In Lactococcus lactis subsp. cremoris FD1, galactose and lactose are both transported and phosphorylated by phosphotransferase systems. Lactose 6-phosphate (lactose-6P) is hydrolyzed intracellularly to galactose-6P and glucose. Glucose enters glycolysis as glucose-6P, whereas galactose-6P is metabolized via the tagatose-6P pathway and enters glycolysis at the tagatose diphosphate and fructose diphosphate pool. Galactose would therefore be a gluconeogenic sugar in L. lactis subsp. cremoris FD1, but since fructose 1,6-diphosphatase is not present in this strain, galactose cannot serve as an essential biomass precursor (glucose-6P or fructose-6P) but only as an energy (ATP) source. Analysis of the growth energetics shows that transition from N limitation to limitation by glucose-6P or fructose-6P gives rise to a very high growth-related ATP consumption (152 mmol of ATP per g of biomass) compared with the value in cultures which are not limited by glucose-6P or fructose-6P (15 to 50 mmol of ATP per g of biomass). During lactose metabolism, the galactose flux through the tagatose-6P pathway \( r_{\text{max}} = 1.2 \, \text{h}^{-1} \) is lower than the glucose flux through glycolysis \( r_{\text{max}} = 1.5 \, \text{h}^{-1} \) and intracellular galactose-6P is dephosphorylated; this is followed by expulsion of galactose. Expulsion of a metabolizable sugar has not been reported previously, and the specific rate of galactose expulsion is up to 0.61 g of galactose g of biomass \( \text{h}^{-1} \) depending on the lactose flux and the metabolic state of the bacteria. Galactose excreted during batch fermentation on lactose is reabsorbed and metabolized when lactose is depleted from the medium. In vitro incubation of galactose-6P (50 mM) and permeabilized cells (8 g/liter) gives a supernatant containing free galactose (50 mM) but no \( P_i \) (less than 0.5 mM). No organic compound except the liberated galactose is present in sufficient concentration to bind the phosphate. Phosphate is quantitatively recovered in the supernatant as \( P_i \) by hydrolysis with alkaline phosphatase (EC 3.1.3.1), whereas inorganic pyrophosphatase (EC 3.6.1.1) cannot hydrolyze the compound. The results indicate that the unknown phosphate-containing compound might be polyphosphate.

The industrial importance of lactococci in milk fermentations is mainly due to the rapid and homolactic fermentation of lactose. Industrial starter strains simultaneously transport and phosphorylate lactose via the phosphoenolpyruvate-dependent phosphotransferase system (PTS), and lactose 6-phosphate (Lac-6P) is subsequently hydrolyzed by phospho-β-galactosidase (P-β-gal) to give glucose and galactose 6-phosphate (Gal-6P) (6, 15, 22, 25, 27, 31). Glucose is catabolized by the Embden-Meyerhof-Parnas (EMP) glycolytic pathway, and Gal-6P is catabolized by the tagatose 6-phosphate (Tag-6P) pathway (5, 7, 22, 24). Nonstarter strains also transport lactose in symport with protons via a permease, and intracellular lactose is hydrolyzed by β-galactosidase to glucose and galactose (5, 8, 22, 24). These sugars are metabolized via the EMP pathway and the Leloir pathway, respectively.

Mutants with defects in lactose transport (9, 13, 16), in lactose or Lac-6P hydrolysis (9, 13), and in metabolism of the glucose and galactose moieties (30) have previously been studied. The present study describes the metabolism of lactose and the constituent sugars (glucose and galactose) by an industrial starter strain which can metabolize both glucose and galactose but exhibits partial expulsion of the galactose moiety during growth on lactose.

