Journal of Industrial Microbiology, 9 (1992) 1–10
Elsevier

SIM 00378

Effect of the levels of dissolved oxygen on the expression of recombinant proteins in four recombinant *Escherichia coli* strains

Xiaoli Li, James W. Robbins, Jr. and Kenneth B. Taylor

Department of Biochemistry, University of Alabama at Birmingham, Birmingham, Alabama, U.S.A.

(Received 23 April 1991; revision received 5 September 1991; accepted 9 September 1991)

Key words: Recombinant culture; Dissolved oxygen; Biomass; Plasmid content

SUMMARY

Four recombinant strains of *Escherichia coli* were examined for the effects of the dissolved oxygen level on the level of biomass, the plasmid content, and the level of recombinant protein at the stationary phase of batch growth. Strains JM101/pYEJ001, and TB-1/pYEJ001 (encoding chloramphenicol acetyltransferase), and strain TB-1/p1034, and TB-1/pUC19 (encoding β-galactosidase) were grown at the constant dissolved oxygen levels of 0, 50, and 100% air saturation, as well as in the absence of dissolved oxygen control. The biomass of all strains under constant aerobic conditions was 12–36 times higher than that under anaerobic conditions, but was the same as or slightly higher than that without dissolved oxygen control. The plasmid content in all strains under aerobic conditions was 2.9–11.7 times higher than that under aerobic conditions. The optimal dissolved oxygen concentration for the specific activity of recombinant proteins was dependent upon the strain. In no strain were constant aerobic conditions optimal. However, because of the effect on biomass, controlled aerobic conditions were optimal for the volumetric activity of recombinant protein in all but one strain.

INTRODUCTION

Oxygen is both essential for the aerobic growth of *Escherichia coli* and, at the same time, the most difficult to supply, because of its low solubility. The rate of oxygen utilization is sufficiently great in cultures of even moderate density that the concentration of dissolved oxygen (DO) may be determined by its transfer rate.

The DO level can be maintained by either an increase in the rate of oxygen transfer (increased vessel pressure, agitation and sparge rates) [5,10] or by a reduction in the oxygen uptake rate in the culture (lower growth temperature or substrate concentration) [4,11]. However, each of the former methods has physical limits and the latter are associated with other potential detrimental effects on productivity.

Although the DO level can be maintained by increased oxygen content of the gas flow [2,7,18], this method is limited by the toxicity of pure oxygen [28]. In the present study, the air stream was supplemented with oxygen by a DO controller so that the desired DO level could be maintained with minimum exposure of the organisms to pure oxygen [24].

Oxygen is an essential nutrient for aerobic organisms, and the dependence of the growth rate on DO concentration follows a Monod saturation dependence [30]. Although the oxygen level affects cellular functions primarily by its effect on the respiratory generation of metabolic energy, *E. coli* is capable of energy generation by either respiratory or fermentative processes [8]. During the transition from aerobiosis to anaerobiosis, the organism loses the function of the TCA cycle as a cycle, and the electron transport chain is altered [8]. Organic compounds, e.g., lactate and ethanol, are used as electron acceptors, and the rate of ATP generation as well as the energy yield per glucose is significantly reduced.

Although lactate, ethanol, acetate, and formate may be produced under both aerobic and anaerobic conditions, they are also subsequently utilized under aerobic conditions. The accumulation of the compounds in high density culture may inhibit the growth [18].

Correlated with the increased grown rate in the presence of oxygen is an increase in both the supply and demand for biosynthetic precursors and ATP. For example, the activity of the hexose monophosphate pathway, which is correlated with the demand for the biosynthesis of nucleic acids and aromatic amino acids, is stimulated at a high level of oxygen [8].

A plasmid is an independent unit of replication, but its replication and gene expression depend on the host for enzymes, energy and biosynthetic precursors. Furthermore, the degradation of plasmid-encoded proteins in *E. coli* requires the continuous production of ATP [12]. As

Correspondence: X. Li, Department of Biochemistry, University of Alabama at Birmingham, Birmingham, AL 35294, U.S.A.

a result, the change in the DO level may affect not only the formation and maintenance of microbial biomass, but also the replication of plasmid and the expression of plasmid gene. One of the aims of the present work was to learn something about how the limited resources are partitioned among these functions.

The effect of the DO level on the productivity of recombinant protein (r-protein) is known for only a small number of strains. Moreover, the effect of the DO level on the plasmid content is previously undocumented. Ryan et al. [25] and Tolentino [29] have observed that the specific activity of recombinant β-lactamase in two *E. coli* strains under anaerobic conditions or at a low rate of aeration was higher than that in the presence of a higher aeration rate.

When the air flow into the medium was interrupted for limited periods during the exponential phase, the plasmid stability in *E. coli* AB1157/pKN401 decreased drastically even under the selective pressure [13]. Arcuri et al. [1] reported that the response to a DO limitation among r-proteins was remarkably variable depending on the cell age and concentration, the growth medium, and the degree of the DO limitation imposed.

Since the existent evidence is somewhat fragmentary and inconclusive with regard to this important parameter, we have investigated the effects of DO on the growth rate, the level of the biomass, the plasmid content, and the r-protein level at the stationary phase in four recombinant strains of *E. coli*. It is hoped that the results will help guide future optimization studies and the formulation of testable hypotheses with respect to microbial mechanisms.

