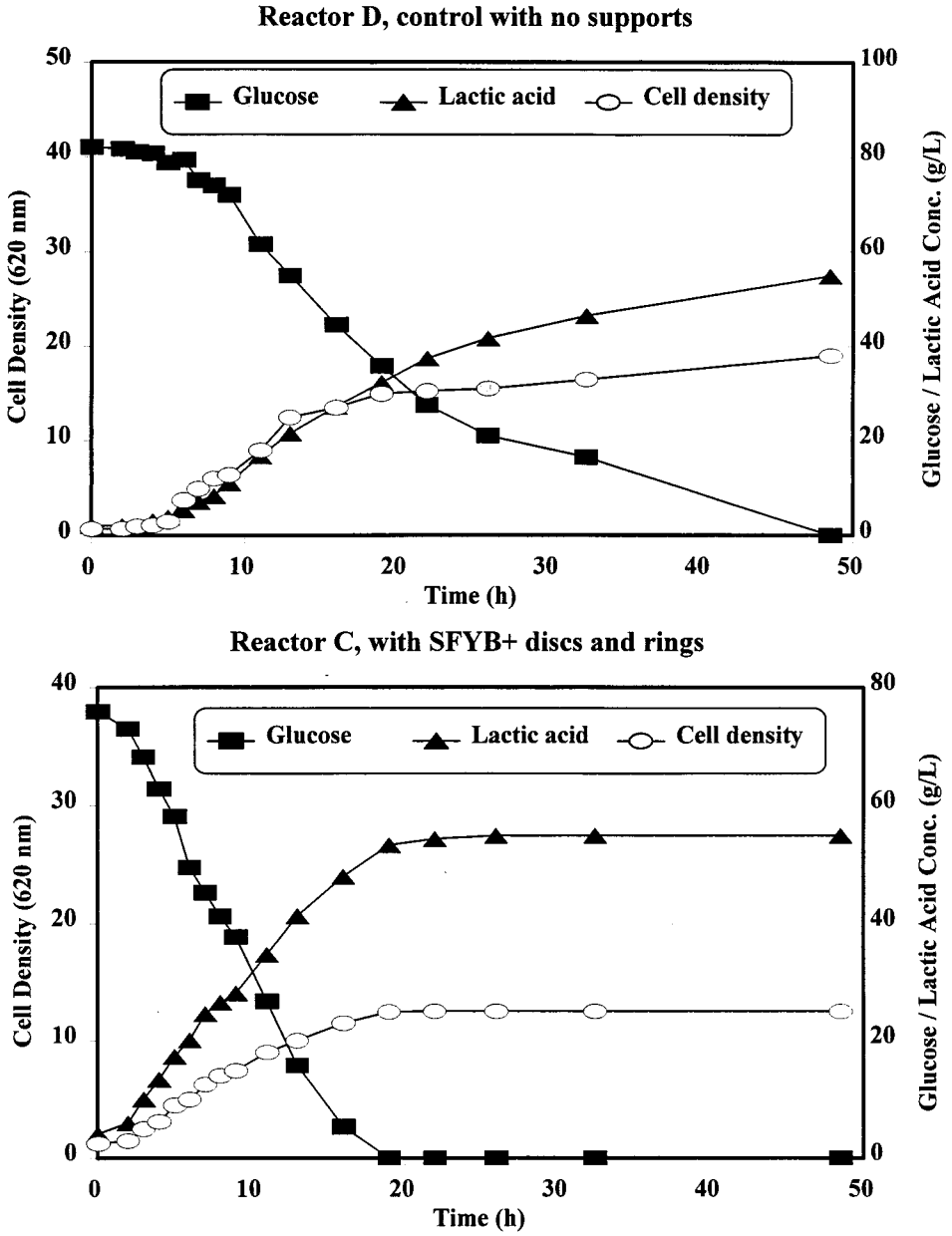

FIG. 2. Lactic acid production, glucose consumption, and cell density of *L. casei* in reactors containing 0.8% yeast extract medium. Reactor D was the control with no supports (top), and reactor C was the PCS bioreactor with SFYB+ discs and rings (bottom). Each curve represents the average for two repeated-batch fermentations. Conc., concentration.

bottom). This observation was also made when we compared the cell densities in reactors B and C with 0.4% (wt/vol) yeast extract medium (Fig. 3, middle). This was not surprising, because the medium cell density did not represent the total cell mass present in the PCS bioreactors, which was made of suspended cells in the medium and immobilized cells on the supports. However, with the 0.2% yeast extract medium (Fig. 3, top), the suspended-cell density in the PCS bioreactor (reactor A) was higher than that in the control (reactor D). This suggested slow release of the complex nutrients from the SYFB+ discs and rings to the suspended-cell population.

