Metabolism and Energetics of *Lactococcus lactis* during Growth in Complex or Synthetic Media

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When *Lactococcus lactis* was grown in various complex or synthetic media, the fermentation of glucose remained homolactic whatever the medium used, with a global carbon balance of about 87%. Moreover, the nitrogen balance was not equilibrated, indicating that some amino acids led to the production of unknown nitrogen-containing carbon compounds while part of the glucose might contribute to anabolic pathways. In minimal medium containing six amino acids, a high concentration of serine was deaminated to pyruvate. This did not occur in more complete media, suggesting the presence of a regulation of this phenomenon by an amino acid. Ammonia produced during serine consumption was partly reconsumed after serine exhaustion. The values for biomass yield and biomass yield relative to ATP (Y_{ATP}), the maximal growth rate, the specific rate of glucose consumption, and the corresponding rate of ATP synthesis all increased with the complexity of the medium, amino acid composition having the most pronounced effect. The Y_{ATP} values were shown to range from 6.6 to 17.6 g of biomass · mol of ATP⁻¹ on minimal and complex media.

Lactic acid bacteria (LAB) are of importance in the food industry, mainly for lactic acid, flavor compound, and bacteriocin production. However, they are characterized by numerous nutritional requirements, especially with regards to nitrogen sources. It has been demonstrated that growth is limited by the availability of essential nutrients, since growth was improved by supplementing the culture media with yeast extract (15), peptone (5), or amino acids (12). Moreover, while certain nutritional requirements appear to be strain dependent, the requirement for certain amino acids, i.e., glutamic acid, methionine, or branched-chain amino acids, is more widespread. This nutritional complexity is such that cultivation of LAB frequently employs complex media (MRS or M17) or milk for applied food research. However, the complex metabolism associated with the use of the numerous compounds included in the rich media is such that the metabolic and energetic behaviors are difficult to characterize.

A defined medium which supports growth of the bacteria at a constant specific growth rate is required for metabolic investigations. The chemically defined medium described by Otto et al. (10) and modified by Poolman and Konings (12) (MCD medium) is used increasingly for the growth of *Lactococcus lactis*, particularly for genetic investigations. While the composition of this medium is certainly defined, neither the composition nor the concentrations of the various components have been optimized. Studies concerning the nutritional requirements of *Lactococcus* (4, 13) or *Lactobacillus* strains (6, 8, 9) have been described previously, although they are incomplete. The first exhaustive nutritional study was reported by Cocaign-Bousquet et al. (3), who proposed the composition of a true minimal medium for the sustained growth of *L. lactis* subsp. *lactis* NCDO 2118 which contained only 15 components, including six amino acids. Different defined media of varying complexity are now available for the growth of this strain, enabling the effects of medium composition on cell behavior to

be precisely assessed. To date, knowledge about both carbon and nitrogen metabolism during growth of LAB in media other than milk is very limited and virtually nonexistent for growth of LAB in defined media.

In the present work, the availability of different defined media for the growth of *L. lactis* (3) was exploited with a view to understanding the metabolic behavior of this bacterium. *L. lactis* subsp. *lactis* NCDO 2118 was cultivated in five different media, one of a complex nature (M17 medium) and four defined media, varying in complexity with from 47 to 15 components. Complete fermentation balances, taking into account the accurate amino acid consumption, growth characteristics, and kinetic behavior of carbon and energy fluxes, were established and compared as a function of the medium composition.

MATERIALS AND METHODS

Organism and culture media. The microorganism used throughout this study was *L. lactis* subsp. *lactis* NCDO 2118, obtained from the collection held at INRA (Jouy-en-Josas, France). The strain was stored at -80°C in MCD medium supplemented with glycerol (20%).

Five different media were used for the growth of *L. lactis*. Growth was undertaken on a reconstituted complex medium similar to M17 medium (18) in which lactose was replaced by glucose. This medium was composed of the following: tryptone (Difco), 5 g/liter; bio-soyane (Biomérieux), 5 g/liter; meat extract (Merck), 5 g/liter; yeast extract (Biomérieux), 2.5 g/liter; ascorbic acid, 0.5 g/liter; MgSO₄ · 7H₂O, 0.25 g/liter; β-glycerophosphate, disodium salt, 19 g/liter. This medium was sterilized by autoclaving (20 min at 121°C) before aseptic addition of glucose (10 g/liter), which had been autoclaved separately.