TABLE 1

Recombinant strains used

	Host strains	
	JM101 Δ(lac-pro), [F′, lacI^q, lacZ ΔM15]	**TB-1** Δ(prAB), [φ80, lacZ ΔM15], F$^-$
Plasmid features	Recombinant strains	
pYEJ001 Amp^r, Cm^r (4.1 kb; the origin of DNA replication derived from pBR322; tac promoter and two lac O for cat gene, IPTG inducible).	**JM101/pYEJ001** CAT, β-lactamase activity measured	**TB-1/pYEJ001** CAT, β-lactamase activity measured
p1034 Amp^r, lacZ (6.7 kb; the origin of DNA replication derived from pBR322; P_L promoter for lacZ gene).		**TB-1/p1034** β-gal activity measured
pUC19 Amp^r, lacP, O, Z′ (2.7 kb; origin of DNA replication derived from pBR322; lac promoter for the lacZ′ gene, IPTG inducible).		**TB-1/pUC19** β-gal activity measured

MATERIAL AND METHODS

Materials

Yeast extract and tryptone were obtained from Difco Laboratories. Molecular weight marker proteins, λDNA *Hin*dIII digest, β-galactosidase (β-gal), β-lactamase, and chloramphenicol acetyltransferase (CAT) were obtained from Sigma Chemical Co. All other reagents and chemicals were of analytical grade. All fermentations were performed in a 16-l fermentor equipped with analog DO and pH controllers (SF116, New Brunswick Scientific).

Strains

Since *E. coli* TB-1 and JM101 (Table 1) both carry the chromosomal deletions of β-gal gene, no active β-gal is made by the host strains. The two strains also produce the repressor *lac*I which suppresses the lac and tac promoters.

Plasmid pYEJ001 carries a CAT gene (Table 1), controlled by two lac operators and a tac promoter. In the *lac*I$^+$ strains, JM101/pYEJ001 and TB-1/pYEJ001, the tac promoter is inducible by isopropyl-β-D-thiogalactopyranoside (IPTG). Plasmid p1034 carries a β-gal gene controlled by a P_L promoter. Plasmid pUC19 carries the regulatory region (lacPO) and the *lacZ′* gene which encodes the α-peptide (N-terminal aa 1–145) portion of β-gal [6]. All the three plasmids carry β-lactamase gene controlled by its native promoter.

Strains JM1-1/pYEJ001 and TB-1/pYEJ001 express recombinant CAT (controlled by a tac promoter). The strain TB-1/p1034 expresses recombinant β-gal (controlled by a P_L promoter) constitutively, since the repressor for P_L promoter is not made in this strain. In TB-1/pUC19, the product of chromosomal M15 deletion β-gal gene is complemented by the pUC19-encoded α-peptide to produce β-gal activity. All the four strains express recombinant β-lactamase. The plasmids were introduced into the cells by $CaCl_2$-mediated transformation [20].

Cell growth

The medium for all experiments was Luria broth (LB), which consisted of (per liter): tryptone, 10 g; yeast extract, 5 g; and NaCl, 5 g; supplemented with 5 g yeast extract. The medium for all strains except TB-1/p1034 contained 0.1 mM IPTG. Ampicillin (60 mg/l) was supplied in the

medium for TB-1/p1034 and TB-1/pUC19 due to the low plasmid stability characteristic of these two strains. The antifoaming agent was MAZU DF60P. The fermentor was inoculated with 1% (v/v) of an overnight culture of the appropriate strain in LB with 60 mg/l ampicillin or 250 mg/l chloramphenicol grown in a shaker at 37 °C. The growth temperature was 37 °C. The pH of the fermentor culture was controlled at 7.2 with NaOH (10 N) and glucose (240 g/l) feeding [23]. The glucose feeding system, which was responsive to the increase in culture pH during growth, served to control pH and to supply the carbon source. The glucose feeding system did not function during anaerobic growth since the increase in culture pH did not occur.

In fermentation without DO control, air was sparged at a rate of 0.6 vol/min. The agitation rate and vessel pressure were set at 500 rpm and 5 psi respectively. The DO probe was set to 100% saturation, before inoculation under these conditions. The DO level dropped to zero during late exponential phase and remained there throughout the remainder of the growth. Cultures without DO control, therefore, were exposed to changing DO conditions.

In fermentations with control of the DO level, the DO probe was calibrated to 100% under the same conditions specified above. The dissolved oxygen was then maintained at a desired level by supplementation of the air stream with oxygen or nitrogen under the control of the DO controller [32]. Anaerobic growth was achieved by sparging the fermentation culture with nitrogen. The oxygen concentration (mg/l) was calculated from the DO saturation data and the Bunsen coefficient [3] in order to determine the K_0 value of TB-1/pUC19. In experiments with JM101/pYEJ001, TB-1/pYEJ001, and TB-1/p1034, data were gathered at three constant DO levels (0, 50 and 100% saturation). However, eight constant DO levels (0, 0.4, 0.7, 2, 10, 27, 50 and 100%) were used for TB-1/pUC19 since a substantial change in the specific β-gal activity was observed at very low DO levels. Cell dry weight was determined as in previous work [19].

