APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1997, p. 2516–2523 Vol. 63, No. 7
0099-2240/97/$04.00+0

Ingredient Selection for Plastic Composite Supports for L-(+)-Lactic Acid Biofilm Fermentation by *Lactobacillus casei* subsp. *rhamnosus*†

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Received 22 July 1996/Accepted 11 April 1997

Plastic composite supports containing 50% agricultural products (oat hulls, soybean hulls, yeast extract, soybean flour, dried bovine erythrocytes, bovine albumin, and/or mineral salts) and 50% (wt/wt) polypropylene were produced by high-temperature twin-screw extrusion. The research employed two half sets of a five-factorial fractional design (2^{5-1}) to evaluate the effects of different agricultural components on the properties of the plastic composite supports and to select the best plastic composite support formulation for lactic acid fermentation. The biofilm population was affected by the contact angle and relative hydrophobicity of the supports ($r = 0.79$ to 0.82). Lactic acid was produced by the suspended cells ($r = 0.96$) and the biofilm on the plastic composite support discs ($r = 0.85$). Incorporation of yeast extract into plastic composite supports enhanced growth of free and attached cells in minimal medium ($P < 0.0001$). The presence of soybean hulls, yeast extract, or mineral salts in plastic composite supports produced less hydrophobic supports ($P < 0.0001$) and enhanced cell attachment ($P < 0.03$). Under all conditions, suspended-cell and polypropylene disc controls gave negligible lactic acid production and cell density. Plastic composite supports containing soybean hulls, yeast extract, soybean flour, bovine albumin, and mineral salts gave the highest biofilm population (2.3×10^9 CFU/g of support), cell density (absorbance of 1.8 at 620 nm), and lactic acid concentration (7.6 g/liter) in minimal medium.

Lactic acid is an organic hydroxy acid that exists in two optically active enantiomers, L-(+) and D-(−) (23). It is widely used by the food industry (as an acidulent, as a preservative, and for stearoyl-2-lactylate synthesis) and by the non-food industry (for polylactic acid, green solvent, and slow release carriers) (6, 16). Polylactic acid-based degradable plastic is a polyester of lactic acid with a projected market of 300 million bushels of corn per year (6).

Lactic acid can be produced chemically from acetaldehyde and hydrogen cyanide or via microbial fermentation. Only microorganisms can produce exclusively the L or D isomer of lactic acid. Presently, lactic acid is produced by batch fermentation (BF) because it exhibits both type I (growth associated) and type II (non-growth associated) fermentation (5). Lactic acid production rates and concentrations can be increased by strain development to obtain high-production mutants (8) or by increasing the cell density in the fermentor (9, 10). Cell immobilization is a common way to increase cell density. However, industrial applications of cell immobilization with calcium alginate beads are few due to 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 (13, 16, 20, 21).

Biofilms are a natural form of cell immobilization that results from microbial attachment to solid supports in submerged environments (2). This increases the cell density and enables the biofilm population to withstand stresses such as pH change and starvation. Attachment of cells on supports to form a biofilm depends largely on the formation of extracellular polysaccharides, surface charge, and hydrophobicity between the solid surface and the microorganisms (3). Studies by Van Loosdrecht et al. (22) demonstrated that measurement of the hydrophobicity of cell surfaces by the contact angle method gave better cell attachment estimation than the hexadecane test and the two-phase (polyethylene glycol and dextran) cell partition method.

Previous studies in our laboratory have successfully proved that biofilm fermentation with chips of plastic composite supports (PCS) containing 75% (wt/wt) polypropylene (PP) and 25% (wt/wt) agricultural material improved lactic acid production. In pure- and mixed-culture continuous fermentation, productivities of 30 and 35 g/liter/h, respectively, were achieved (9). In addition, PCS chips were shown to be effective in long-term (more than 2 months) repeated-batch lactic acid biofilm fermentation with both pure and mixed cultures (10). However, medium channelling and clumping of cells among the PCS chips interfered with mixing, pH control, and, ultimately, lactic acid production. To overcome these problems, new disc-shaped PCS with a central large hole for easy flow of medium were produced for this study. We demonstrated that lactic acid production correlated highly with suspended-cell density, biofilm population, and hydrophobicity of the new PCS discs.

MATERIALS AND METHODS

PCS. PCS discs that contained 50% (wt/wt) PP and 50% (wt/wt) agricultural materials were produced by high-temperature extrusion in a twin-screw corotat-

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† Journal paper no. J-16944 of the Iowa Agriculture and Home Economics Experiment Station, Ames, project no. 3253.

TABLE 1. Composition of the PCS used

Support	Ingredients of extrusion mixture (% [wt/wt])						
	PP[a]	Hull (S[b] or O[c])	Soybean flour (F[d])	Yeast extract (Y[e])	Dried bovine RBC (R[f])	Dried bovine albumin (B[g])	Mineral salts (+[h])
S	50	50, S					
OF	50	40, O	10				
OY	50	40, O		10			
SFY	50	40, S	5	5			
OR	50	40, O			10		
SFR	50	40, S	5		5		
SYR	50	40, S		5	5		
OFYR	50	35, O	5	5	5		
O+	50	50, O					+
SF+	50	40, S	10				+
SY+	50	40, S		10			+
OFY+	50	40, S	5	5			+
SR+	50	40, S			10		+
OFR+	50	40, O	5		5		+
OYR+	50	40, O		5	5		+
SFYR+	50	35, S	5	5	5		+
OB	50	40, O				10	
SFB	50	40, S	5			5	
SYB	50	40, S		5		5	
OFYB	50	35, O	5	5		5	
SB+	50	40, S				10	+
OFB+	50	40, O	5			5	+
OYB+	50	40, O		5		5	+
SFYB+	50	35, S	5	5		5	+
PP (control)	100						