Defined synthetic media were also used for the growth of the strain. The standard synthetic medium used for the preparation of inocula and for reference culture was the MCD medium containing 47 components, including 18 amino acids and 14 vitamins, described by Otto et al. (10) and modified by Poolman and Konings (12). Synthetic media described by Cocaign-Bousquet et al. (3) for the growth of *L. lactis* NCDO 2118 (MS10 and MS13 media) were also used. The MS10 medium containing 27 components has the same amino acid composition as that of the MCD medium but was simplified with regards to vitamin, nucleotide, and salt composition. The MS13 medium, containing 15 components, differs from the MS10 medium in that the amino acid composition reduced to six amino acids. A modified MS13 medium was also used. This medium (MS14 medium) had different amino acid concentrations to avoid amino acid depletion during culture and the following composition: glucose, 20 g/liter; KH₂PO₄, 9 g/liter in tube cultures or 3 g/liter in pH-regulated fermentors; K₂HPO₄, 7.5 g/liter in tube cultures or 2.5 g/liter in pH-regulated fermentors; MgCl₂ · 6H₂O, 0.2 g/liter; pyridoxamine, 5 mg/liter; biotin, 10 mg/liter; nicotinic acid, 1 mg/liter; Capantothenate, 1 mg/liter; riboflavin, 1 mg/liter; glutamic acid, 0.6 g/liter; isoleucine, 0.33 g/liter; leucine, 0.66 g/liter; methionine, 0.12 g/liter; serine, 2.08

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FIG. 1. Growth of *L. lactis* on MCD (A and B) and MS13 (C and D) media. (A and C) Symbols: ◆, biomass; ■, glucose; □, lactate. (B and D) Symbols: ●, formate; ○, acetate; △, ethanol.

g/liter; valine, 0.33 g/liter. These media were prepared from concentrated individual stock solutions stored at 4°C after filtration, except for the cysteine solution, which was freshly prepared. The media (pH 6.6) were sterilized by filtration through cellulose nitrate membranes (0.22- μ m pore size; Sartorius) directly into the sterilized (20 min at 121°C) culture vessel.

Culture conditions. The fermentations were carried out under strictly anaerobic conditions in 2-liter glass fermentors (Sétic Génie Industriel, Toulouse, France) or in anaerobic tubes for inoculum cultures, at a temperature of 30°C and an agitation speed of 250 rpm. The bacteria were grown under a controlled gas environment by flushing both the vessel and the medium with nitrogen. The medium in the fermentor was aseptically gassed (30 min) immediately before inoculation and maintained under an N₂ atmosphere at a positive pressure of 10³ Pa. Cultures in the fermentor were maintained at pH 6.6 by the automatic addition of 5 N KOH.

Inoculation was at 2% with a high-optical-density (optical density at 580 nm = 2.0) culture on MCD medium with a high concentration of phosphates ([K₂HPO₄] = 15 g/liter; [KH₂PO₄] = 18 g/liter) to buffer acid production; the culture was incubated overnight at 30°C in a butyl-stoppered 250-ml shake flask under N₂. Precultures were washed twice with sterile phosphate buffer (100 mM, pH 6.6) to avoid carryover of essential nutrients and resuspended in the same buffer. All cultures were carried out in triplicate and repeated if experimental variation exceeded 5%.

Analytical methods. Bacterial growth was monitored by spectrophotometric measurements at 580 nm and calibrated against cell dry weight measurements. Cells were harvested by filtration on 0.45- μ m-pore-size nylon membranes, washed with 2 volumes of deionized water, and dried to a constant weight at 60°C under a partial vacuum (200 mm of Hg [ca. 26.7 kPa]). A change of 1 U of optical density was shown to be equivalent to 0.31 g of dry matter · liter⁻¹. The biomass formula, determined by elemental analysis at ENSC (Toulouse, France), was

C_{4.63}H_{7.89}O_{2.35}N_{1.0} on M17, MCD, and MS10 media and C_{5.05}H_{9.20}O_{2.77}N_{1.0} on MS13 and MS14 media, with molar masses of 129 and 140 g · mol⁻¹, respectively, used to convert cell dry weights into molar cell carbon concentrations.

The carbon dioxide concentration in the gas phase was determined by gas chromatography with a Porapak Q column followed by a molecular sieve maintained at 40°C, with helium as the carrier gas and catharometer detection.