The cell density in reactor D dropped abruptly from 12.5 to 5 absorbance units (2.5-fold) when its medium yeast extract concentration was reduced from 0.4 to 0.2% (wt/vol) (Fig. 3, top and middle). This suggested that the amount of complex nutrients in the extracellular medium in reactor D was not enough for the normal performance of *L. casei*. When the medium yeast extract concentration in the PCS bioreactor was decreased from 0.4 to 0.2% (wt/vol), there was just a 1.4-fold decrease (10 to 7.2 absorbance units) in the final cell density. This further suggested that the suspended *L. casei* in the PCS bioreactor received complex nutrients from the leachate of the SFYB+ discs and rings.

With all yeast extract concentrations, reactors A, B, and C (PCS bioreactors) outperformed reactor D (control) in regard to lactic acid production (Fig. 4). The lag times of reactors B and C (11 and 9 h, respectively) were 3 and 6 times shorter than that of reactor D, respectively. The PCS bioreactors, at all

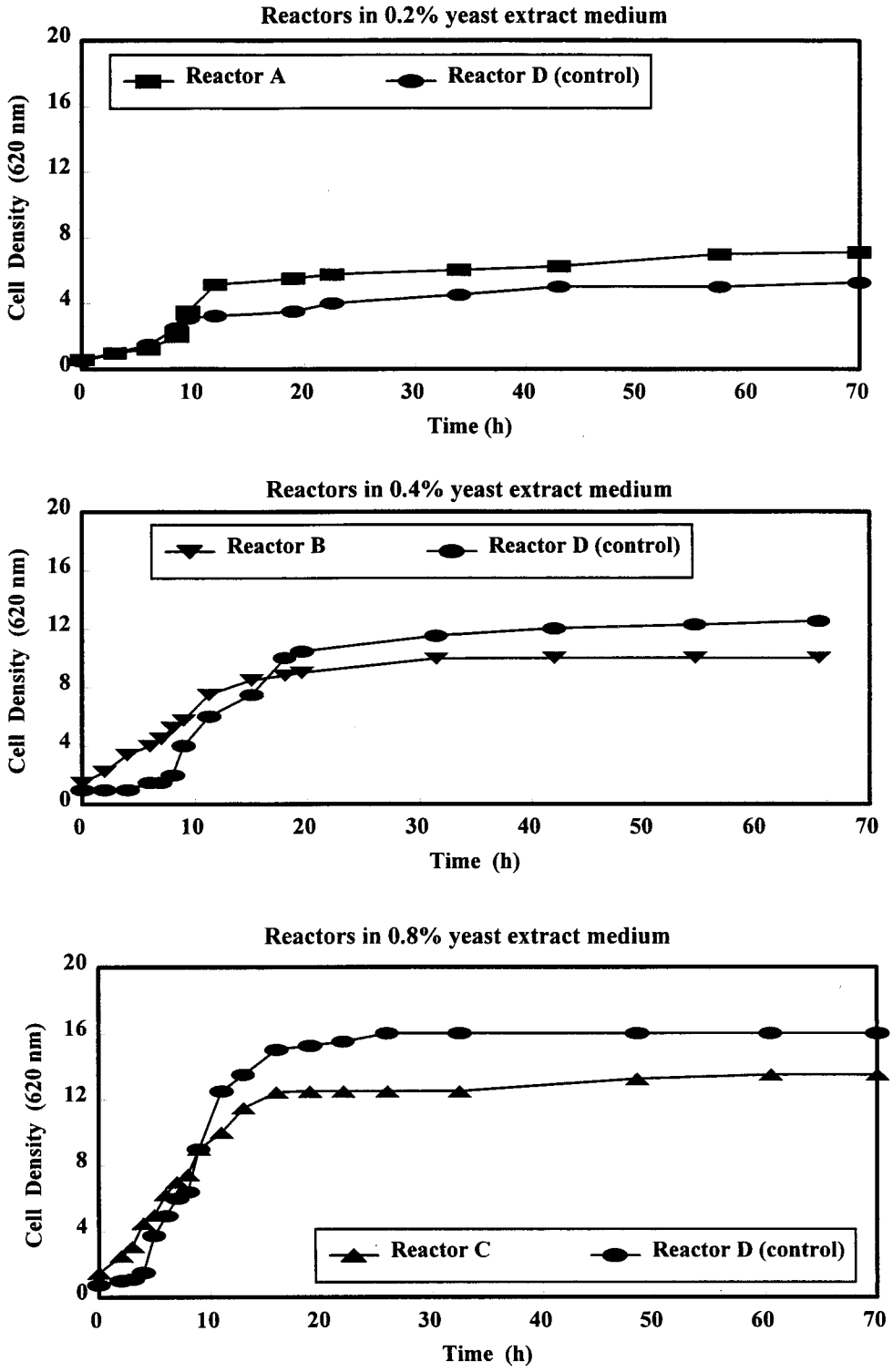

FIG. 3. *L. casei* cell density in control (reactor D) and PCS (reactors A, B, and C) bioreactors with various levels of yeast extract. Each curve represents the average for two repeated-batch fermentations.

yeast extract concentrations, increased the maximum lactic acid productivity by 40 to 70%. Moreover, the total fermentation times of reactors A, B, and C were 1.4, 2.1, and 2.6 times shorter than those of the control, respectively. Furthermore, the yields of reactors A and C were 24 and 6% higher than the yield of the control, respectively. Indeed, the performance of reactor D with 0.8% yeast extract medium was only equivalent to the performance of reactor B (PCS bioreactor with 0.4%

