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Sigma Factors as Potential Targets to Enhance Recombinant Protein Expression

Laura Pohlen[1] | Emily García[2] | Luz María Martínez[2] | Noemí Flores[2] | Jochen Büchs[3] | Guillermo Gosset[2] | Alvaro R. Lara[4]

[1]Department of Chemistry and Biotechnology, FH Aachen University of Applied Sciences, Jülich, Germany | [2]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, México | [3]Chair of Biochemical Engineering (AVT.BioVT), RWTH Aachen University, Aachen, Germany | [4]Department of Biological and Chemical Engineering, Aarhus University, Aarhus, Denmark

Correspondence: Guillermo Gosset (guillermo.gosset@ibt.unam.mx) | Alvaro R. Lara (alvaro.lara@bce.au.dk)

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ABSTRACT

The transcriptional factors control the expression of many genes and represent an important layer of complexity in cell factories. However, the effect of individual sigma factor deletions from a biomanufacturing perspective has not been addressed. In this contribution, growth, green fluorescence protein (GFP) expression, and oxygen consumption of *Escherichia coli* BW25113 strains with individual inactivation of each sigma factor were characterized under various conditions. Specific growth rate, specific GFP fluorescence, and fluorescence emission rates were compared in a mineral media and in lysogeny broth at two temperatures. *rpoD* has been reported to be lethal for *E. coli*; however, the evaluated *rpoD* mutant did not exhibit major growth defects in the mineral medium. This is attributed to the presence of a second copy of *rpoD* in this strain. GFP was expressed at three different induction levels in a mineral and LB media. The *fliA* mutant was the best producer in the mineral medium, whereas the *rpoD* mutant overperformed the other strains in LB medium. This suggests that a lower *rpoD* gene dosage is positive for the performance of the cell factory in a complex medium. In cultures at 20°C, the *rpoS* mutant exhibited the greatest recombinant expression. To our knowledge, this is the first systematic study evaluating the potential of sigma factor deletion for improving recombinant protein production.

1 | Introduction

Bacterial RNA polymerases require subunits, named sigma (σ) factors to recognize specific promoters and start the transcription process. In *Escherichia coli*, the "housekeeping" and essential σ^{70} (*rpoD*) controls the expression of many genes expressed during exponential growth (Shimada et al. 2014). Alternative σ-factors have been described in *E. coli*, σ^{54} (*rpoN*), σ^{38} (*rpoS*), σ^{32} (*rpoH*), σ^{28} (*fliA*), σ^{24} (*rpoE*, which is an essential gene), and σ^{19} (*fecI*). Cho and coworkers reconstructed a genome-scale network of the σ-factors and reported 4724 σ-factor specific promoters (Cho et al. 2014). While the σ-factors control the expression of many genes, whether such genes are dispensable for the cell factory under production conditions remains largely unstudied, but there are some reports suggesting that σ-factor deletions can be useful to improve cellular or production performance. For instance, RpoS is known as a general stress response or stationary phase transcriptional factor that controls the expression of approx. 500 genes (Gottesman 2019). It has been shown that the deletion of *rpoS* increases the production of recombinant protein in chemostat cultures of *E. coli* at low dilution rates (Chou et al. 1996), as well as putrescine and 1-pronanol in fed-batch cultures (Qian et al. 2009; Jun Choi et al. 2012). Sigma factor RpoH has been

used as chaperone to increase the production of lipoxygenase in *E. coli* (Pang et al. 2022). When *E. coli* is exposed to heterogeneous substrate availability, similar to the conditions in an industrial scale bioreactor, the expression level of thousands of genes changes. Löffler and coworkers showed that 89% of the differentially expressed genes under heterogeneous substrate availability were under control of sigma factors (Löffler et al. 2016). The genes controlled by *rpoS* were particularly affected by heterogeneous conditions. Bafna-Rührer and coworkers cultured *E. coli rpoS* and *fliA* mutants under short periods of glucose starvation and found that the *rpoS* deletion improved the biomass yield, but *fliA* deletion worsened it (Bafna-Rührer et al. 2024).