[a] PP resins (Quantum USI Division, Cincinnati, Ohio).
[b] S, ground (20-mesh), vacuum-dried (48 h at 110°C; 30 in. of mercury) soybean hulls (Cargill Soy Processing Plant, Iowa Falls, Iowa).
[c] O, ground (20-mesh) oat hulls (Ralston Foods, Cedar Rapids, Iowa).
[d] Defatted soybean flour (Archer Daniels Midland, Decature, Ill.).
[e] Ardamine Z; Champlain Industries Inc.
[f] American Protein Corp., Ames, Iowa.
[g] American Protein Corp.
[h] Per kilogram, 2 g of sodium acetate, 1.2 g of $MgSO_4 \cdot 7H_2O$, and 0.06 g of $MnSO_4 \cdot 7H_2O$.

ing Brabender PL2000 extruder (model CTSE-V; C.W. Brabender Instruments, Inc., South Hackensack, N.J.). The ingredients and formulation of each PCS type are listed in Table 1. Materials to be extruded were first mixed in a separate container on a weight basis before being poured into the extruder hopper. The mixture was extruded (11 and 15 rpm for soybean hull and oat hull PCS, respectively) in the form of a continuous tube through a medium pipe die (3.2-mm inside diameter and 12.7-mm outside diameter [o.d.]) with barrel temperatures of 200, 220, and 200°C and a die temperature of 167°C. The barrel exhaust vent was plugged because the release of moisture at the die was essential for producing a porous support. Composite tubing was extruded onto a steel rod and air cooled slowly without fanning. Tubes with an o.d. of 10 to 11 mm were then cut into discs. PP discs were bored out of a PP sheet (3.5 mm thick) with cork borers with a 7-mm inside diameter and 11-mm o.d. The bulk density, weight per disc, and extrusion mixture moisture content for each PCS type and PP disc are listed in Table 2.

Hydrophobicity of *Lactobacillus casei* and PCS discs. The relative hydrophobicity of each PCS disc type was determined by measuring the contact angles by the sessile drop technique (1). A drop of deionized water (20 μl) was deposited on the cut surface of the disc (Fig. 1). The surface and the drop were photographed within 1 s of application, and the contact angle (θ) of each water droplet on the disc's cut surface was measured.

The relative hydrophobicity of *L. casei* was determined by measuring the contact angles of deionized water droplets deposited on a cell lawn within 1 s of application (Fig. 1) (7, 19). The cell lawn was prepared by filtering 300 ml of *L. casei* culture (18 h in lactic acid fermentation [LAF] medium with 8% [wt/vol] glucose [9]) with a 0.45-μm-pore-size cellulose triacetate filter (HAWP; Millipore Corporation, Bedford, Mass.). The initial moisture contents of different cells lawns were normalized by placing the filters with cells in a petri dish containing glycerol-agar (1% [wt/vol] in water containing 10% [vol/vol] glycerol) for 3 h (19).

Bacterial culture preparation. *L. casei* subsp. *rhamnosus* (ATCC 11443) is a homofermentative L-(+)-lactic acid bacterium. Stock cultures were maintained in *Lactobacillus* MRS broth (Difco Laboratories, Detroit, Mich.) at 4°C with monthly transfers to fresh medium. Ten milliliters of an active *L. casei* culture (18 h in MRS broth at 37°C) was inoculated into 100 ml of LAF 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]) (9), which was then incubated for 18 h at 37°C. Centrifugation (16,300 × g, 20 min) followed by a rinsing step with 100 ml of minimal medium (MM) (2 g of glucose per liter in mineral salts solution with no yeast extract) was used to remove LAF medium from the active cells. The rinsed active cells were then resuspended into 100 ml of sterilized MM and used in the BF studies.

BF studies. BF without pH control was used to characterize the performance of each PCS blend. Suspended cells and PP discs were used as controls. PCS or PP discs (5 g) were sterilized dry (45 min at 121°C) in a 50-ml screw-cap cultured tube. The sterilized discs were aseptically transferred into a dilution bottle containing 20 ml of sterilized MM and soaked at 37°C in duplicate for 24 h. After decanting of the initial soaking solution, each dilution bottle was refilled with 20 ml of sterilized MM, inoculated with 0.2 ml of active *L. casei* culture (18-h culture), and incubated in a 37°C water bath for 48 h.