Concentrations of glucose and fermentation products in the fermentation broth were measured by high-pressure liquid chromatography with a Bio-Rad HPX87H⁺ column and the following conditions: a temperature of 50°C, the solvent H₂SO₄ (5 mM), a flow rate of 0.5 ml · min⁻¹, and refractometer detection. Ethanol concentration measurements were also carried out by isocratic gas chromatography on a Porapak-Q packed column at 190°C with a flame ionization detector (Intersmat GC).

Concentrations of amino acids in the medium were determined with an Ami-Quant 1090 high-pressure liquid chromatograph (Hewlett-Packard) after derivatization by *ortho*-phthalaldehyde in the presence of 3-mercaptopropionic acid, separation with a C₁₈ column, and spectrophotometric detection at 338 nm. Ammonia concentrations were determined in filtered culture samples with an ammonia-selective electrode (Orion).

RESULTS AND DISCUSSION

Growth and amino acid consumption. Whatever the medium used, growth started immediately after inoculation and continued until the complete exhaustion of glucose from the medium (Fig. 1A and C). Both the amino acid consumption and the biomass formation were a function of the composition

TABLE 1. Stoichiometric coefficients, expressed relative to 100 mM glucose consumed, established on the complex medium and the synthetic media used for *L. lactis* culture

Medium	Stoichiometric coefficient (mM)								
	Glucose	Amino acids ^a	Biomass ^b	Lactate	Formate	Acetate	Ethanol	Pyruvate	NH ₃
M17	100	ND ^c	25.6	170.0	12.0	5.0	4.0		ND
MS10	100	21.5	20.3	165.6	11.0	3.9	4.4		ND
MS13	100	16.2	11.3	171.2	7.0	3.9	2.8		1.8
MS14	100	28.2	9.9	175.0	16.1	13.2	0.0	2.9	13.5

^a The average elemental amino acid compositions were C_{4.69}H_{9.76}O_{2.38}N_{1.4} on MS10 medium, C_{4.62}H_{10.01}O_{2.54}N_{1.0} on MS13 medium, and C_{3.84}H_{8.43}O_{2.84}N_{1.0} on MS14 medium.

^b The elemental biomass formulas were C_{4.63}H_{7.89}O_{2.35}N_{1.0} on M17, MCD, and MS10 media and C_{5.05}H_{9.20}O_{2.77}N_{1.0} on MS13 and MS14 media.

^c ND, not determined.

of the medium (Table 1). Effectively, growth on MCD and MS10 media with the same amino acid compositions led to the synthesis of similar quantities of biomass and the consumption of correspondingly equal quantities of amino acids (Table 1). Even the levels of relative consumption of individual amino acids were similar for the media (Table 2).

Growth on M17 medium led to a higher yield of biomass relative to glucose consumption than in the media mentioned above, but the precise consumption of amino acids could not be determined in this medium due to its complexity. In contrast, growth on MS13, which contained only 6 amino acids, was less efficient than that on media containing 18 amino acids, the final biomass concentrations being approximately twofold lower. The use of individual amino acids was higher in this medium than it was in more complex media (Table 2). Hence, the ratio of amino acid consumption to biomass formation was higher in MS13 medium than in more complete media (Table 1), indicating that not only were the nonessential amino acids being synthesized but also a part of the carbon from the consumed amino acids may be associated with the production of unidentified fermentation products. Growth ceased after about 9.5 h of cultivation (Fig. 1C), although both glutamate and serine were exhausted prior to this (Fig. 2). Serine was con-

sumed in high amounts under these conditions. About 1.8 mM ammonia was produced during the period of serine consumption, and furthermore, a significant part of this ammonia, about 1 mM of the 1.8 mM, was reconsumed once serine had been exhausted. This ammonia consumption was observed only when serine was exhausted from the medium, and no ammonia consumption occurred when ammonium salts were added to the culture medium during the initial period of serine consumption (data not shown). Moreover, the strain has previously been shown to be unable to grow in a modified MS13 medium containing ammonium but lacking serine (3). Hence, the question remains as to how growth proceeds at the expense of ammonia consumption in the absence of apparently essential amino acids.