Enzymatic assays

The activity of β-gal was determined as in previous work [19]. The assay method for CAT was adopted from Shaw [27]. The cells at the stationary phase were collected by centrifugation at 3500 rpm for 30 min. The pellet was washed and suspended in the volume of 0.05 M Tris buffer, pH 7.5, in 0.145 M NaCl, to give a standard turbidity (OD_{650} = 1). The resulting suspension was subjected to two cycles of freezing and thawing, and three pulses of sonic oscillation of 20 s each (32 kc, 40% power, ice water cooling), with a sonic dismemberator (Model 300, Dynatech Laboratories, Chantilly, VA). Finally, toluene was added to the cell lysate to give a final concentration of 5% (v/v). The assay mixture contained 0.3 ml of 1.0 M Tris buffer, pH 7.8; 0.3 ml of 1 mM acetyl CoA; 0.3 ml of 1 mM 5,5′-dithiobis-2-nitrobenzoic acid; 1.425 ml of deionized water; and 0.375 ml of the cell lysate. The reaction was initiated by the addition of 0.3 ml of 1 mM chloramphenicol to the mixture and allowed to react for 1 min at 25 °C. Then, the reaction mixture was filtered with a 0.2 μ syringe filter, to remove cells, and the increase in absorbance at 412 nm was measured. The reference cuvette contained the reaction mixture without chloramphenicol. One unit of CAT was the amount that acetylates 1 μmol of chloramphenicol per min under the conditions specified.

The cell extract for β-lactamase assay was prepared as described by Weber et al. [31]. The enzyme in the crude extract was assayed spectrophotometrically by determination of the rate of hydrolysis of cephalothin as described by Ryan et al. [25]. One unit of β-lactamase was defined as the amount of the enzyme than decreased one OD_{255} unit per min at 25 °C.

Determination of the plasmid content

Plasmid DNA in culture samples taken at stationary phase was prepared by an alkaline lysis method [21]. The volume of culture corresponding to 1 mg of dry biomass was used for each preparation. The plasmid DNA was linearized by *Eco*RI digestion. The method for the deter-

TABLE 2

Effect of the DO concentration on strain JM101/pYEJ001

DO concentration (%)	Dry weight (g/l)	Growth rate (h^{-1})	Plasmid content (mg/g cell)	Specific CAT ($UX10^{-3}$/g cell)	Volumetric CAT ($UX10^{-3}$/l)	β-lactamase (U/g cell)
0	0.20	0.07	0.49	643.4	128.7	661
50	7.20	0.63	1.31	589.9	4247.1	729
100	6.94	0.47	1.19	630.7	4377.2	760
Uncontrolled	6.94	0.63	1.24	544.2	3777.0	605

TABLE 3

Effect of the DO concentration on strain TB-1/pYEJ001

DO concentration (%)	Dry weight (g/l)	Growth rate (h^{-1})	Plasmid content (mg/g cell)	Specific CAT ($UX10^{-3}$/g cell)	Volumetric CAT ($UX10^{-3}$/l)	β-lactamase (U/g cell)
0	0.44	0.07	0.047	505.4	222.4	364
50	4.00	0.40	0.29	1091.2	4364.8	488
100	5.12	0.41	0.19	1127.3	5772.0	553
Uncontrolled	3.87	0.40	0.25	1044.0	4040.4	525

mination of plasmid content was adopted from that of Koizumi [14]. The linearized plasmid DNA (4–8 μl) was separated by electrophoresis (100 V, 1–2 h) on 1% agarose gel. The running buffer was 30 mM Tris, pH 8.8; 20 mM Na acetate; 1 mM EDTA; and 0.5 μg/ml ethidium bromide. A known amount of λDNA *Hin*dIII digest was analyzed simultaneously with plasmid samples as a marker and standard. The DNA band was photographed under UV light with a Polaroid type 55 film. Each lane of the negative was scanned with a video densitometer (BioRad, Richmond, CA) coupled to a recording integrator (HP3392A) to determine the peak area of each DNA band. The peak area of band of the standard DNA corresponding to the fragments of 9.4 or 6.6 kb were plotted against the amount of each of the λDNA fragments to construct a calibration curve. The amount of plasmid DNA in each lane was determined by incorporation of the peak area into the calibration curve. The preparation of plasmid DNA was repeated 3 times and the determination of each plasmid sample was repeated for 3–6 times. The plasmid content was expressed as mg plasmid/g dry biomass.

Stability of plasmid

The plasmid retention rate at stationary phase is the fraction of viable cells that express the recombinant phenotype [17]. The culture samples of TB-1/p1034 and TB-1/pUC19 were plated on LB plates coated with 40 μl of 0.1 M IPTG and 40 μl of 2% X-gal. The recombinant cells produced blue colonies whereas host cells appeared white on these plates. The cells of JM101/pYEJ001 and TB-1/pYEJ001 were plated on LB plates (about 100 colonies/plate), and the colonies were then replicated with sterile toothpicks onto LB plates containing 250 mg/l chloramphenicol. The colonies that survived the selection were counted as the recombinant cells.

Growth rate

The specific growth rate (h^{-1}) was the slope of the curve plotting culture turbidity (OD_{650}) and time during the exponential phase.

SDS polyacrylamide gel electrophoresis

The cell crude extract was prepared as described by Mizukami et al. [22]. The extract was analyzed by discontinuous SDS electrophoresis on 7.5 and 12% polyacrylamide gel [16].