**Materials and Methods**

Microorganism and growth conditions. Lactococcus lactis subsp. cremoris FD1 is an ordinary starter strain which was obtained from the Danish Institute of Dairy Research, Hillerød, Denmark (closed since 1990). The medium contained a mixture of equal amounts of yeast extract and casein peptone (YECP) and 1.25 ml of salt solution (0.23 M NaH₂PO₄, 1.15 M NH₄Cl, 58 mM MgSO₄, 58 mM KCl) per g of YECP. The concentrations of YECP and sugar are stated in the figure legends. The inoculum was prepared as described previously (3). The fermentation media were inoculated with 1 mg of biomass per liter, and the bacterium was grown without aeration in laboratory fermentors (working volume, 0.7 to 1.8 liters). The temperature was controlled at 30°C, and the pH was controlled at 6.30 by addition of 2.00 M NaOH.

Analytical methods. The concentrations of glucose, lactic acid, and biomass were measured on-line by three flow injection analyzers as described previously (1). The substrate addition rate and the acid production rate were gravimetrically measured on-line with electronic balances (Sartorius F32000S-D2 and ES100P). The volumetric acid production rate (under conditions of homofermentative metabolism) was calculated from the measured NaOH addition rate and the culture volume. The specific lactic acid formation rate and specific growth rate were calculated as described previously (3). Lactose was determined by measurement of the amount of glucose (2) liberated by quantitative hydrolysis with β-galactosidase (no. G-5625, Sigma). Galactose was measured in a flow
injection analyzer similar to the one used for measurement of glucose (2) except that galactose oxidase (no. G-3385 [Sigma]; 150 U immobilized on a 1-m nylon tube) was used and the buffer consisted of 10 mM KH₂PO₄ and 2.5 mM K₂Fe(CN)₆ (pH 6.3). Galactose oxidase also oxidizes lactose, but the concentration of lactose (in grams per liter) must be 27 times higher than the concentration of galactose for the same signal (peak height) in the analyzer. When galactose was measured in samples which also contained lactose, the measurements were corrected for the small signal due to lactose (galactose oxidase does not oxidize Gal-6P).

Preparation of disrupted cells. Disrupted cells were prepared by washing the bacteria twice in 50 mM Tris-HCl–10 mM MgCl₂ (pH 7.0) and disrupting them in a ball mill (21) at 4°C for 40 min. Permeabilized cells were prepared by using the same washing procedure, resuspending the cells in 2 ml of buffer (1.5 g of biomass per liter), adding 100 μl of acetone-toluene (9:1, vol/vol), and swirlingly mixing for 2 min at 30°C.

Measurement of P, P \(_{\text{p}}\), and \(P_{\text{g}}\). \(P_{\text{g}}\) was measured by reaction with molybdate and reduction of the formed phosphomolybdate by ascorbic acid to minimize the signal from phosphate esters (14). The molybdate reagent was prepared immediately prior to use by dissolving 1.25 g of ammonium heptamolybdate and 10 ml of HClO₄ (70%, w/v) to 100 ml and finally adding 25 ml of ascorbic acid (10 g/liter). To 100 μl of sample containing less than 1 mM \(P_{\text{g}}\) was added 2 ml of molybdate reagent, and the \(A_{250}\) was measured after 30 min.

Assay for \(\beta\)-galactosidase. The sample (disrupted or permeabilized cells) was added to either a solution (5 ml) of 2 mM \(\alpha\)-nitrophenyl-\(\beta\)-D-galactopyranoside (ONPG) (no. N-1127; Sigma) or a solution of 10 mM lactose in 50 mM Tris-HCl–10 mM MgCl₂ (pH 7.0) at 30°C. With ONPG as substrate, an equal volume of 1 M Na₂CO₃ was added to centrifuged samples and the \(A_{420}\) was determined. With lactose as substrate, the released glucose in the centrifuged samples was determined by flow injection analysis (2).

Assay for \(P\)-\(\beta\)-gal. The assay for \(P\)-\(\beta\)-gal was the same as the \(\beta\)-galactosidase assay except that ONPG-6-phosphate (ONPG-6P) (no. N-4013; Sigma) was used instead of ONPG.