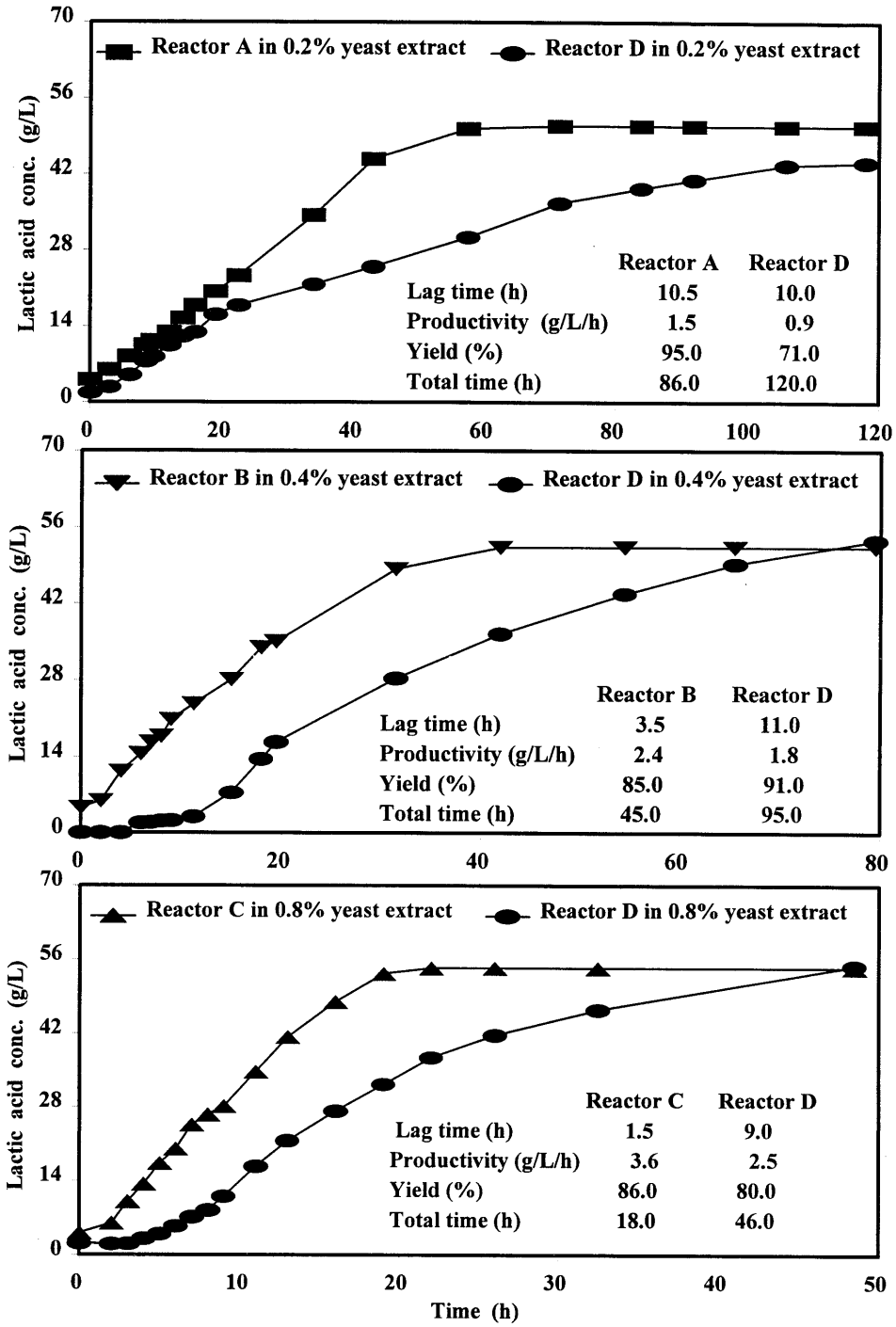

FIG. 4. *L. casei* lactic acid production in control (reactor D) and PCS (reactors A, B, and C) bioreactors with various levels of yeast extract. Each curve represents the average for two repeated-batch fermentations. conc., concentration.

yeast extract medium). This showed the advantage of using medium with reduced complex nutrients in PCS bioreactors for commercial batch fermentation.

The biofilm population on the surface of the SFYB+ discs and rings also contributed to the overall bioreactor performance. The biofilm populations on the outer surfaces of the SFYB+ discs and rings in reactors A, B, and C were 7.1×10^9, 8.5×10^9, and 2.4×10^{10} CFU/g of support, respectively (see Fig. 6). As expected, the biofilm population increased with the medium yeast extract concentration. This was further demonstrated by SEM micrographs (Fig. 5), where the biofilm on the interior surface of the SFYB+ in reactor C (Fig. 5C and D) appeared significantly denser than that in reactor A (Fig. 5E and F). The biofilm cells of *L. casei* on the outer surface of the SFYB+ were present mostly in the streptobacillus form rather than the usual nonfilamentous bacillus form (Fig. 5A and B).