The MS14 medium was constructed on the basis of the MS13 medium but with different amino acid concentrations to avoid nutritional depletion. Effectively, under these conditions, more glutamate was consumed than it was in MS13 medium, and serine was consumed in a very much higher amount, which is associated with a high level of NH₃ production (Table 2): for 19 mM serine consumed, 13.5 mM ammonia was recovered in the medium. In the MS14 medium, ammonia was produced throughout the fermentation. This observation confirmed the correlation observed for the MS13 medium between serine exhaustion and ammonia consumption. The estimated concen-

TABLE 2. Amino acid consumption and ammonia production expressed relative to the consumption of 100 mM glucose, during growth of *L. lactis* subsp. *lactis* NCDO 2118 on the synthetic media

Substance consumed or produced	Concn (mM) on medium		
	MS10	MS13	MS14
Ala	1.72		
Arg	0.71		
Asn	1.09		
Gln	3.48		
Glu	-1.32 ^a	1.24	2.30
Gly	1.38		
His	0.27		
Ile	1.52	1.88	1.94
Leu	2.72	4.37	3.20
Lys	1.23		
Met	0.43	0.45	0.44
Phe	0.54		
Ser	4.03	6.22	19.0
Thr	1.52		
Trp	0.09		
Tyr	0.60		
Val	1.45	2.02	1.37
Ammonia	ND ^b	1.0	13.5

^a Concentration of produced glutamate.

^b ND, not determined.

FIG. 2. Amino acid consumption and ammonia profile during growth of *L. lactis* on MS13 medium.

TABLE 3. Elemental and coenzyme balances, growth and energetic parameters, and specific rates obtained during culture of *L. lactis* on various media

Medium	Balance				Biomass yield (g · mol of glucose ⁻¹)	ATP yield (mol · mol of glucose ⁻¹)	Y_{ATP} (g of biomass · mol of ATP ⁻¹) ^c	μ_{max} (h ⁻¹)	q_s^{max} (mmol · g ⁻¹ · h ⁻¹)	q_{ATP}^{max} (mmol · g ⁻¹ · h ⁻¹)	Y_{ATP}^{max} (g · mol of ATP ⁻¹) ^d
	Total C ^a	Catabolic C ^b	NADH	N							
M17		90.0	89.1		33.0	1.87	17.6	1.10	25.0	40.0	27.5
MCD	87.7	87.0	86.9	71.9	28.5	1.78	16.0	0.80	24.5	42.1	19.0
MS10	87.4	86.5	87.2	67.4	26.2	1.75	15.0	0.60	20.6	34.7	17.3
MS13	86.9	89.0	88.4	80.1	14.6	1.82	8.0	0.23	15.7	27.4	8.4
MS14	87.8	96.0	87.5	83.0	12.8	1.94	6.6	0.20	14.8	26.1	7.7

^a C balance calculated between glucose and amino acid consumption and biomass plus product formation.^b C balance calculated between glucose consumption and product formation.^c Global Y_{ATP} calculated as the ratio of biomass yield to ATP yield.^d Maximal Y_{ATP} calculated as the ratio of μ_{max} to q_{ATP}^{max} .

tration of serine consumed for anabolic functions, e.g., the serine consumed minus the ammonia produced, was about 5 mM, a value nearly identical for both MS13 and MS14 media, since quite similar biomass concentrations were formed on the two media. The apparently high ratio of amino acid consumption to biomass production (Table 1) was directly related to this important consumption of serine. Since serine was deaminated, it can be postulated that it was converted into pyruvate and ammonia by the serine dehydratase (or the serine deaminase) enzyme. The observation that pyruvate was a product of this fermentation agreed with this hypothesis. Since this high consumption of serine was found for MS13 and MS14 media, although not for MCD and MS10 media, it seems probable that this aspect of serine metabolism was regulated by the amino acid composition of the medium.

Product formation. Whatever the medium used for the growth of *L. lactis*, from the complex M17 medium to the minimal MS14 medium, the metabolism of glucose remained homolactic, with lactic acid being produced at a yield of about 1.7 mol · mol⁻¹ (Table 1). Among the minor products currently observed in LAB, CO₂ did not occur, indicating that pyruvate dehydrogenase was not active in these culture conditions, certainly due to the strictly anaerobic environment used. The same observation can be made for acetolactate synthase activity, since none of the products derived from acetolactate, i.e., acetoin or butanediol, was observed despite some accumulation of pyruvate in the minimal medium. However, some pyruvate was converted by the pyruvate formate lyase into formate plus acetyl coenzyme A (acetyl-CoA), the latter being transformed into a mixture of acetate and ethanol (Fig. 1B and D). It should be noted that the sum of acetate and ethanol production was never as high as the amount of formate produced, indicating that some acetyl-CoA was involved in other metabolic reactions. No ethanol was produced in the MS14 medium, while some pyruvate appeared in the fermentation broth (Table 1). Moreover, lactate, formate, and acetate were produced in higher amounts in MS14 medium than they were in the other media, and this increase correlates well with the high serine consumption. From these results, it appears that besides performing its essential anabolic function, serine was also transformed into pyruvate, a part of it being accumulated in the medium and a major part being converted into the normal products of pyruvate metabolism. Ethanol was not produced during the serine consumption phase, indicating that the reducing equivalent equilibrium did not necessitate the reduction of acetyl-CoA to ethanol. Confirmation of this was found with the MS13 medium, where no ethanol was produced during the period of serine consumption, while a shift from