Assay of Gal-6P hydrolase activity. Permeabilized cells (8 g/liter) were incubated with 50 mM Gal-6P (no. G-2270; Sigma) in 50 mM Tris-HCl–5 mM MgCl₂ (pH 7.0) at 30°C. After 90 min the sample was centrifuged and the clear supernatant was analyzed for galactose and for \(P\). The reaction product was subjected to hydrolysis by alkaline phosphatase (no. P-6772; Sigma) in diethanolamine buffer as described by the supplier. Likewise, the susceptibility of the reaction product to inorganic pyrophosphatase (no. I-1643; Sigma) (from bakers yeast) was assayed in the Tris-MgCl₂ buffer for 3 h at 25°C with 2 U of enzyme per ml.

RESULTS

Abbreviations. Apr, volumetric acid production rate (milli-moles per liter per hour); \(D\), dilution rate (reciprocal hours); \(k_{\text{ATP},}\) non-growth-associated ATP consumption (millimoles of ATP per gram of biomass per hour); \(K_{\text{Gal}},\) Michaelis-Menten saturation constant for galactose uptake (grams per liter); LA, lactate concentration (grams per liter); \(r_{\text{Gal}},\) specific rate of galactose uptake (grams per gram of biomass per hour); \(r_{\text{Gal,exp}},\) specific rate of galactose efflux from cells (grams per gram of biomass per hour); \(r_{\text{Gal,max}}\) maximum specific rate of galactose metabolism (grams per gram of biomass per hour); \(r_{\text{Gal,met}},\) specific rate of galactose metabolism (grams per gram of biomass per hour); \(r_{\text{Glc,met}},\) specific rate of glucose metabolism (grams per gram of biomass per hour); \(r_{\text{A},\text{L}}\), specific rate of lactate acid formation (grams per gram of biomass per hour); \(r_{\text{Lac},\text{H}_{2}O},\) specific rate of lactate uptake (grams of Lac·H₂O per gram of biomass per hour); \(Y_{\text{ATP}},\) growth-associated ATP consumption (millimoles of ATP per gram of biomass); \(r_{\text{Lac,flux}}\), specific growth rate (reciprocal hours).

Batch fermentations. In a batch fermentation with lactose as the only sugar (Fig. 1), growth is rapid and the metabolism is homofermentative; i.e., lactic acid is the only end product. However, galactose accumulates in the medium during metabolism of lactose, and as the lactose is depleted at \(t = 12.6\) h, growth stops and the accumulated galactose is slowly metabolized into lactic acid by a first-order reaction. No glucose could be detected in the medium at any time during the fermentation. During the whole fermentation until the depletion of lactose, the galactose accumulated in the medium is proportional to the lactose which has been metabolized, as expressed by the equation Galactose (moles per liter in medium) = 0.20 \(\times\) Lactose (moles per liter metabolized). The relative standard deviation (RSD) of the slope is 2%, and the coefficient of correlation is 0.992 (dilution of the medium by NaOH addition to keep pH constant has been taken into account). Thus, lactose is phosphorylated, transported into the cells, and hydrolyzed to give free glucose and Gal-6P. Intracellular glucose is phosphorylated by glucokinase or by mannose-PTS (30) and enters the EMP pathway. The proportionality constant in the above equation shows that as long as there is lactose in the medium, 80% (mol/mol) of the Gal-6P flux enters the Tag-6P pathway and the remaining 20% (mol/mol) is dephosphorylated and excreted back into the medium.

It is observed from the logarithmic plot of the galactose concentration in Fig. 1 that galactose is metabolized by apparent first-order kinetics after the lactose has been consumed. If the overall uptake and metabolism of galactose are governed by a Michaelis-Menten-type kinetics, the saturation constant \(K_{\text{Gal}}\) must be larger than the maximum extracellular galactose concentration (~0.7 g/liter). From Fig. 1 one obtains \(r_{\text{Gal,exp}}/K_{\text{Gal}} = 0.24\) liter g of biomass \(^{-1}\) h \(^{-1}\) (RSD 1%). From the lactate acid production rate (Apr) data a slightly higher value is obtained, but the data are less accurate than the galactose concentration measurements.