Despite the physiological relevance of the σ-factors, there is not, to the best of our knowledge, a systematic study on the effect of individual knockouts on the growth and expression of recombinant protein under different culture conditions. Here, the performance of the individual σ-factors mutants was characterized in a mineral medium and in lysogeny broth (LB), at 20°C and 37°C in microbioreactors with online measurement of the oxygen transfer rate (OTR), biomass, and NADH or GFP fluorescence signals. Such experimental setting allows detailed online data acquisition, and the information obtained is equivalent to the obtained in larger cultures. It has been demonstrated that cultures in microtiter plates can be successfully scaled to stirred bioreactors of several L volumes by choosing the appropriate scale-up criterion (Seletzky et al. 2007; Kensy et al. 2009; Funke et al. 2010; Wewetzer et al. 2015; Ihling et al. 2020).

The impact of individual sigma factor deletion was first evaluated in a buffered mineral medium supplemented with 4 g L^{-1} glucose. The strains were cultured at 37°C, except for the *rpoH* mutant, which did not grow at this temperature. The growth profiles for the strains were similar, exhibiting a clear relation between biomass accumulation, OTR, and NADH fluorescence (Figure 1a). Notwithstanding, the NADH fluorescence remained constant during the stationary phase for all strains except for the *rpoS* mutant, in which a slow but steady increase was observed until 18 h of culture. Possibly, the lack of control of genes related to the stationary phase promoted some metabolic activity using intracellularly available metabolite pools, affecting the redox state of the cell. Apart from the *fliA* and *fecI* mutants, which grew at the same rate as the WT, the other sigma factors deletions decreased the growth rate between 13% and 30% (Figure 1b). Biomass synthesis was similar for all the strains, with small differences for *rpoN* and *rpoS* mutants (Figure 1b). The specific NADH fluorescence intensity was 25% lower for the *rpoD* and *rpoS* mutants, which suggests a decreased energy metabolism in these genotypes. The accumulated oxygen transferred to the liquid was lower for all the mutants except *rpoN* (Figure 1b). This can be related to decreased protein formation in the mutants, which results in ATP savings. In turn, deletion of the *rpoN* gene affects nitrogen metabolism by limiting the uptake of ammonium into the cell, which may explain the reduced growth of this mutant. Thus, the *rpoN* mutants may perceive nitrogen limitation, increasing the activity of the glutamine synthetase, which increases the ATP demand by 14%, compared to nitrogen-excess conditions (Reitzer and Schneider 2001). This may contribute to an oxygen demand similar to that of the wild-type, and higher than the rest of the mutants. Interestingly, the accumulated oxygen transfer rate for the *fecI* and *fliA* mutants decreased by 18% and 11%, respectively, compared to the WT strain, despite all three exhibiting similar growth rates (Figure 1b). The lower oxygen consumption by the *fliA* mutant is probably the consequence of the absence of flagellum synthesis and flagellum rotation, which are highly energy-demanding processes (Schavemaker and Lynch 2022). This may result in less energy being required and, thus, less oxygen consumed by the cells. However, the reasons for reduced oxygen consumption by the *fecI* mutant remain elusive.

The growth of the *rpoD* mutant was unexpected, since σ^{70} is considered an essential gene (Goodall et al. 2018). Examination of the mutant genome revealed the presence of a second copy of the *rpoD* gene (Supporting Information S1: Figure S1), in agreement with a previous report for strain BW25113 (Yamamoto et al. 2009). The *rpoD* expression, relative to the housekeeping gene *ihfB* was determined for the mutant and WT strains by RT-qPCR. The results are depicted in Supporting Information S1: Figure S2. The *rpoD* expression level in the mutant strain was approx. 20% lower than in the WT ($n = 7$, $p < 0.07$). The expression of the duplicate *rpoD* appears to be insufficient to retain the growth rate at the same value as the WT in the mineral medium (Figure 1b). Because of the essentiality of *rpoD*, no further attempt to delete the second copy was made in this work. On the contrary, the mutant strain with only one *rpoD* copy was used for the following experiments.