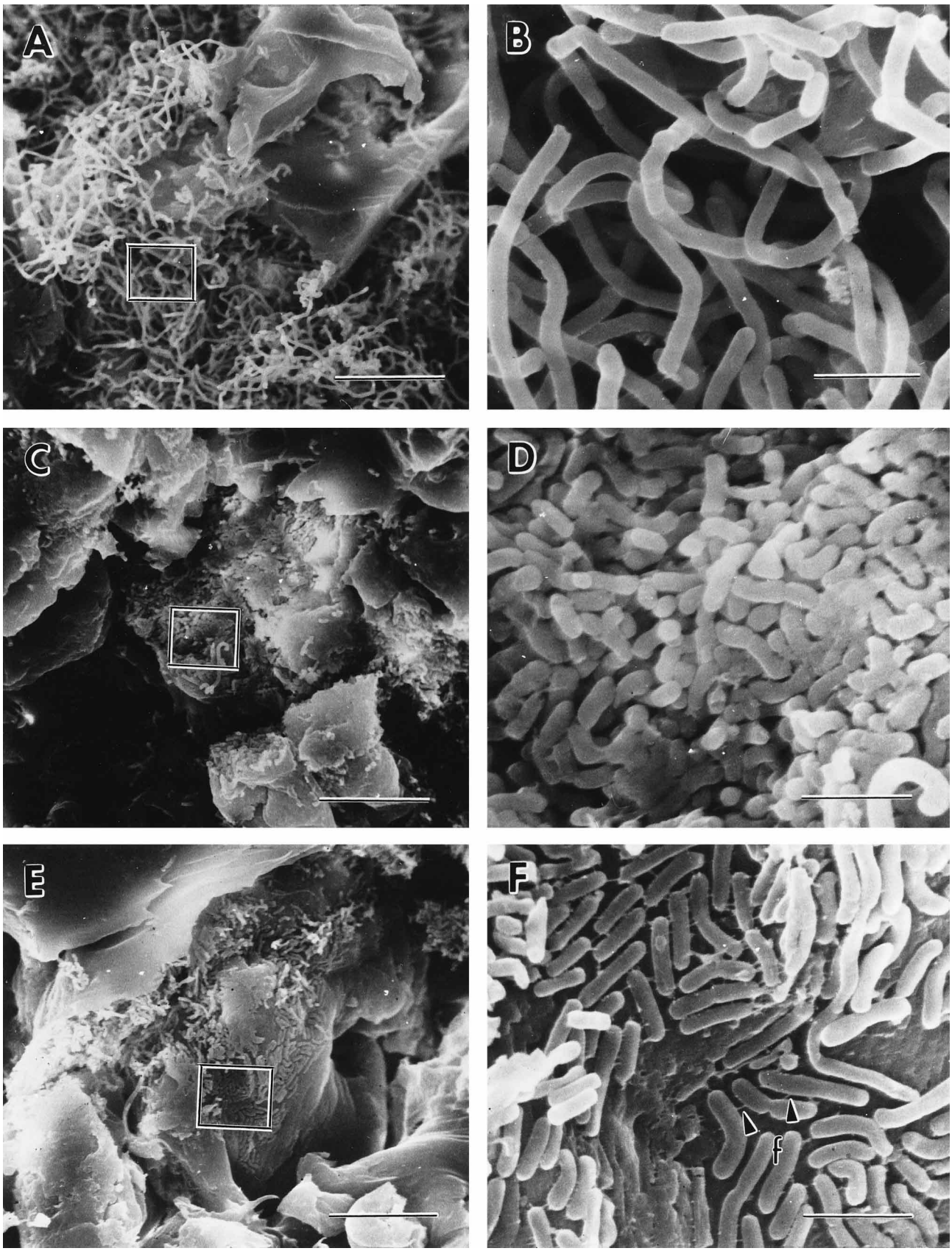

FIG. 5. SEM micrographs of *L. casei* on the exterior or interior surfaces of SFYB+ in media with different yeast extract concentrations. (A) *L. casei* biofilms formed on the exterior surface of SFYB+ in 0.8% yeast extract medium. Bar, 20 μm. (B) Enlargement of boxed area in panel A to show the long-rod filamentous morphology of *L. casei*. Bar, 3 μm. (C) *L. casei* biofilms formed on the interior surface of SFYB+ in 0.8% yeast extract medium. Bar, 20 μm. (D) Enlargement of boxed area in panel C to show the short-rod filamentous morphology of *L. casei*. Bar, 3 μm. (E) *L. casei* biofilms formed on the interior surface of SFYB+ in 0.2% yeast extract medium. Bar, 20 μm. (F) Enlargement of the boxed area in panel E to show the fibrillar network (f) formed from the EPS of *L. casei* biofilms. Bar, 3 μm.