The fermented medium was aseptically decanted and evaluated for lactic acid

TABLE 2. Physical properties of PCS

Support[a]	Bulk density (g/cm^3)[b]	Wt (mg/disc)[b]	Support contact angle (°)[c]	Extrusion mixture water (%)[d]
S	0.24	204	106	0.7
OF	0.23	159	99	2.4
OY	0.21	150	94	2.4
SFY	0.25	182	102	0.6
OR	0.21	161	102	2.6
SFR	0.26	176	104	1.0
SYR	0.23	179	112	1.1
OFYR	0.21	150	103	2.8
O+	0.22	185	101	2.8
SF+	0.26	197	104	0.8
SY+	0.25	179	93	1.1
OFY+	0.24	182	95	3.0
SR+	0.25	170	104	1.2
OFR+	0.21	164	92	2.1
OYR+	0.20	170	100	3.2
SFYR+	0.25	174	101	1.2
OB	0.23	200	103	2.0
SFB	0.25	197	99	0.8
SYB	0.25	181	100	0.9
OFYB	0.20	164	93	3.3
SB+	0.25	154	101	1.0
OFB+	0.22	182	92	2.3
OYB+	0.21	176	88	2.4
SFYB+	0.25	168	93	1.0
PP (control)	0.21	217	97	

[a] See Table 1.
[b] Values are averages from two replicates.
[c] Values are averages from three replicates.
[d] Percent moisture was obtained by a convection drying method. Values are means from two replicates.

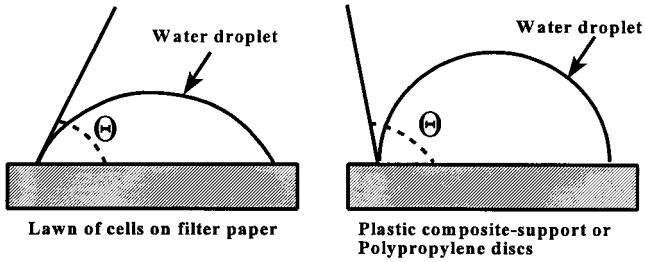

FIG. 1. Shape of deposited water droplet on PCS or PP discs and on *L. casei* cell lawn for contact angle determination.

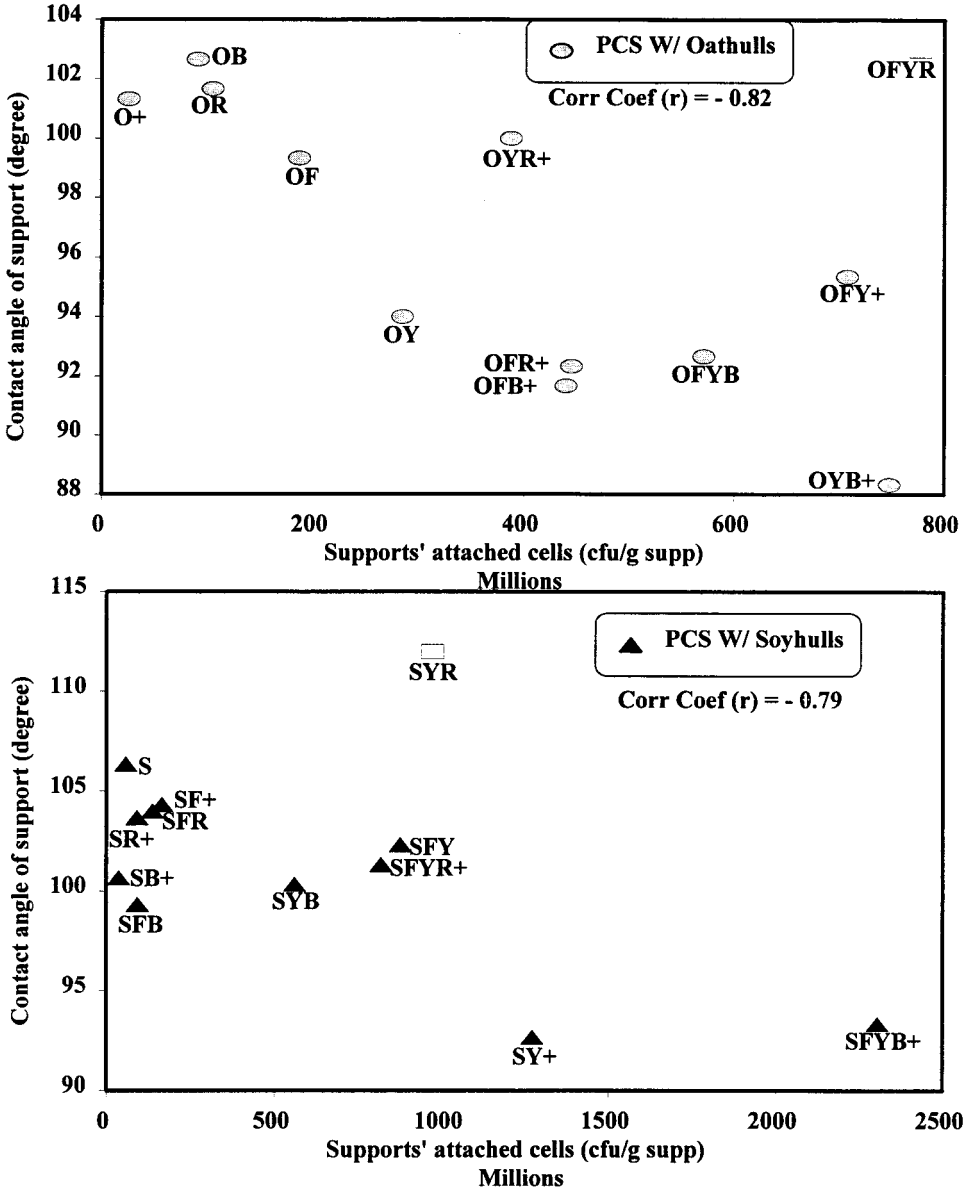

FIG. 2. Relationship between contact angle and *L. casei* biofilm population on various support (supp) surfaces during batch fermentation. The least significant difference ($P < 0.05$) of contact angle and bacterial attachment were 4.3° and 0.58×10^9 CFU/g of support, respectively. The empty squares were not included in the calculation of *r*. See Table 1 for support compositions.