RESULTS AND DISCUSSION

Effect of the DO level on the level of biomass

Anaerobic growth reduced significantly the level of biomass at stationary phase in all four strains (Tables 2–5). Since the culture pH did not increase during anaerobic growth, as it did during aerobic growth, the glucose feeding system did not function under the former

TABLE 4

Effect of the DO concentration on strain TB-1/p1034

DO concentration (%)	Dry weight (g/l)	Growth rate (h^{-1})	Plasmid content (mg/g cell)	Specific β-gal ($UX10^{-3}$/g cell)	Volumetric β-gal ($UX10^{-3}$/l)
0	0.23	0.02	1.16	18.6	4.3
50	3.70	0.36	3.34	11.9	44.0
100	3.90	0.35	3.24	17.5	68.3
Uncontrolled	2.57	0.21	2.58	96.1	247.0

TABLE 5

Effect of the DO concentration on strain TB-1/pUC19

DO concentration (%)	Dry weight (g/l)	Growth rate (h^{-1})	Plasmid content (mg/g cell)	Specific β-gal ($UX10^{-3}$/g cell)	Volumetric β-gal ($UX10^{-3}$/l)
0	0.33	0.04	0.16	331.5	109.4
0.4	2.53	0.26	0.32	173.6	439.2
0.7	2.68	0.32	0.48	59.8	160.3
2	3.83	0.36	0.52	34.8	133.3
10	4.80	0.50	0.80	35.3	169.4
27	4.74	0.51	0.97	36.0	170.6
50	4.80	0.50	1.19	35.0	168.0
100	4.83	0.50	1.87	36.5	176.4
Uncontrolled	4.67	0.51	1.28	26.5	123.8

condition. Therefore, the limitation of glucose may also contribute to the effects of anaerobic growth.

In experiments in which the aerobic level of DO was increased from 50 to 100% saturation, the biomass concentration in JM101/pYEJ001, TB-1/p1034, and TB-1/pUC19 remained constant (Tables 2, 4, and 5), whereas that in TB-1/pYEJ001 increased (Table 3). Although the biomass at stationary phase was constant over the range of 50–100% DO in strain TB-1/pUC19, there was a gradual increase from anaerobic to 50% saturation (Table 5).

With uncontrolled DO, the biomass level at stationary phase was either the same as (JM101/pYEJ001 and TB-1/pUC19) or slightly less than (TB-1/pYEJ001 and TB-1/p1034) that at a DO level of 100%.

Effect of the DO level on the growth rate

The specific growth rate under anaerobic conditions was only 6–17% of that at the constant aerobic levels in all strains (Tables 2–5). In aerobic experiments, the growth rates of TB-1/pYEJ001, TB-1/p1034, and TB-1/pUC19 were constant, whereas the growth rate of JM101/pYEJ001 decreased by 25% at 100% DO.

The growth rate of TB-1/pUC19 increased with the concentration of DO in accordance with the Monod model (Fig. 1) [30] ($\mu_{max} = 0.496 \pm 0.026\ h^{-1}$ and $K_0 = 0.038 \pm 0.0094$ mg/l).

The growth rate without DO control was the same as that at the constant aerobic DO levels in JM101/pYEJ001, TB-1/pYEJ001, and TB-1/pUC19, whereas the growth rate of TB-1/p1034 with uncontrolled DO was 40% lower than that at constant aerobic conditions. The results with TB-1/p1034 suggest that the growth rate, as well as the biomass concentration, was limited by oxygen under uncontrolled DO conditions.

Effect of the DO level on the plasmid stability

Selective pressure by antibiotics was exerted on all strains in the inoculum development, and during large scale fermentations in two of the strains: TB-1/p1034 and TB-1/pUC19. Since the plasmid retention at stationary phase never fell below 98% in any of the experiments, the previously reported influence of DO on plasmid stability [13] is apparently dependent on the strain.

Effect of the DO level on the plasmid content

Anaerobic growth resulted in a reduction of the plasmid content in all four strains (Tables 2–5).

Under constant aerobic conditions (50–100% DO), the plasmid content remained the same in JM101/pYEJ001 and TB-1/p1034. However, it decreased somewhat with increased DO in TB-1/pYEJ001, and increased continuously over the DO range from 0.4 to 100% in TB-1/pUC19 (Table 5).

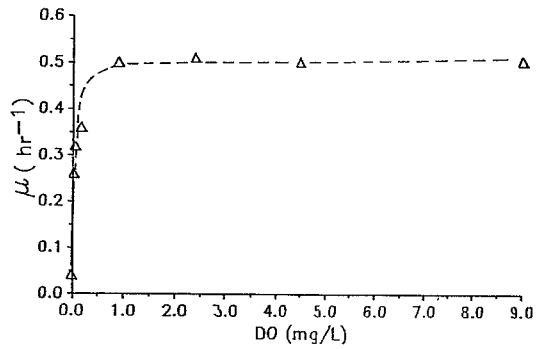

Fig. 1. Experimentally determined growth rate for TB-1/pUC19 (Δ) as function of the dissolved oxygen concentrations. The data were fitted to the Monod equation (---) using a non-linear regression program to estimate the values of μ_{max} and K_0.