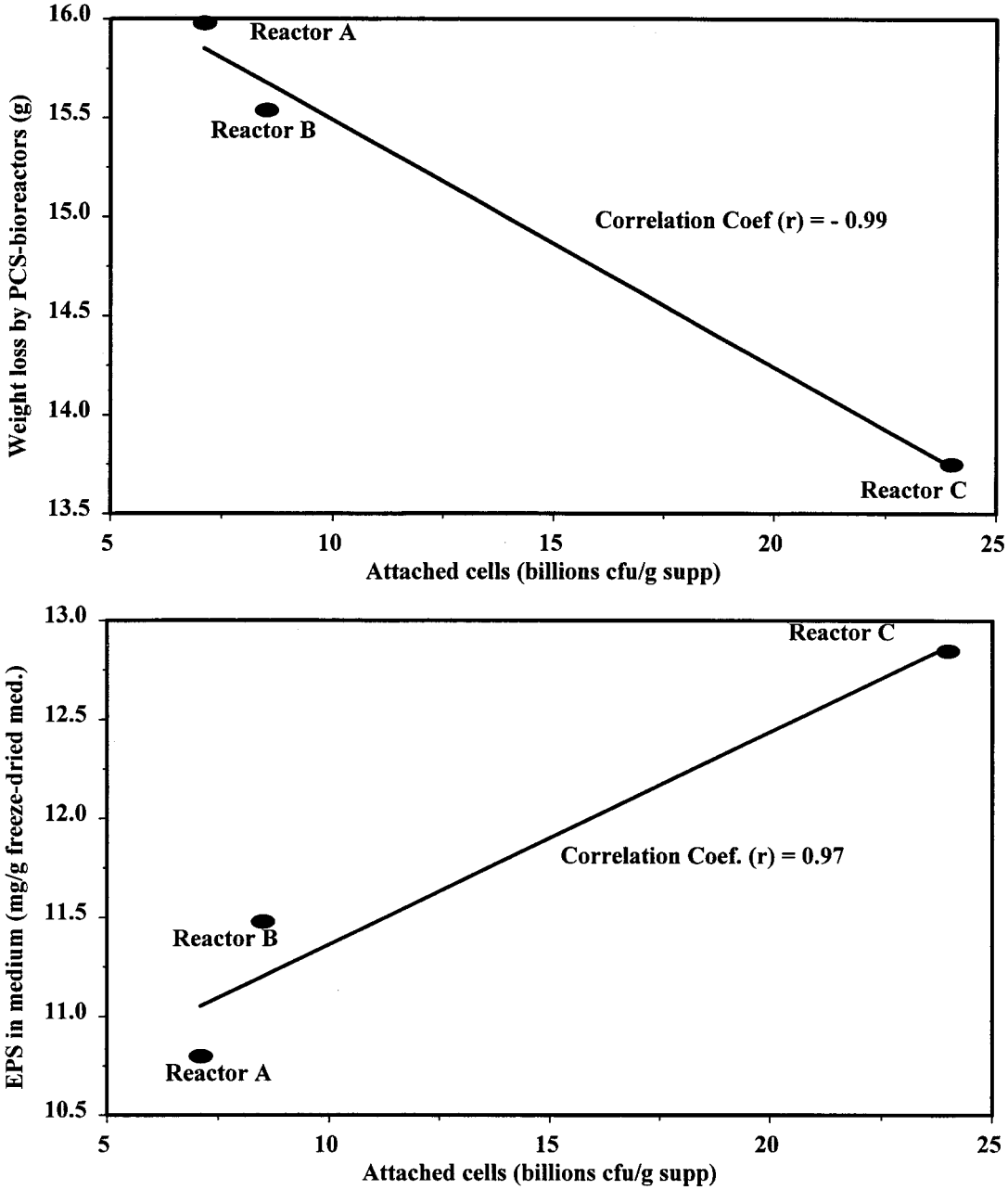

FIG. 6. (Top) Weight loss by SFYB+ discs and rings in the PCS bioreactors versus the biofilm populations of reactors A, B, and C. (Bottom) EPS in the media of the PCS bioreactors versus the biofilm populations of reactors A, B, and C. The standard errors for biofilm population, weight loss, and EPS in the medium was 6.4 billion CFU/g of support (supp), 1 g, and 0.5 mg/g of freeze-dried medium (med.), respectively ($P < 0.05$).

This might be a result of the hydraulic stress experienced by the biofilm cells (1). Typical fibrillar extracellular materials derived from EPS of the biofilm, as observed by Leppard and Bakke (cited in reference 1) and Ho et al. (8), were observed with the SFYB+ discs and rings in reactor A (Fig. 5F). The relative amounts of EPS present in the culture media of reactors A, B, and C were 11, 12, and 13 mg/g of freeze-dried medium, respectively. The standard error obtained from SAS analysis of variance for the EPS in the reactors was 0.5 mg/g of freeze-dried medium. This showed that the amount of EPS present in the reactor varied significantly along with the change in the yeast extract concentration in the reactor's medium. The high correlation between the