The expression of recombinant protein was tested in the mineral medium within all strains using the green fluorescence protein (GFP) as a model. The *gfp* gene was coded in a high copy-number plasmid under transcriptional control of the *lac* promoter. GFP expression was induced with 0.1, 0.2, or 0.3 mM IPTG. Culture profiles are shown in Figure 2. For a given strain, a higher GFP fluorescence is related to a lower maximal OTR (Figure 2a), which can be connected to a higher metabolic burden (Ladner et al. 2017; Mühlmann et al. 2018). The growth rate of the WT and *rpoS* mutant strains was not affected by the induction level (Figure 2b). In contrast, the growth rate of the other mutant strains using 0.3 mM IPTG was lower than the corresponding value at 0.1 mM IPTG (Figure 2b). In comparison to the WT strain, the growth rates of the *rpoD*, *rpoS,* and *fliA* mutants were considerably lower. The accumulated biomass was affected slightly, showing only small decreases for mutants other than *rpoN* (Figure 2b). Remarkably, the deletion of sigma factor genes resulted in strongly decreased GFP expression, compared to the WT, being *fliA* the only exception. The *fliA* mutant attained approx. Three and fourfold higher maximal and specific GFP fluorescence, respectively, than the WT strain. Moreover, despite the growth rate reduction of the *fliA* mutant, the specific GFP fluorescence emission rate in cultures of this mutant was between 1.8 and 3-fold greater than for the WT strain (Figure 2b). When induced with 0.2 and 0.3 mM IPTG, all the mutants exhibited higher accumulated oxygen transfer rates compared to the corresponding values of the WT (Figure 2b). Interestingly, the greatest accumulated oxygen transfer rate was seen in cultures of the *rpoS* mutant induced with 0.2 mM IPTG, even though under such conditions, this strain grew slower, produced less biomass, and expressed less GFP than the other strains (Figure 2b). This results in higher stoichiometric oxygen demand