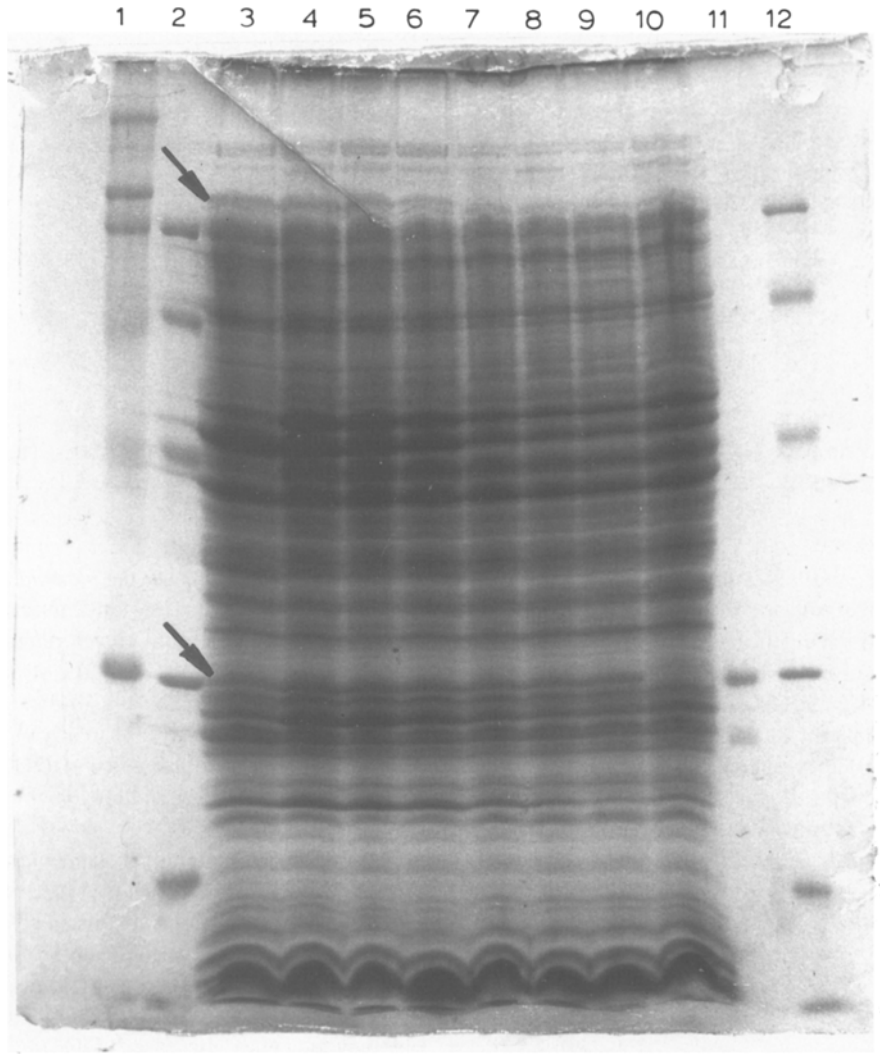

Fig. 2. The extract of equal amount of TB-1/pUC19 cells was subjected to 12% SDS–PAGE and stained with Coomassie brilliant blue. Lane 1, high molecular mass standards (29, 45, 66, 97, 116 and 205 kDa); lane 2 and 12, low molecular mass standards (16, 24, 33, 47, 84 and 110 kDa); lane 11, β-lactamase standard; lanes 3–7, cells grown at the DO levels of 0, 0.4, 0.7, 2, and 10% air saturation, respectively; lane 8, cells grown without DO control; lane 9, cells grown at a DO level of 100% saturation; lane 10, host cells grown without DO control. The arrows indicate the bands of β-gal and β-lactamase.

The plasmid content with no DO control was the same as that at a DO level of 50% in JM101/pYEJ001, TB-1/pYEJ001, and TB-1/pUC19. However, in TB-1/p1034, the plasmid content, as well as the biomass and growth rate, was lower with no DO control than that at 50% DO.

The fact that the plasmid content of JM101/pYEJ001 was higher than that of TB-1/pYEJ001 (Tables 2 and 3) at all DO levels demonstrates a host effect on the plasmid content. Furthermore, the plasmid content of the former was associated with a greater absolute increase with the switch from anaerobic conditions. These results suggest that the latter strain (TB-1/pYEJ001) allocated a higher proportion of its logistic resources to the formation of microbial biomass than does the former. Lancaster et al. [15] also observed the strong dependence of copy number on the host strain used. However, the relevant host-dependent factors are not completely identified, and little is known about how the rate of synthesis and degradation of control elements for plasmid replication are coordinated with the physiology of the host under various logistic conditions.

In the recombinants with the same host strain: TB-1/pYEJ001, TB-1/p1034, and TB-1/pUC19, plasmids pYEJ001 (4.1 kb), p1034 (6.7 kb) and pUC19 (2.7 kb) share the same host strain and an origin of DNA replica-

tion derived from pBR322. The response of the plasmid content to the DO level, therefore, is more likely a result of the logistic effects on replication itself than of logistic or regulatory effects on regulation of the initiation process of plasmid replication.