Pulse of lactose to the chemostat. Addition of lactose to
chemostat cultures limited by glucose or by lactose resulted in rapid constant-rate metabolism of the added lactose while a considerable fraction of the formed intracellular Gal-6P appears in the fermentation medium as free galactose (Fig. 2). Hence, the enzymes involved in uptake and metabolism of lactose are present in cells grown on glucose, i.e., the lactose-PTS and the enzymes of the Tag-6P pathway. No free glucose could be detected in the fermentation medium during the pulse experiment. Within 27 min of the pulse addition (Fig. 2), all lactose had been metabolized and the accumulated galactose was then slowly metabolized for the next ≈2 h at virtually constant biomass concentration (1.19 g/liter). The acid production rate and the galactose concentration during this phase both decay exponentially, and from either one of these curves one finds the rate constant \( r_{\text{Gal}} = 1.45 \) liters g \(^{-1}\) h \(^{-1}\) (RSD 2%). The corresponding rate constant from an experiment with a lactose pulse to a lactose-limited chemostat culture was estimated to be \( r_{\text{Gal}}/K_{\text{Gal}} = 0.80 \) liter g \(^{-1}\) h \(^{-1}\) (RSD 1%).

**Step change of dilution rate in the chemostat.** The expulsion of galactose during metabolism of lactose must imply that in lactose-limited continuous cultures free galactose is also present in the medium. At \( D = 0.40 \) h \(^{-1}\) the bacteria could not even metabolize all the lactose (Fig. 3); i.e., the culture was limited by some component(s) in the N source. A total of 330 mg of galactose per liter had accumulated in the medium, and when the dilution rate was decreased to 0.10 h \(^{-1}\) the galactose concentration increased to 500 mg/liter during metabolism of the excess lactose. When the lactose concentration had decreased to less than 10 mg/liter (at \( t = 7 \) h), the galactose concentration started to decrease as a result of metabolism and washout from the fermentor, both first-order processes at the low galactose concentration. The net rate of galactose metabolism at \( t = 7 \) to 15 h is given by \( r_{\text{Gal}} = 0.19 \times \text{Gal} \) (h \(^{-1}\)); i.e., \( r_{\text{Gal}}/K_{\text{Gal}} = 0.16 \) liter g \(^{-1}\) h \(^{-1}\). When steady state was attained at \( D = 0.10 \) h \(^{-1}\), the galactose concentration had decreased to ca. 25 mg/liter.

**Pulse of galactose to the chemostat.** To estimate the saturation constant of galactose uptake, we added a pulse of galactose (4.8 g/liter) to a lactose-limited culture at a low dilution rate (Fig. 4). The specific rate of galactose consumption \( (r_{\text{Gal}} = 0.5r_{\text{Gal}}) \) was fitted to a Michaelis-Menten expression (Lineweaver-Burk plot) to give \( K_{\text{Gal}} = 0.95 \) g/liter (RSD 1%) and \( r_{\text{max}} = 0.85 \) h \(^{-1}\) (RSD 6%); the coefficient of correlation is 0.9985. The extra galactose flux due to the simultaneous metabolism of lactose \((0.47 \) h \(^{-1}\)) should be added to the estimated maximum galactose uptake to give the maximum flux through the Tag-6P pathway, i.e., \( r_{\text{Gal,max}} = 0.85 + 0.47/2 = 1.09 \) h \(^{-1}\). No detectable lactose accumulated in the medium after addition of galactose. Since the biomass concentration increased after the galactose addition, the fraction of glucose which is used for biomass precursors also had increased. This was expected since galactose metabolism can

---

**FIG. 2.** Pulse of lactose to a glucose-limited chemostat \((D = 0.10 \) h \(^{-1}\), with a feed composition of 7 g of glucose per liter and 10 g of YECP per liter). The substrate addition was stopped when the pulse was added \((t = 0)\). The measured volumetric acid production rate \((x)\) and concentrations of lactose \(\cdot H_2O\) (■) and galactose (▲) are shown together with the estimated total galactose concentration \(\sum\) (sum of free and lactose bound [+]).