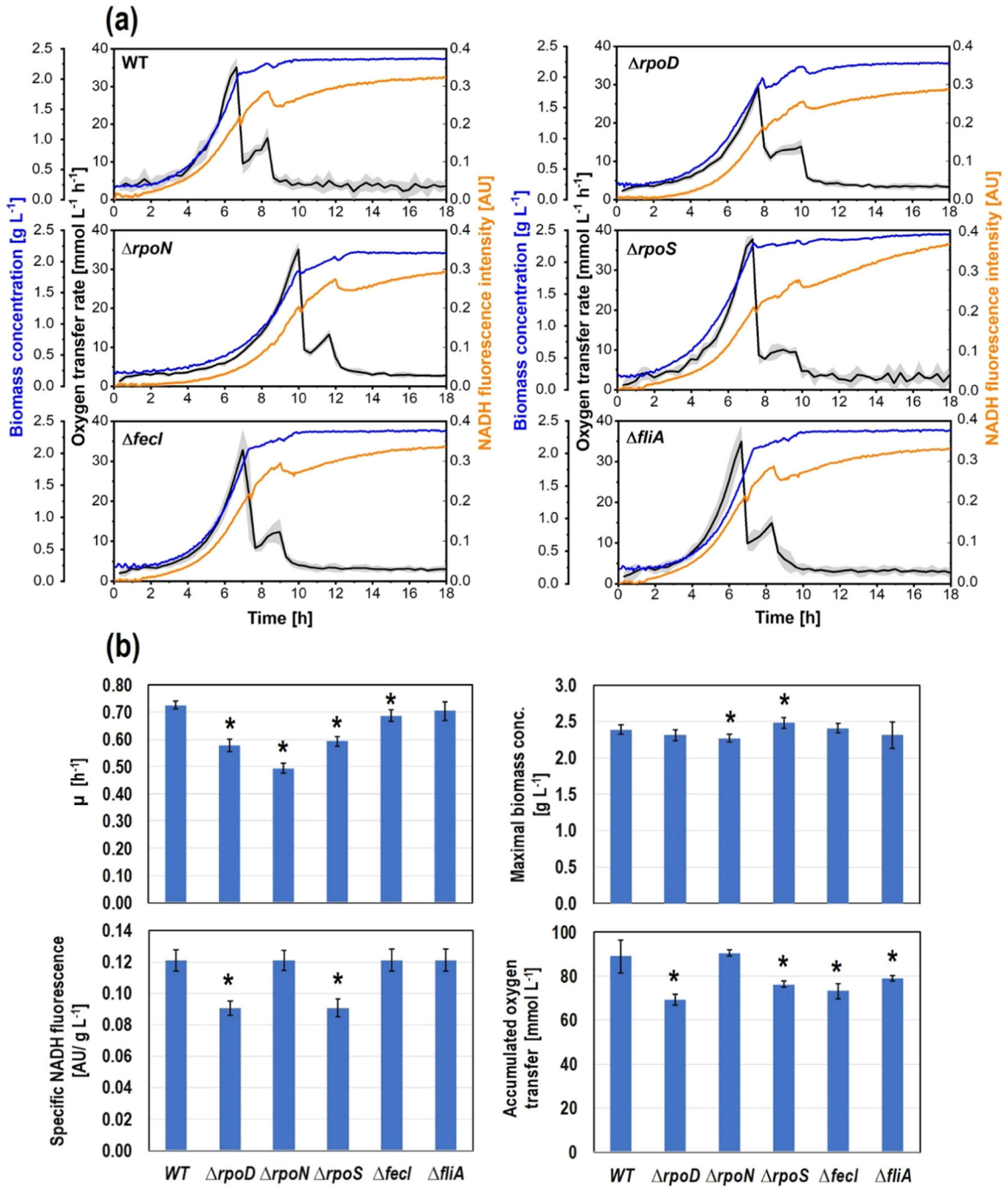

FIGURE 1 | (a) Culture profile of the untransformed strains in a mineral medium. Biomass concentration was calculated from the online monitored scattered light intensity. The oxygen transfer rate and NADH fluorescence were also monitored. Shaded bands indicate the standard deviation between replicates. (b) Main culture parameters. Specific growth rate (μ) and NADH fluorescence were calculated over the exponential growth phase. * Indicates significant difference ($n = 4$; $p < 0.05$), compared to the WT strain.

to oxidize glucose to CO_2 for cellular maintenance during the longer fermentation times of this strain. In contrast, in cultures of the *fliA* mutant induced with 0.2 mM IPTG, the accumulated oxygen transfer was the lowest among all the mutants, while the GFP-specific fluorescence and emission rate was the highest (Figure 2b).

There is no clear relationship between the observed parameters. In general, the effects were more evident for the *rpoS* and *fliA* mutant strains. The positive effect observed in the *fliA* mutant can be attributed to better resource stewardship, due to less energy spent in motility-related protein synthesis. Similar effects have been observed by deleting genes coding for the transcriptional dual regulator FlhC, which is the main regulator of flagellum synthesis and swarming migration (Han et al. 2021; Lara et al. 2024). Ziegler and coworkers also reported an increase in the production of a recombinant protein in a strain lacking *fliA* and some operons related to motility (Ziegler et al. 2021). In the case of the *rpoS* mutant, it is known that flagellar synthesis is upregulated, when the *rpoS* gene is deleted (Dong and Schellhorn 2009), which may