The fact that the aerobic plasmid content was much higher in TB-1/p1034 and TB-1/pUC19 than that in TB-1/pYEJ001 demonstrates the dominant role played by selection pressure. Furthermore, the plasmid copy number (data not shown) in the former two strains (TB-1/p1034 and TB-1/pUC19) which had identical selection conditions were substantially the same. The logistic burden placed on the host cell by the plasmid [17,25,26] was demonstrated by the inverse correlation in the two strains of the plasmid content with both the biomass level and the specific growth rate (Tables 4 and 5).

Effect of the DO level on the r-protein level at stationary phase

In two strains (JM101/pYEJ001 and TB-1/p1034), the switch from anaerobic to constant aerobic conditions resulted in little change in the specific activity in r-proteins (CAT, β-lactamase and β-gal), whereas in TB-1/pYEJ001, the aerobic specific activity of CAT was twice that under anaerobic conditions.

Surprisingly, the specific activity of β-gal in TB-1/pUC19 under anaerobic conditions was higher than that under all aerobic conditions. It is probable that the observed reduction in the chromosome-encoded component of β-gal protein (Fig. 2) at levels of DO above zero was responsible for at least part of the lower β-gal activity. The reduction is unlikely due to known glucose repression since the highest glucose concentration in the medium during the feeding never exceeded 0.033% (data not shown), which was much lower than the glucose concentration (0.2%) that produces the repression [9]. Although the recombinant α-fragment of β-gal could not be identified on the electrophoresis gel, because the lack of a standard sample, the fact that the recombinant β-lactamase protein level was also decreased with increased DO concentration (Fig. 2), even under conditions of ampicillin selection, suggests that the production of the α-fragment was also affected. A similar increase in β-lactamase level during anaerobic growth of *E. coli* c600/pKN401 has been observed by Tolentino et al. [29]. In addition, Ryan et al. [25] found that the specific β-lactamase activity increased and the cell mass concentration decreased with a reduction in the air flow rate. The latter workers suggested that a reduction in the growth rate at lower aeration rate resulted in increased allocation of resources for the synthesis of recombinant β-lactamase.

When the DO level was increased from 50 to 100%, the specific activity of r-protein was not substantially changed in all strains.

In the absence of DO control, the specific activity of the r-proteins in three strains (JM101/pYEJ001, TB-1/pYEJ001, and TB-1/pUC19) remained the same as that with a constant aerobic DO level. However, in TB-1/p1034, the specific activity of β-gal with uncontrolled DO was 5–8 times higher than that at all the constant DO levels. Furthermore, analytic PAGE confirmed the accumulation of a much larger amount of the enzyme protein in cells of the latter strain grown with no DO control (Fig. 3).

In order to test the hypothesis that a high DO level leads to an increased rate of degradation of β-gal, cultures of TB-1/p1034 at stationary phase were switched from the normal aeration conditions (with no DO control) to 100% DO. That the activity of β-gal was found to be stable (97% of the activity remained) for at least 3 h after the switch (data not shown), suggests that the high level of oxygen did not affect the degradation rate of the protein during that period of time. Therefore, the low level of β-gal at the constant DO levels must be the result of the effect of oxygen on the specific rate of synthesis. The results may be explained by the hypothesis that the plasmid replication is suppressed under anaerobic conditions, whereas the gene expression is increased when the DO concentration becomes low. Therefore, the specific activity under uncontrolled DO conditions represents a combination of the two. The accumulation of plasmid is favored, when the oxygen supply is sufficient (during early exponential phase), whereas the gene expression is favored as the DO level drops at the later stage of growth. Thus, the cells at both aerobic and anaerobic constant DO levels produced less β-gal than the cells grown with no DO control.

That strain JM101/pYEJ001 had 10 times more plasmid under anaerobic conditions than did strain TB-1/pYEJ001, but had only 1.3 times the specific CAT activity (Tables 2 and 3) confirms the hypothesis that logistics are more limiting than plasmid content under these conditions. Furthermore, in the switch to aerobic conditions, TB-1/pYEJ001 still lagged in the plasmid content, but had two times as much CAT activity as did JM101/pYEJ001. This latter contrast substantiates the relative importance of logistics over plasmid content and the relatively greater allocation of logistic resources to r-protein production than to biomass production in the former strain than in the latter. Furthermore, the response of r-protein production is dependent upon the nature of the plasmid and of the r-protein itself. For example, the level of r-protein in TB-1/pYEJ001 and TB-1/p1034 was virtually unaffected by DO variation during constant aerobic growth, whereas the level of r-protein in TB-1/pUC19, which has the same host, was inversely correlated with the DO level.

Although the specific activity of r-protein in anaerobic growth is somewhat determined by the specific host and