amount of EPS and the biofilm population of each reactor ($r = 0.97$) (Fig. 6, bottom) demonstrated that the amount of EPS in the medium was also a good indicator of the biofilm population on the supports.

The weight losses of the PCS after 66 days of lactic acid repeated-batch biofilm fermentation in reactors A, B, and C were 16.0, 15.5, and 13.8 g/reactor, respectively (Fig. 6, top). The maximum weight losses due to agricultural product leaching after the 17 batches of repeated-batch fermentation should be similar among the three PCS bioreactors, since they con-

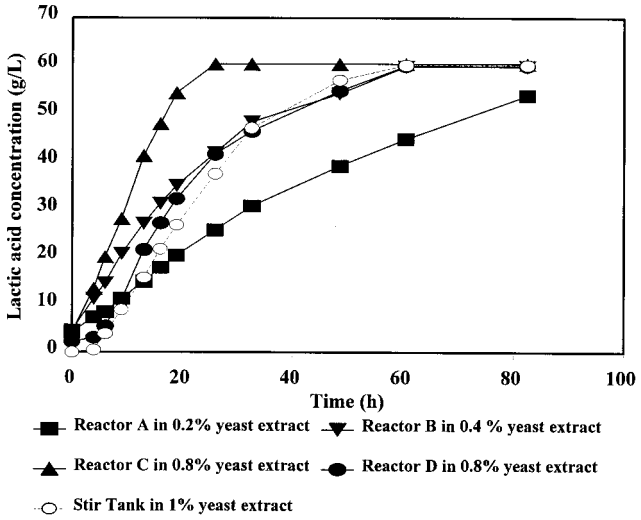

FIG. 7. Lactic acid production by *L. casei* in PCS bioreactors (reactors A, B, and C), a control bioreactor (reactor D), and a 1-liter Braun stir tank fermentor with various yeast extract concentrations. Each curve represents the average for two repeated-batch fermentations.

tained the identical weight of SFYB+. Hence, the factor contributing to the smaller weight loss by the PCS bioreactors at different yeast extract concentrations was probably due to the biofilm population. This was supported by the high correlation between weight loss and biofilm population for reactors A, B, and C ($r = -0.99$) (Fig. 6, top).