produced, glucose consumed, and suspended-cell density (by absorbance at 620 nm). L-(+)-Lactic acid and D-glucose concentrations were analyzed with a high-performance liquid chromatograph (Waters, Milford, Mass.) equipped with a Waters model 401 refractive index detector and an Aminex HPX-87H column (300 by 7.8 mm) (Bio-Rad Chemical Division, Richmond, Calif.) with 0.012 N H_2SO_4 as the mobile phase. Bacterial growth in LAF medium was monitored by measuring the absorbance at 620 nm with a Spectronic 20 spectrophotometer (Milton Roy Co., Rochester, N.Y.).

Viable biofilm population on the supports. A modification of the method of Dickson and Koohmaraie (12) was used to enumerate the relative biofilm population on the PCS. Five discs (both PCS and PP) were aseptically removed from each BF bottle and transferred into a dilution bottle containing 100 ml of sterilized peptone water. Each dilution bottle was then vigorously shaken for 5 s. Most researchers consider bacteria in a biofilm to be those that are strongly attached to surfaces (11, 14, 17). Hence, the goal of the shaking process was to remove loosely attached bacteria or to remove free cells in the water film on the support surface. The rinsed supports were then aseptically transferred into a screw-cap culture tube with 9-ml of sterilized peptone water and 5 g of sterilized sand. The culture tube was subsequently vortexed vigorously at 30-s intervals for a total of 1.5 min. The culture tube medium was then serially diluted (10^5 to 10^8)

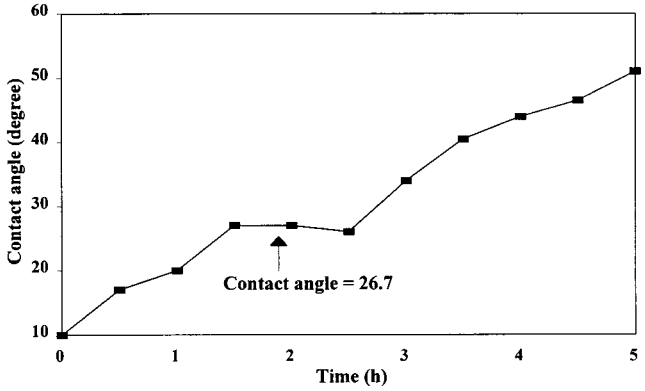

FIG. 3. Relationship between contact angle of water droplet and drying time of *L. casei* cell lawns.

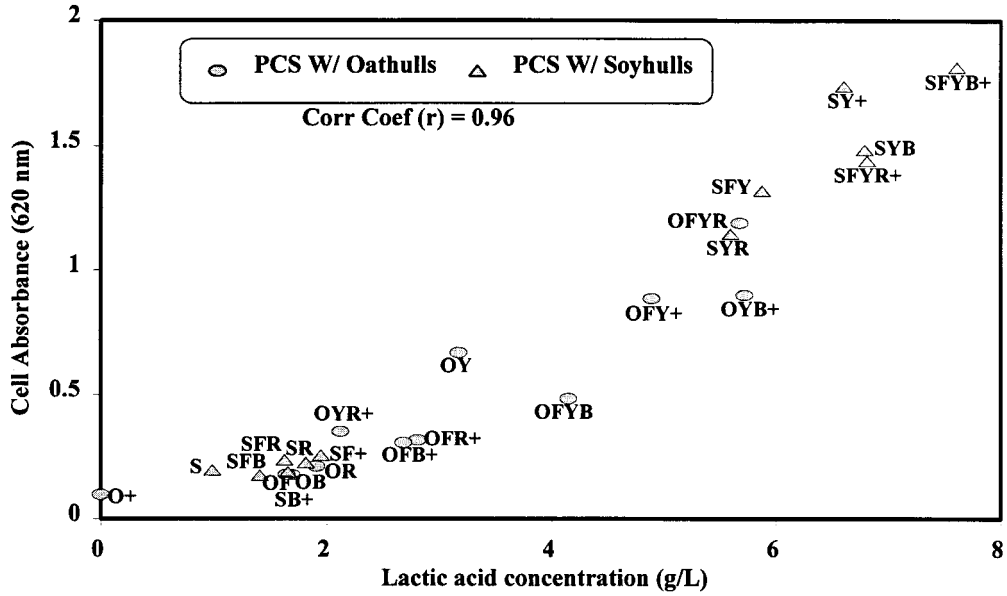

FIG. 4. Relationship between *L. casei* suspended-cell density in MM and lactic acid production during BF. The least significant difference ($P < 0.05$) of cell density and lactic acid concentration were absorbance of 0.58 and 2 g/liter, respectively. See Table 1 for support compositions.

into sterilized peptone water. CFU were determined for each tube by using *Lactobacillus* MRS agar spread plates in duplicate. Finally, the five sand-stripped discs from each BF bottle were rinsed with water, convection oven dried (70°C, 24 h), and weighed. The weights of the dried discs were used to calculate the CFU per gram of support for each type of PCS.