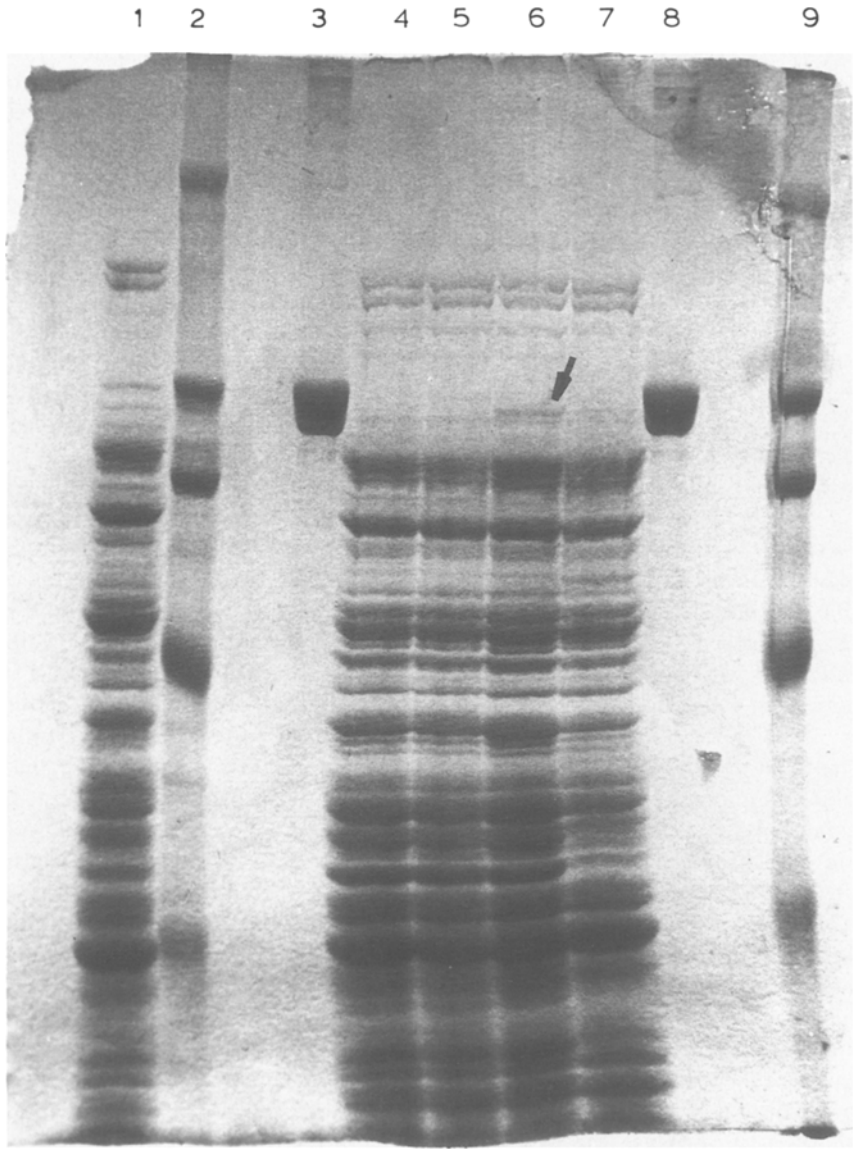

Fig. 3. The extract of equal amount of cells of TB-1/p1034 was subjected to 7.5% SDS–PAGE. Lane 1, host cells grown without DO control; lanes 2, 9, molecular mass standards (30–200 kDa); lanes 3, 8, β-gal standard; lane 4, cells grown at a DO level of 100% saturation; lane 5, cells grown at a DO level of 50% saturation; lane 6, cells grown without DO control; lane 7, cells grown anaerobically. The arrow indicates the position of recombinant β-gal.

plasmid, the volumetric activity under anaerobic conditions was lower than that under aerobic in all strains, due to the low anaerobic biomass concentration. However, the optimum conditions for the volumetric r-protein activity are determined by the host and plasmid. For example, although in TB-1/p1034 uncontrolled DO resulted in a moderate biomass level, the maximum volumetric β-gal activity was achieved due to high β-gal specific activity. In TB-1/pUC19, the β-gal activity and the biomass responded to the DO level in the opposite directions. Therefore, the maximum volumetric β-gal activity was achieved at a low DO level (0.4%), which gave intermediate levels of both biomass and β-gal specific activity.

REFERENCES

1 Arcuri, E.J., K. Turner, D. Sharr and B. Okita. 1988. The influence of dissolved oxygen limitation upon the accumulation of heterogeneous proteins in *Escherichia coli*. SIM News 4: 33.

2 Bailey, F.J., J. Blankenship, J.H. Condra, R.Z. Maigetter and R.W. Ellis. 1987. High-cell-density fermentation studies of a

recombinant *Escherichia coli* that expresses atrial natriuretic factor. J. Ind. Microbiol. 2: 47–52.

3 Brauer, H. 1985. In: Biotechnology (Brauer, H., ed.), pp. 161, VCH, Weinheim, F.R.G.

4 Bauer, S. and E. Zin. 1976. Dense growth of aerobic bacteria in a bench-scale fermentor. Biotechnol. Bioeng. 18: 81–94.

5 Clark, T.A., T. Hesketh and T. Seddon. 1985. Automatic control of dissolved oxygen tension via fermentor agitation speed. Biotechnol. Bioeng. 27: 1507.

6 Chambers, S.P., S.E. Prior, D.A. Bartow and N.P. Minton. 1988. The pMTL nic⁻ cloning vectors. Gene 68: 139–149.

7 Cutayar, J.M. and D. Poillon. 1989. High cell density culture of *E. coli* in a fed-batch system with dissolved oxygen as a substrate feed indicator. Biotechnol. Lett. 11: 155–160.

8 Doelle, H.W. 1981. In: Biotechnology (Rehm, H.-J. and G. Reed, eds.), pp. 196, Verlag Chemie, Weinheim, F.R.G.

9 Epstein, W., L.B. Rothman-Denes and J. Hesse. 1975. Adenosine 3′,5′-cyclic monophosphate as mediator of catabolic repression in *Escherichia coli.* Proc. Natl. Acad. Sci. USA 72: 2300.