The lactic acid production curve for reactor D with 0.8% yeast extract medium was similar to that for the B-Braun Biostat-M stir tank fermentor with 1% yeast extract medium (Fig. 7). This showed that our customized bioreactor had pH control and medium mixing ability equivalent to those of a standard bench-top continuous-stir tank fermentor. In addition, it also indicated that the suspended cells of *L. casei* could perform lactic acid fermentation in an environment with a lower yeast extract concentration (0.8% instead of 1%).

Effects of medium recycling rate and starting glucose concentration. The recycling rate of the customized bioreactors affected the pH-controlling ability of the reactors with supports (Fig. 8). The maximum lactic acid productivities of reactors B and C were positively correlated with their medium recycling rates (except for recycling rates of <1.5 cycles/h). In contrast, no significant effect of the medium recycling rate on the lactic acid productivity of reactor D (control) was observed for all recycling values (standard error of maximum lactic acid productivity, 0.2 g/liter/h [$P < 0.05\%$]) (Fig. 8). This was probably because the diffusion of substrates, products, and complex nutrients into and out of the PCS was highly dependent on the movement, mixing, and recycling rate of the medium.

Reactors B, C, and D demonstrated optimum starting glucose concentration peaks at 100, 60 to 80, and 80 g of glucose per liter, respectively. Reactor C had its highest maximum lactic acid productivity (4.3 g/liter/h) at a starting glucose concentration of 100 g/liter, whereas reactor D, in 0.8% (wt/vol) yeast extract medium, had its highest maximum lactic acid productivity (2.8 g/liter/h) at a starting glucose concentration of 80 g/liter (Fig. 9). This again illustrated the high stress tolerance of the biofilms. Reactor B had its greatest maximum productivity (3.0 g/liter/h) at a starting glucose concentration of 60 to 80 g/liter. This suggested that the reduction in complex nutrients in the medium would lower the ability of *L. casei* to withstand the

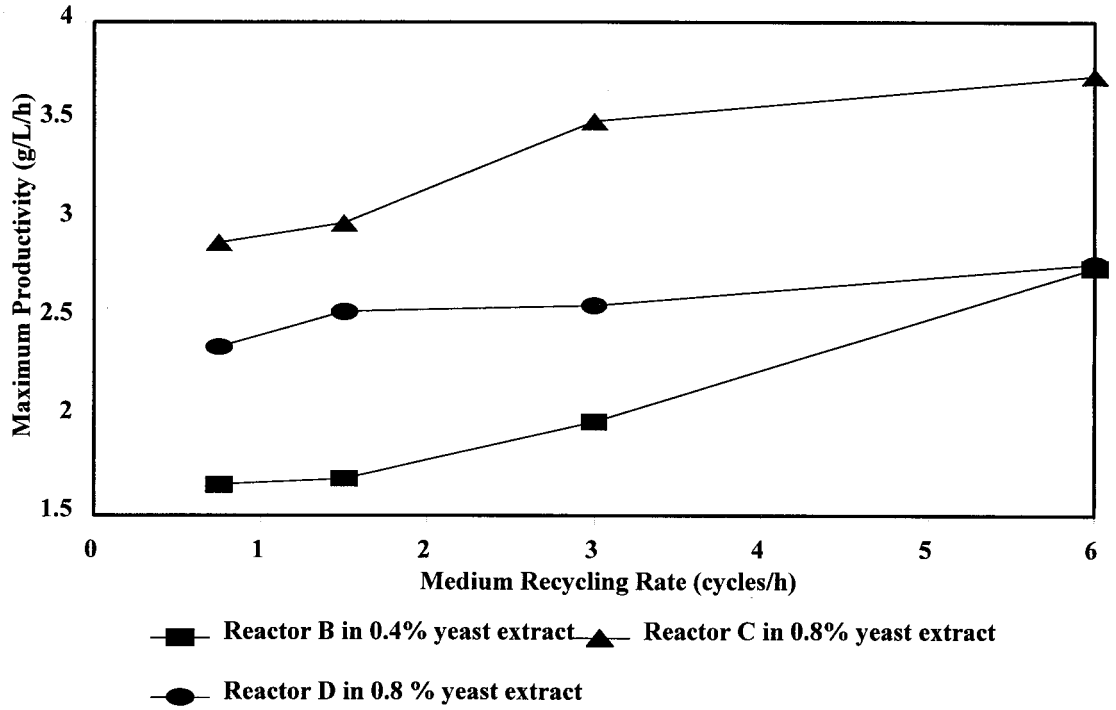

FIG. 8. Effects of medium recycling rate on maximum lactic acid productivity of *L. casei* under various fermentation conditions. The standard error for maximum productivity among the different treatments was 0.2 g/liter/h ($P < 0.05$).