SEM. Two supports were retrieved aseptically from the dilution bottle and immediately fixed with 4% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) overnight at 4°C. Fixed samples were washed three times in the same buffer at room temperature and postfixed in 1% osmium tetroxide with shaking for 1 h at 4°C. Postfixed samples were again washed three times in the same buffer and then dehydrated through a graded ethanol series (50, 70, 75, 80, 85, 90, 95, 100, 100, and 100% [wt/vol]). The dehydrated samples were then critical point dried with a hexamethyldisilazine (HMDS) solution series (30-min intervals of 1:1 [vol/vol] HMDS–100% ethanol, 100% HMDS, 100% HMDS, and 100% HMDS) at room temperature with shaking. Samples were allowed to dry overnight inside a solvent hood in a crack-lid petri dish containing HMDS-saturated filter paper at room temperature. Scanning electron microscopy (SEM) micrographs of gold-coated critical-point-dried supports were taken with a JSM-35 scanning electron microscope (JEOL, Tokyo, Japan) at 25 kV.

Statistical analysis. Two half sets of a five-factorial fractional design (2^{5-1}) (4) were used to evaluate the effects of oat hulls, soybean hulls, soybean flour, yeast extract, dried bovine erythrocytes (RBC), dried bovine albumin, and mineral salts on the characteristics of PCS in all of the tests performed. The five factors evaluated in the first half set were hulls (oat hulls or soybean hulls), soybean flour, yeast extract, dried bovine RBC, and mineral salts. The five factors studied in the second half set were hulls (oat hulls or soybean hulls), soybean flour, yeast extract, dried bovine albumin, and mineral salts. In each half set, 16 treatments were examined in duplicate. The data from each test were analyzed by least significant difference and analysis of variance by using the Statistical Analysis System package (version 6.03) (SAS Institute, Inc., 1985).

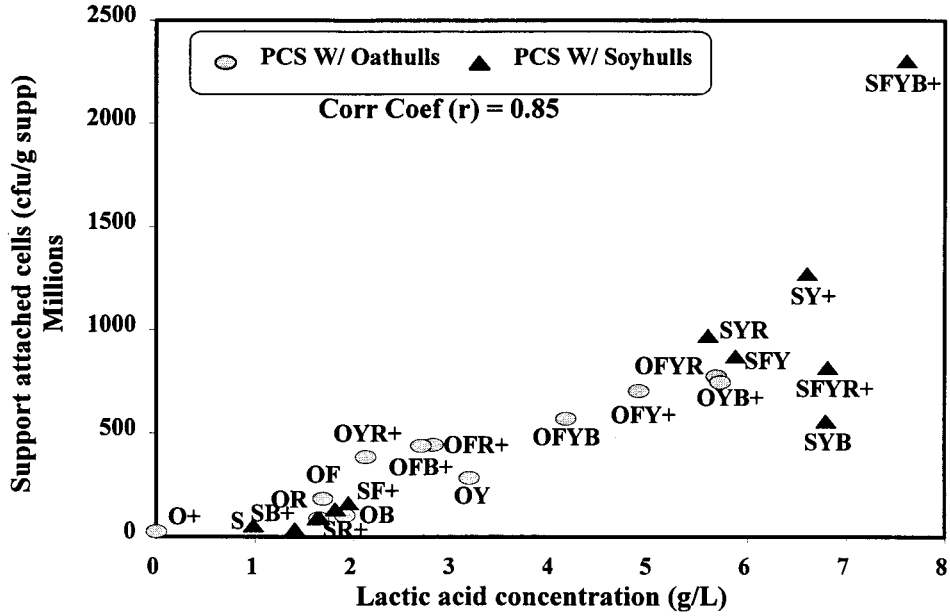

FIG. 5. Relationship between *L. casei* biofilm population on supports (supp) and lactic acid production during BF. The least significant difference ($P < 0.05$) of viable cells attached on supports and lactic acid concentration were 5.7×10^8 CFU/g of support and 2 g/liter, respectively. See Table 1 for support compositions.