**FIG. 3.** Step change of the dilution rate \((t = 3.6 \) h\) from 0.40 to 0.10 h \(^{-1}\) in a chemostat \((\text{feed contained } 7 \text{ g of lactose }\cdot\text{H}_2\text{O per liter and } 10 \text{ g of YECP per liter})\). The acid production rate \((x)\), volumetric \((-\), specific) and the concentrations of biomass \((+), \text{lactose }\cdot\text{H}_2\text{O} \text{ (■), galactose (▲), and lactic acid (LA) (□) are shown.}

**FIG. 4.** Pulse addition of 4.8 g of galactose per liter to a lactose-limited chemostat \(D = 0.1 \) h \(^{-1}\) \((\text{feed contained } 7 \text{ g of lactose }\cdot\text{H}_2\text{O per liter and } 10 \text{ g of YECP per liter})\). The acid production rate \((x)\), volumetric \((-\), specific) and the concentrations of biomass \((+), \text{galactose (▲), and lactic acid (LA) (□) are shown. The line beneath the galactose measurements is a simulation of the galactose concentration \((K_{\text{Gal}} = 0.93 \) g/liter, \(r_{\text{max}} = 0.85 \) h \(^{-1}\)). The concentration of lactose was always below the detection limit \((5 \text{ mg/liter})\).
supply the cells with metabolic energy (via lactic acid formation) and thereby reduce the amount of glucose which has to be catabolized via the EMP pathway.

**Fed-batch fermentation.** The major part of the biomass is synthesized from the YECP substrate, and if biomass precursors (intracellular Glc-6P or Fru-6P) are continuously supplied during a batch fermentation with galactose as the (main) energy source and YECP as the (main) carbon and nitrogen source, a high growth rate may still be possible. The fed-batch experiment of Fig. 5 proves the validity of this hypothesis. Initially the medium contained the N source (20 g of YECP per liter) and galactose (15 g/liter), whereas lactose was slowly fed to the medium during the fermentation. In this way the glucose part of lactose served as biomass precursor while galactose supplied the cells with metabolic energy (lactic acid formation). Indeed, the effect of the slow lactose addition is substantial growth of the bacterium, in gross contrast to the absence of growth in a similar fermentation without addition of lactose. A plot of the specific lactic acid formation rate versus the specific growth rate at the same time—a so-called phase-plane plot—shows that growth occurs in two energetically distinct phases (Fig. 6). Since 1 mol of ATP per mol of lactic acid is formed in glycolysis, the slope and intercept in this plot are the growth-related \( \gamma_{\text{ATP}} \) and the non-growth-related \( k_{\text{ATP}} \) ATP consumption, respectively. Linear regression gives the following two sets of parameters: for \( \mu > 0.10 \text{ h}^{-1}, Y_{\text{ATP}} = 20 \text{ mmol g}^{-1} \text{ h}^{-1} \) and \( k_{\text{ATP}} = 15 \text{ mmol g}^{-1} \text{ h}^{-1} \); for \( \mu < 0.10 \text{ h}^{-1}, Y_{\text{ATP}} = 152 \text{ mmol g}^{-1} \text{ h}^{-1} \) and \( k_{\text{ATP}} = 1.6 \text{ mmol g}^{-1} \text{ h}^{-1} \). When \( \mu > 0.10 \text{ h}^{-1} \) both the growth-related and the non-growth-related ATP consumptions are typical of the values found for a culture which is limited neither by energy nor by carbon source, e.g., the start of a batch fermentation with 20 g of glucose per liter and 20 g of YECP per liter (4). The very high \( Y_{\text{ATP}} \) and the correspondingly low \( k_{\text{ATP}} \) for \( \mu < 0.10 \text{ h}^{-1} \) is, however, never seen in a glucose-YECP or lactose-YECP batch culture, in which typically \( Y_{\text{ATP}} \) increases from 15–20 to about 50 mmol/g at a certain \( \mu \) value. Only in a culture grown on fructose and YECP was an equally high \( Y_{\text{ATP}} \) measured after the break in

**FIG. 5.** Fed-batch fermentation where lactose·H₂O is slowly added at a constant rate (0.19 g/h) to an inoculated medium containing 15 g of galactose per liter and 20 g of YECP per liter. The acid production rate (×, volumetric; –, specific) corrected for the decreasing volume due to sample removal, and the concentrations of biomass (+) and lactic acid (□) are shown with the estimated specific growth rate (×), calculated by numerical differentiation of the biomass-versus-time curve.