acetate to ethanol production was induced soon after serine exhaustion (Fig. 1D).

The coenzyme balance calculated from the known metabolic pathways producing or consuming reducing equivalents was shown to be constant for each medium, with an average recovery value of 87.5%. While some of the reduced coenzymes were no doubt consumed during anabolic reactions, the production of unidentified compounds may also have involved the consumption of reducing equivalents to equilibrate the coenzyme balance. The global carbon balance calculated by taking into account glucose and amino acid consumption converted into biomass and known products was also constant whatever the medium used (Table 3). On the other hand, the catabolic carbon balance calculated between glucose consumption and product formation, which was higher in MS14 medium than in the other media, confirms that serine catabolism led only to classical identified products of *L. lactis* derived from pyruvate.

With regards to the nitrogen balance, a large deficit was observed on all media, when taking into account amino acids, ammonia, and nitrogen included in the biomass. This indicates that some of the unidentified fermentation products contain nitrogen and were most probably produced from amino acids, consequently equilibrating both the carbon and nitrogen recoveries. Therefore, if the partial metabolism of amino acids contributes to the carbon imbalance, this implies that a part of the glucose carbon may be contributing to the synthesis of biomass.

Growth yields and energetics. The growth yield relative to the glucose consumed was a function of the medium composition (Table 3). The richer the medium, the higher the growth yield observed. Poor yields were obtained on MS13 and MS14 media, while twofold-higher values were observed on MS10 medium, the only difference in medium composition being the amino acid content. Growth yield on MCD medium was not very different from that observed on MS10 medium, the difference between these two media being the mineral salts, vitamin, and nucleotide compositions. Finally, a better growth yield was logically obtained on the complex M17 medium, although the increase in yield compared to that of the MCD medium was not very significant.

Taking into account the homofermentative pathway of glucose metabolism by *L. lactis*, the amounts of ATP synthesized by substrate-level phosphorylation are 1 mol · mol of lactate⁻¹, 1 mol · mol of formate⁻¹, and 1 mol · mol of acetate⁻¹. Other possible mechanisms of ATP synthesis, like ATP production associated with lactate efflux, were possibly present in our bacterium but were not quantified. However, the contribution of this mechanism in global ATP production during our