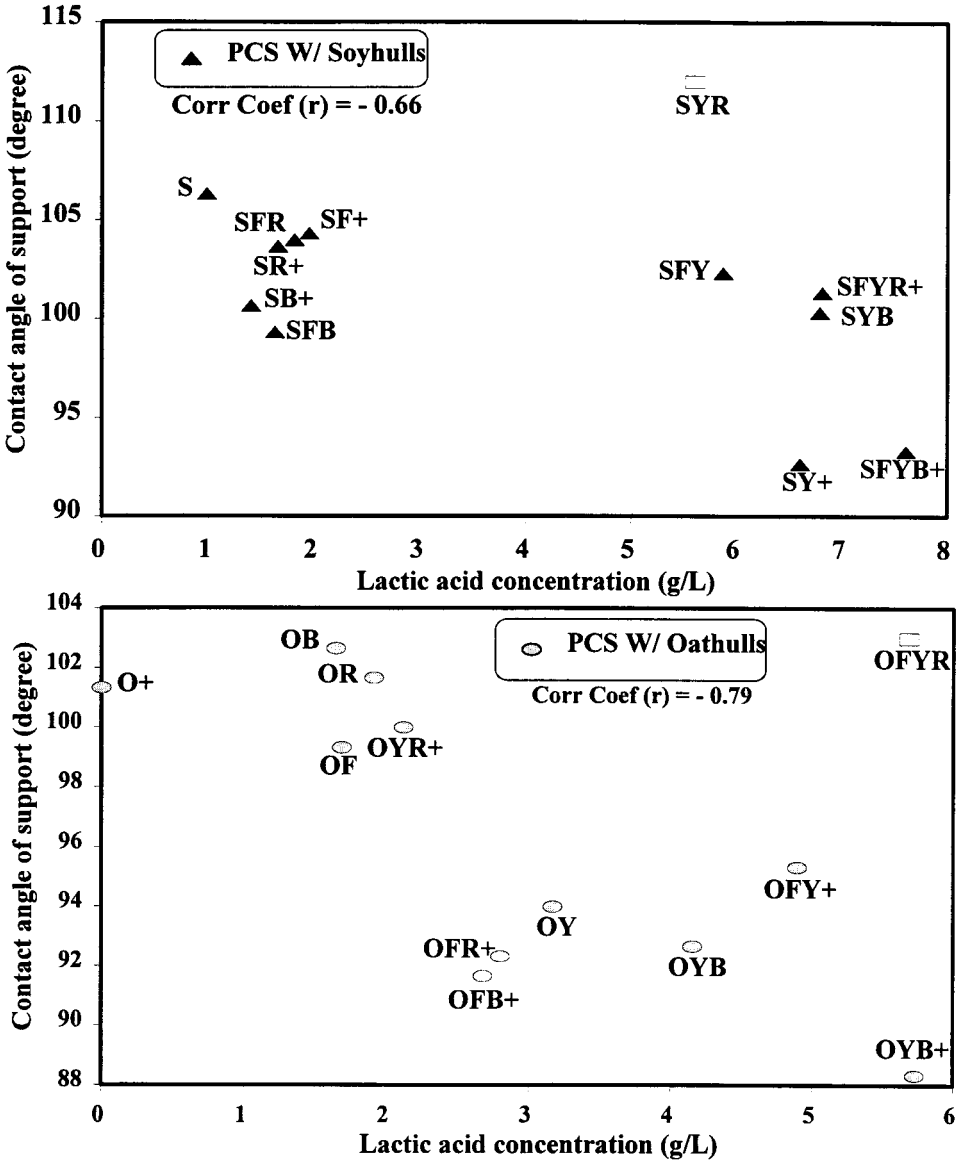

FIG. 6. Relationship between *L. casei* contact angle and lactic acid production during BF. The least significant difference ($P < 0.05$) of contact angle and lactic acid concentration were 4.3° and 2 g/liter, respectively. The open squares were not included in the calculation of *r*. See Table 1 for support compositions.

RESULTS

Effects of agricultural ingredients on the contact angle of supports. The contact angle range for the PCS and PP discs (Table 2) was 88 to 112° and 93 to 99°, respectively, which indicated that all supports possessed a hydrophobic surface. Statistical analysis showed that, individually, soybean hulls, yeast extract, or mineral salts ($P < 0.0001$) decreased the contact angle (hydrophobicity) of the supports, whereas oat hulls ($P < 0.0001$), dried bovine RBC ($P < 0.005$), or dried bovine albumin ($P < 0.0002$) increased the hydrophobicity of the supports ($P < 0.005$).

When dried bovine RBC interacted with yeast extract, the average contact angle for PCS with dried bovine RBC was increased ($P < 0.0001$) by 4°. This indicated that the yeast extract contact angle reduction effect was being masked by the overall hydrophobic nature of dried bovine RBC. Only mineral salts could overcome the hydrophobic nature of the dried bovine RBC, and they reduced ($P < 0.05$) the average contact angle of dried bovine RBC with PCS by 6°.

In contrast, the average contact angle of PCS containing dried bovine albumin was lowered by 5° ($P < 0.05$) when soybean flour, yeast extract, or mineral salts were added. Hence, SFYB+ and OYB+ (Table 1) had the lowest contact angle among the soybean hull- and oat hull-containing PCS, respectively (Fig. 2).

Relative hydrophobicity effects of supports and bacteria on biofilm formation. As the bacterial contact angle curve (Fig. 3) plateaued temporarily at 1.5 to 2.5 h, the contact angle, θ, of *L. casei* was determined to be 26.7°. Because θ was less than 40°, *L. casei* was considered to be hydrophilic (19). Van Loosdrecht et al. (22) concluded that hydrophobic bacteria will adhere to hydrophobic surfaces more readily than hydrophilic bacteria. Therefore, the hydrophilic *L. casei* should attach more readily to the less hydrophobic (smaller contact angle) PCS with soy-