10 Flynn, D.S. and M.D. Lilly. 1967. A model for the control of the dissolved oxygen tension in microbial cultures. Biotechnol. Bioeng. 9: 515–531.

11 Gleiser, I.E. and S. Bauer. 1981. Growth of *E. coli* W to high cell density by oxygen level linked control of carbon source concentration. Biotechnol. Bioeng. 23: 1015.

12 Goldberg, A.L. and S.A. Goff. 1986. The selective degradation of abnormal proteins in bacteria. In: Maximizing Gene Expression (Reznicoff, W. and L. Gold, eds.), pp. 287–314, Butterworth Publishers, Stoneham.

13 Hopkins, D.J., M.J. Betenbaugh and P. Dhurjati. 1987. Effects of dissolved oxygen shock on the stability of recombinant *Escherichia coli* containing plasmid pKN401. Biotechnol. Bioeng. 29: 85–91.

14 Koizumi, J., Y. Monden and S. Aiba. 1985. Effects of temperature and dilution rate on the copy number of recombinant plasmid in continuous culture of *Bacillus stearothermophilus* (pLP11). Biotechnol. Bioeng. 27: 721–728.

15 Lancaster, M.J., R.J. Sharp, J.R. Court, I.D. McEntee, R.G. Melton and R. Sherwood. 1989. Production of cloned carboxypeptidase G2 by *Escherichia coli*: genetic and environmental considerations. Biotechnol. Lett. 10: 699–704.

16 Laemmli, U.K. 1970. Cleavage of structure proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680.

17 Lee, G.M., K.B. Son, S.K. Rhee and M.H. Han. 1986. Plasmid maintenance and growth of recombinant *Saccharomyces cerevisiae* producing hepatitis B virus surface antigen. Biotechnol. Lett. 8: 385–390.

18 Lee, Y.L. and H.N. Chang. 1988. High cell density continuous culture of *Escherichia coli* producing penicillin acylase. Biotechnol. Lett. 10: 787–792.

19 Li, X., J.W. Robbins, Jr. and K.B. Taylor. 1990. The production of recombinant beta-galactosidase in *Escherichia coli* in yeast extract enriched medium. J. Ind. Microbiol. 5: 85–94.

20 Maniatis, T., E.F. Fritsch and J. Sambrook. 1982. Transformation of *Escherichia coli* by plasmid DNA. In: Molecular Cloning, pp. 249–255, Cold Spring Harbor Laboratory, New York, NY.

21 Maniatis, T., E.F. Fritsch and J. Sambrook. 1982. Rapid isolation of plasmid or bacteriophage DNA. In: Molecular Cloning, pp. 365–373, Cold Spring Harbor Laboratory, New York, NY.

22 Mizukami, T., Y. Komatsu, N. Hosoi, S. Itoh and T. Oka. 1986. Production of active human interferon-B in *Escherichia coli.* Biotechnol. Lett. 9: 605–610.

23 Robbins, J.W., Jr. and K.B. Taylor. 1989. Optimization of *Escherichia coli* growth by controlled addition of glucose. Biotechnol. Bioeng. 34: 1289–1294.

24 Rollins, M.J., S.E. Jensen and D.W.S. Westlake. 1988. Effect of aeration of antibiotic production by *Streptomyces clavuligerus.* J. Ind. Microbiol. 3: 357–364.

25 Ryan, W., S.J. Parulekar and B.C. Stark. 1989. Expression of B-lactamase by recombinant *Escherichia coli* strains containing plasmids of different sizes – effects of pH, phosphate, and dissolved oxygen. Biotechnol. Bioeng. 34: 309–319.

26 Seo, J.-H. and J.E. Bailey. 1985. Effects of recombinant plasmid content on growth properties and cloned gene product formation in *Escherichia coli.* Biotechnol. Bioeng. 27: 1668–1674.

27 Shaw, W.V. and R.F. Brodsky. 1968. Characterization of chloramphenicol acetyltransferase from chloramphenicol resistant *Staphylococcus aureus.* J. Bacteriol. 95: 28–36.

28 Stainer, R.Y., J.L. Ingraham, M.L. Wheelis and P.R. Painter. 1986. In: The Microbial World, pp. 210, Prentice-Hall, New York.

29 Tolentino, G.J. and K.-Y. San. 1988. Plasmid maintenance and gene expression of a recombinant culture under aerobic and anaerobic conditions. Biotechnol. Lett. 10: 373–376.

30 Wang, D.I.C., C.L. Cooney, A.L. Demain, P. Dunnill, A.E. Humphrey and M.M. Lilly. 1978. In: Fermentation and Enzyme Technology, pp. 91, Wiley, New York.

31 Weber, A.E. and K.-Y. San. 1987. Presistence and expression of the plasmid pBR322 in *Escherichia coli* K-12 cultured in complex medium. Biotechnol. Lett. 11: 757–760.

32 Yegneswaran, P.K., M.R. Gray and D.W.S. Westlake. 1988. Effects of reduced oxygen on growth and antibiotic production in *Streptomyces clavuligerus.* Biotechnol. Lett. 10: 479–484.