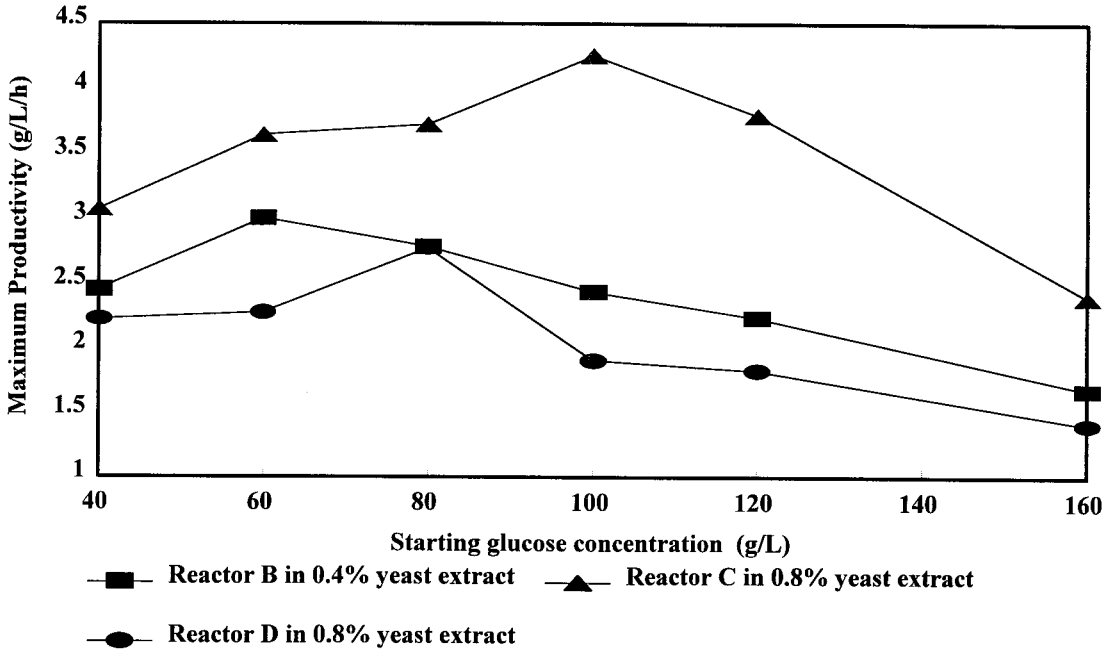

FIG. 9. Effect of starting glucose concentration on maximum lactic acid productivity of *L. casei* under various fermentation conditions. The standard error for starting glucose concentration among the different treatments was 0.3 g/liter/h ($P < 0.05$).

inhibitory effect of a high starting glucose concentration. This was supported by the decreasing trend demonstrated by the maximum lactic acid productivity of reactor A (1.8, 1.7, 1.5, and 1.1 g/liter/h corresponding to starting glucose concentrations of 2, 4, 6, and 8%, respectively). Substrate inhibition by high starting glucose concentrations was also reported by Goncalves et al. (6).

Based on the results presented here, it was clear that the use of SFYB+ discs and rings shortened the lactic acid fermentation lag phase time and total fermentation time, increased the suspended- and immobilized-cell populations, enhanced maximum productivity, and improved *L. casei* tolerance to high starting glucose concentrations and to low medium yeast extract concentrations. The shorter batch fermentation time resulting from PCS biofilm fermentation should lower the overall cost of production of lactic acid. The performance of reactor B (fermentation in 0.4% [wt/vol] yeast extract medium) suggested the potential use of inexpensive complex nutrients (e.g., corn-steep liquor or soybean flour, etc.) with PCS in lactic acid batch fermentation, which might enhance the downstream recovery of lactic acid. Hence, employing the SFYB+ in commercial repeated-batch biofilm lactic acid fermentation deserves strong consideration.

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