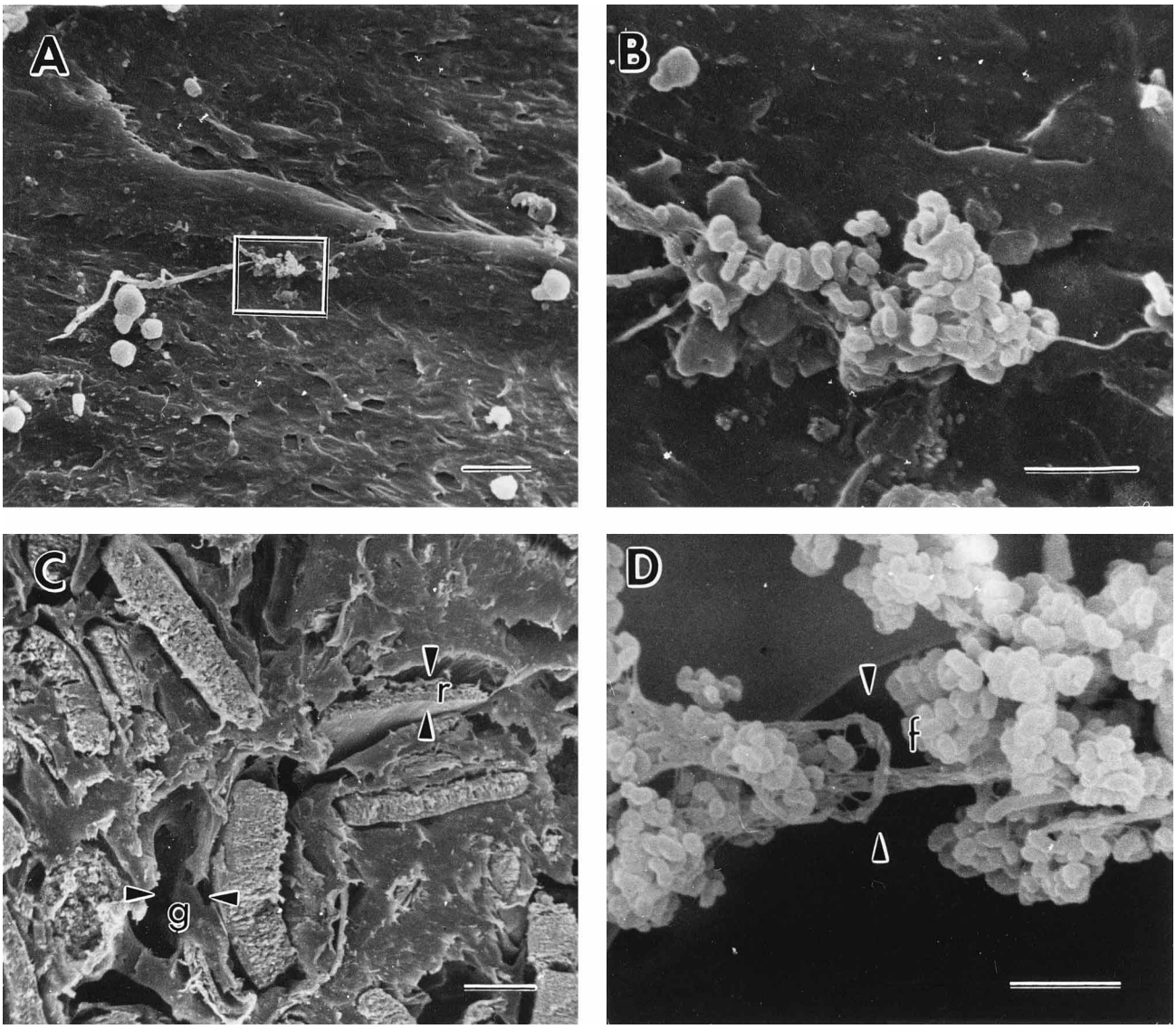

FIG. 7. SEM micrographs of the outer surfaces of the PCS and PP discs with *L. casei* biofilms in MM. (A) Smooth outer surface of PP discs. Bar, 25 μm. (B) Enlargement of the boxed area in panel A to show a small cluster of *L. casei*. Bar, 5 μm. (C) Rough outer surface of OYR+ discs with grooves (g) and ridges (r). Bar, 180 μm. (D) Surface of OYR+ discs enlarged to show large clusters of *L. casei* with fibrillar networks (f) formed from the exopolysaccharides of the biofilms. Bar, 5 μm.

bean hulls, yeast extract, and mineral salts than to the more hydrophobic PCS with oat hulls, dried bovine RBC, and dried bovine albumin.

This postulation was confirmed by the biofilm populations of the various supports. The contact angles of PCS with soybean hulls and oat hulls had a positive correlation (correlation coefficient [r] = 0.79 and 0.82, respectively) with the biofilm population (Fig. 2). All supports with soybean hulls ($P < 0.03$), yeast extract ($P < 0.0001$), or mineral salts ($P < 0.007$) had increased biofilm populations. In addition, PCS with soybean hulls had a biofilm population (5.7×10^8 to 23.0×10^8 CFU/g of support) higher than that of the PCS with oat hulls (2.6×10^8 to 7.5×10^8 CFU/g of support).

Soybean flour, yeast extract, and mineral salts had a greater hydrophobicity reduction effect on the PCS with dried bovine albumin than on the PCS containing dried bovine RBC. Hence, dried bovine albumin-containing PCS with soybean hulls, yeast extract, and mineral salts had a less hydrophobic surface and might be expected to have a greater biofilm population. This was supported by the results for SFYB+, which had the greatest biofilm population (2.3×10^9 CFU/g of support) among all of the supports (Fig. 2).

SYR and OFYR were the two outlying PCS blends that did not follow the positive contact angle and biofilm population correlation. In both cases, despite the supports' high contact angle and more hydrophobic nature, a greater biofilm population was observed. This might be due to the leaching property of yeast extract, which compensated for the hydrophobic nature of the supports.

In all instances, PP discs had the fewest average viable cells attached on their surfaces (4.1×10^4 CFU/g of support), although their average contact angle, 96.7°, was less than those of most of the PCS (Table 2). This was due to the lack of complex-nutrient leaching and/or to the porosity of the PP discs.