batch cultures would not have been very important since it normally operates significantly only at the beginning of the culture (16, 17). Moreover, our results were examined comparatively for the various media used, and the phenomena contributing to ATP synthesis other than substrate-level phosphorylation were neglected in all of the media. From the product yields obtained during the fermentation, the global yield of ATP production can be calculated. Since the fermentation was homolactic on each medium, ATP production varied only from 1.75 to 1.94 mol · mol of glucose consumed⁻¹ (Table 3). Hence, the biomass yield relative to ATP, i.e., Y_{ATP} , in grams of biomass per mole of ATP, evolved like the biomass yield relative to glucose did. Values ranging from 6.6 on the minimal medium to 17.6 on the complex medium were calculated. These values are consistent with those currently available for the growth of anaerobic bacteria, as shown in the values cited by Benthin et al. (1), ranging from 8.6 to 24.4 g · mol⁻¹ for various microorganisms, or when compared with the values obtained by Salou et al. (14) for *Leuconostoc oenos*. However, they were average values calculated throughout the fermentation from the global yield values. The specific rates of growth, substrate consumption, and product formation, and hence ATP synthesis, were maintained at their maximal values for the vast majority of the culture and decreased gradually thereafter (data not shown), as might be expected from the toxic effect of some metabolic products (7). Therefore, a difference can be observed in the Y_{ATP} values calculated as global values for the entire duration of the culture or calculated from the maximal parameters and corresponding to the period of the culture in which the kinetic parameters were constant and maximal. The maximal values of Y_{ATP} ($Y_{\text{ATP}}^{\text{max}}$) observed during the early exponential phase of growth were calculated as the ratio of the maximal specific growth rate (μ_{max}) to the maximal specific rate of ATP formation ($q_{\text{ATP}}^{\text{max}}$). The $Y_{\text{ATP}}^{\text{max}}$ values were not very different than the average Y_{ATP} values for the synthetic media, indicating that Y_{ATP} was maintained at a nearly constant value in these media. On the other hand, the $Y_{\text{ATP}}^{\text{max}}$ value was much higher than the corresponding average value in the complex medium. This indicates that the efficiency of growth was maximal in fresh medium and declined progressively. Benthin et al. (1) reported an identical observation since in a complete energetic study of the growth of *Lactococcus cremoris* FD1, Y_{ATP} varied during the different stages of a batch fermentation from a value near the theoretical maximal value at the onset when no nutritional limitation was present to a much lower value later in the culture. Moreover, Y_{ATP} values were higher in a complex medium than in a defined medium. In the second part of the culture, the calculated values were 20 and 8.3 g · mol⁻¹ for complex and defined media, respectively. Another study, by Benthin and Villadsen (2), with the same strain cultivated in a medium containing yeast extract, reported a Y_{ATP} value of 26 g · mol⁻¹ maintained throughout the fermentation. On the other hand, Salou et al. (14) demonstrated that the growth of *L. oenos* in a complex medium had a constant efficiency whatever the energetic substrate used, with a Y_{ATP} value of about 10 g · mol⁻¹. The values obtained here confirm that the nutritional richness of the medium has a profound effect on growth efficiency.

Carbon and energy fluxes. The maximal specific rates of glucose consumption (q_s^{max}) were found to be different on the various media tested, varying from 15 to 25 mmol · g⁻¹ · h⁻¹. The corresponding rates of ATP synthesis ($q_{\text{ATP}}^{\text{max}}$) calculated from the specific rates of product formation varied in the same proportions (Table 3). Moreover, the maximal specific rates of growth were found to be related linearly to the rates of ATP synthesis, at least for the four defined media (Fig. 3). This

FIG. 3. Relationship between the maximal specific rates of glucose consumption (q_s^{max}) (●) and ATP formation ($q_{\text{ATP}}^{\text{max}}$) (○) and the growth rate during cultures of *L. lactis* on various media.

correlation indicates that a delicate balance is maintained between anabolism and catabolism, thereby avoiding the necessity to activate energy-wasting reactions. This correlation has often been taken as an indication that energy availability limits growth (19), as formalized in the following equation proposed by Pirt (11): $q_{\text{ATP}} = \mu/Y_{\text{ATP}}^{\text{max}} + m_{\text{ATP}}$, where m_{ATP} is the coefficient of energy maintenance, which corresponds to the energy required when no growth occurs (q_{ATP} when $\mu = 0$). The m_{ATP} can be estimated to be 21 mmol of ATP · g⁻¹ of biomass · h⁻¹ whatever the medium used, a high value compared to the rate of ATP generation, accounting for about 79% of the energy produced on the minimal medium and about 47% of that produced on MCD medium. However, similar values of non-growth-related ATP consumption were observed with *L. cremoris* (1, 2).

The linear relationship was not maintained on the complex medium in which the q_{ATP} appeared to be underestimated compared to the observed growth rate (Fig. 3). As shown previously when comparing Y_{ATP} and $Y_{\text{ATP}}^{\text{max}}$ values, the energy balance on M17 medium at the beginning of the fermentation was clearly different, and other mechanisms of ATP production should have been involved during this phase of growth in complex medium.

Another important comment needs to be made in view of the observed rates of glucose consumption on the various media. Effectively, this rate was not constant but decreased as the medium composition was simplified. Thus, the concept that the substrate uptake rate can be adequately modeled by classical Monod-type kinetics irrespective of the nutritional or physico-chemical environment needs to be reexamined. It is becoming apparent that the uptake rate for a given substrate is subject to complex regulation involving feedback and signalling processes controlling uptake capacity as a function of the metabolic and energetic constraints imposed on the microorganism. Thus, the apparent growth limitation by energy supply most probably reflects a limiting anabolic flux and the regulation of energy-producing catabolic pathways to avoid wasteful production of metabolic energy.

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