**FIG. 6.** Phase-plane plot of the specific lactic acid production rate versus the specific growth rate during the fed-batch fermentation shown in Fig. 5. Two distinct phases are observed, and the lines are plotted by using the estimated bioenergetic parameters (see text).

the phase plot (3, 4). Comparison of Fig. 5 and 6 shows that after an initial exponential growth phase (\( t < 4 \text{ h}, \mu \approx 0.65 \text{ h}^{-1} \)), a second growth phase with rapidly decreasing \( \mu \) is entered. The breakpoint in the phase-plane plot occurs at about \( t = 10 \text{ h} \). Apparently, the culture is able to grow at the same energetic conditions all the way through the first two growth phases, i.e., after the (hitherto unknown) component(s) in the YECP which supports the high growth rate has become nearly exhausted.

**Enzyme assays.** No β-galactosidase activity could be detected with either ONPG or lactose as substrate in the spent medium, in cell extracts, or in permeabilized cells taken from batch or continuous cultures on lactose. Furthermore, a suspension of washed cells readily metabolized ONPG with simultaneous expulsion of o-nitrophenol. The presence of P-β-gal in cell extracts and in permeabilized cells was verified by their ability to hydrolyze ONPG-6P. Hence, L. lactis subsp. cremoris FD1 cells exclusively apply the lactose-PTS for transport and phosphorylation of lactose (and ONPG), followed by hydrolysis of Lac-6P (and ONPG-6P) by P-β-gal. Galactose which accumulates in the medium therefore is a result of dephosphorylation of intracellular Gal-6P and release of free galactose into the medium.

To assess the mechanism responsible for expulsion of galactose, we assayed permeabilized cells for hexose-6-phosphate: phosphohydrolase (Hex-6Pase) activity (29) by using Gal-6P as substrate. In a supernatant from an assay which contained 50 mM Gal-6P and 8 g of permeabilized cells per liter, galactose was quantitatively recovered (48 to 50 mM) but no P₆ (less than 0.5 mM) was liberated. Phosphate was not precipitated and thus removed from the supernatant with the cells, since it was quantitatively liberated in the supernatant by hydrolysis with alkaline phosphatase. The permeabilized cells cannot be the origin of the compound which binds phosphate, since 6.25 mmol of phosphate is bound per g of permeabilized cells in the assay, i.e., a theoretical maximum molar weight of 160.

**DISCUSSION**

The metabolism of lactose and galactose by L. lactis subsp. cremoris FD1 is different from that observed with other starter strains of L. lactis subsp. cremoris (6, 22, 24). In strain FD1 galactose is exclusively transported and phosphorylated via phosphotransferase systems (Lac-PTS or Gal-PTS) and subse-

Downloaded from http://aem.asm.org on October 29, 2017 by guest
and expulsion when the specific lactose concentrations are used only for energy formation, the galactose permease or an enzyme in the Leloir pathway must also be missing.

FIG. 7. Key reactions for uptake and metabolism of galactose for biomass synthesis and for energy formation in lactococci. The PTS produces Gal-6P, which is a gluconogenic metabolite, whereas the galactose permease produces free intracellular galactose, which is an ordinary glycolytic substrate. FDPase is not present in *L. lactis* subsp. *crenaris* FDI, and since galactose can be used only for energy formation the galactose permease or an enzyme in the Leloir pathway must also be missing.

When the specific rate of metabolism of lactose is compared with the specific rate of galactose expulsion (Fig. 8; Table 1), it is observed that the rate of expulsion of galactose depends on both the lactose flux and the metabolic state of the cells, i.e., on the concentrations of enzymes expressed at the particular growth conditions. The two pulse experiments illustrate that when the cells are grown on lactose (lactose-limited culture), the rate of galactose expulsion is lower than when the cells are grown on glucose (glucose-limited culture). Also, during the lactose pulse to the glucose-limited culture, the fraction of the galactose which is expelled from the cells decreases.