BF of lactic acid. The lactic acid concentration in the BF was highly associated with the cell density in MM ($r = 0.96$) (Fig.

4) and the biofilm population ($r = 0.85$) (Fig. 5). These results indicated that the lactic acid production in all instances was determined by both the suspended-cell population and the immobilized-cell (biofilm) population on the supports. Negligible lactic acid concentrations and cell absorbances were observed in the suspended-cell controls and in the PP disc controls under all conditions.

Because the biofilm population of PCS was affected by their relative hydrophobicities, lactic acid production was expected to be influenced also by the contact angle of the supports. As indicated in Fig. 6, the lactic acid concentration and contact angle of soybean hull-containing and oat hull-containing PCS had correlation coefficients of 0.66 and 0.79, respectively. Statistical analysis of the influence of PCS agricultural ingredients on the suspended-cell density and lactic acid production showed effects comparable to those on contact angle and biofilm population. Soybean hulls ($P < 0.0009$) and yeast extract ($P < 0.0001$) increased cell absorbance in the MM, and soybean hulls ($P < 0.02$), yeast extract ($P < 0.0001$), and soybean flour ($P < 0.02$) enhanced lactic acid production.

The impact of soybean hulls, yeast extract, and soybean flour on the suspended-cell density (absorbance at 620 nm) of dried bovine albumin-containing PCS was greater than that for the dried bovine RBC-containing PCS. Consequently, SYB and SFYB+ had cell absorbances of 1.5 and 1.8, respectively, whereas SYR and SFYR+ had cell absorbances of only 1.1 and 1.4, respectively (Fig. 4). Similarly, *L. casei* produced 6.8 and 7.6 g of lactic acid per liter in the presence of SYB and SFYB+, respectively, whereas only 5.6 and 6.8 g of lactic acid per liter were produced in SYR and SFYR+, respectively (Fig. 5).

SEM. SEM photographs illustrated that the hulls were well mixed and that agricultural materials were spread among the PP matrix in the PCS (Fig. 7C). This produced a network with grooves, ridges, and pits (Fig. 7C), whereas the PP discs had relatively smooth and flat surfaces (Fig. 7A). The agricultural material increased the surface area and provided regions sheltered from hydraulic shear forces for bacterial attachment on the PCS surfaces. These observations matched those made by Massol-Deya et al. (18) and also partly explained why the less hydrophobic PP discs did not result in a higher cell attachment. SEM micrographs also indicated that the PCS with yeast extract had denser and larger cell clusters (Fig. 7D) than PP discs (Fig. 7B). Furthermore, extensive electron-opaque fibrillar networks were observed (Fig. 7D). This suggested the production of exopolysaccharides by the *L. casei* biofilm. The exopolysaccharide network as observed in SEM micrographs paralleled that observed by Leppard and Bakke, which also showed the presence of 5-nm electron-opaque fibrils in biofilms of *Pseudomonas aeruginosa* (cited in reference 2).

DISCUSSION

Our results strongly indicate that PCS, besides being able to provide surfaces and shelters from hydraulic shear forces for biofilm formation, were also able to supply complex nutrients for the fastidious growth of *L. casei* (15). The contact angle of *L. casei* was within the range of *Thiobacillus* and *Bacillus* contact angles (26.8° and 32.6°, respectively) as reported by Van Loosdrecht et al. (22). SEM micrographs indicated that hull addition was necessary for increasing the PCS surface area and providing regions sheltered from hydraulic shear forces by forming porous networks with grooves and ridges.

Of the two types of hulls evaluated, soybean hulls outperformed oat hulls by being able to lower PCS hydrophobicity and by having better lactic acid production. This result was similar to that obtained by Demirci et al. (9) with PCS chips (25% [wt/wt] agricultural materials), which demonstrated that soybean hull-containing PCS had a better yield (93.6%) and higher productivity (0.8 g/liter/h) than oat hull-containing PCS (yield, 90.6%; productivity, 0.64 g/liter/h) in continuous lactic acid fermentation with *L. casei*.

Mineral salts were another ingredient that played an important role in reducing the hydrophobicity of PCS and enhancing attachment of cells on the supports. It was the only ingredient that could overcome the hydrophobic nature of dried bovine RBC.

Among the other ingredients, yeast extract was the most outstanding additive. Yeast extract lowered the hydrophobicity of the PCS dramatically (except in the presence of dried bovine RBC) and increased both the biofilm and the MM cell density, which in turn greatly enhanced lactic acid production. The favorable effects of yeast extract were a result of its hydrophilicity and solubility. This is an important factor because it indicates the possibility of lactic acid fermentation in minimal or reduced-complex-nutrient medium, which in turn would lower the overall fermentation cost significantly.

Among all of the PCS evaluated, SFYB+ had the greatest counts of viable attached cells, the highest lactic acid concentration and suspended-cell density in the MM BF, and a relatively small contact angle. All of these results indicated that SFYB+ possessed the greatest potential to be used as a nutrient carrier and biofilm support for commercial production of lactic acid in medium with reduced complex nutrients.

ACKNOWLEDGMENTS

This research was supported by Hatch Act and State of Iowa funds, the Iowa Corn Promotion Board, the ISU Center for Crops Utilization Research, and the Iowa Agriculture and Home Economics Experiment Station.

Special thanks go to Bruce Wagner with the ISU Bessey Microscopy Facility for SEM photographs.

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