Estimated ratio $r_{gal,ext}/K_{gal}$ varies considerably (0.16 to 1.45 liters g⁻¹ h⁻¹) between the experiments, and this is attributed to the very different growth conditions used. However, the estimates (0.80 to 1.45 liters g⁻¹ h⁻¹) from experiments in which the culture had been sugar limited are much larger than the estimates (0.16 to 0.24 liter g⁻¹ h⁻¹) from cultures which had been growing at a saturating lactose concentration.

Despite the Tag-6P pathway being constitutive, its flux capacity is not constant, and this indicates that synthesis of the rate-limiting enzyme(s) is affected by the sugar (lactose or glucose). The maximum flux capacity of the Tag-6P pathway is 1.09 to 1.17 h⁻¹ as found during growth on lactose (Fig. 1 and 4). From the present work it is not possible to determine whether the limiting enzyme is Gal-6P isomerase, Tag-6P kinase, or tagatose 1,6-diphosphate aldolase. Together with the lactose-PTS these enzymes are encoded by the same operon on a plasmid in *L. lactis* (10, 33). The defective Tag-6P pathway could be caused by an insufficient concentration of one of the three enzymes or by an exceedingly large $K_{m}$, of one of these enzymes.

Expulsion of galactose may be a detoxification process which protects the cell against an inhibitory intracellular concentration of Gal-6P or galactose. Detoxification by dephosphorylation and expulsion of nonmetabolizable phosphorylated sugar analogs, e.g., 2-deoxy-D-glucose and methyl-β-D-thiogalactopyranoside, in lactococci is well known (19, 28, 31, 32). The novelty of the results described here is that a metabolizable sugar phosphate is dephosphorylated and excreted into the medium. Expulsion of a nonmetabolizable free sugar (the galactose moiety of lactose) is also well known from lactococci unable to metabolize galactose, in *Streptococcus thermophilus* (12, 18, 23) and in *Lactobacillus bulgaricus* (11, 18). There are two known mechanisms of dephosphorylation of sugar phosphates in lactococci: simple hydrolysis and transphosphorylation. Hex-6Pase hydrolyzes many sugar phosphates and liberates the sugar and P₆ (29). In vitro measurements amply illustrate that dephosphorylation and expulsion may be coupled by transphosphorylation (exchange group translocation) with an incoming sugar (17, 19, 20, 30). Transphosphorylation has been shown in vitro to be catalyzed by the Lac-PTS in several different microorganisms including *L. lactis* (19, 20, 31), by fructose-PTS in *Bacillus subtilis* (17), and by mannose-PTS in *Salmonella typhimurium* (20).
Since the dephosphorylation of Gal-6P by permeabilized cells proceeds without the formation of P, and in the absence of a free sugar, the dephosphorylation occurs by a mechanism which is not direct hydrolysis or transphosphorylation. P, may be liberated from the reaction products by alkaline phosphatase but not by inorganic pyrophosphatase, and this indicates that polyphosphate may be produced during galactose dephosphorylation. Formation of an organic phosphate-containing compound would require a large amount of the phosphate acceptor, which is not present in the small mass of permeabilized cells. An exact identification of the phosphate-containing compound has not been made, but the results suggest that it is polymerized phosphate. This is interesting since lactococci are not considered to produce polyphosphates.

The complex energetic pattern characteristic of the fed-batch fermentation in which growth on galactose is made possible by a small feed of lactose has to be studied further, but a tentative explanation for the breakpoint in Fig. 6 may be advanced, as follows. Since a constant feed rate of lactose is maintained throughout the fermentation, biomass precursors are in excess at the start of the fermentation when the biomass concentration is low, whereas towards the end of the fermentation the lactose supply is insufficient to support growth of the large amount of biomass. The two sets of parameters describing the growth energetics thus reflect the transition from N-source-limited growth to biomass-precursor-limited growth, where growth is energy sufficient in both phases. A more comprehensive description of the growth energetics of L. lactis subsp. cremoris FDI is given elsewhere (4).

REFERENCES