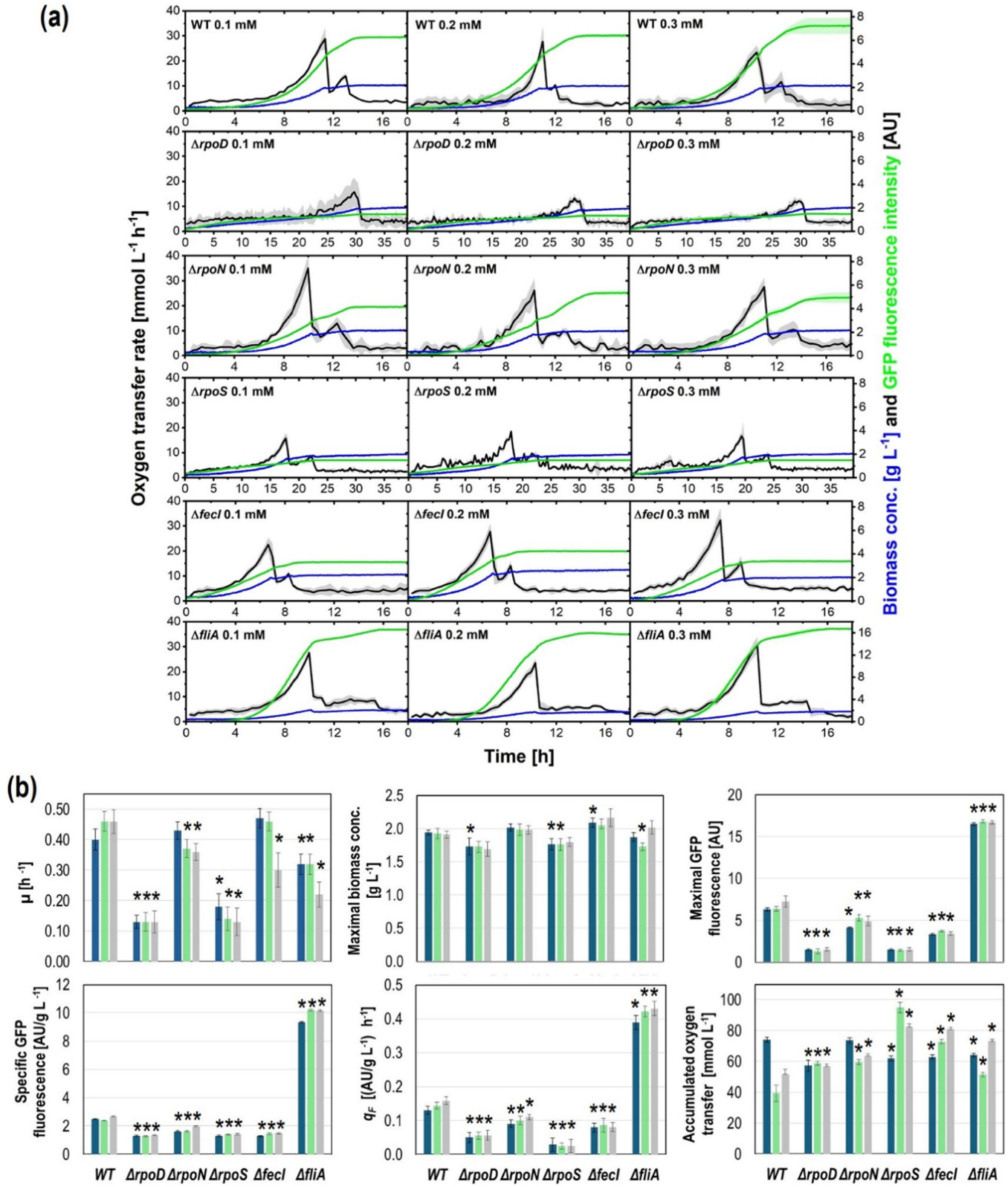

FIGURE 2 | (a) Culture profile of the strains bearing the plasmid pV21 in mineral medium at 37°C. GFP expression was induced with 0.1, 0.2, or 0.3 mM IPTG from inoculation. Biomass concentration was calculated from online monitored scattered light intensity. The oxygen transfer rate and GFP fluorescence were also monitored. Shaded bands indicate the standard deviation between replicates. (b) Main culture parameters using 0.1 (blue bars), 0.2 (green bars), or 0.3 (grey bars) mM IPTG. Specific growth rate (μ), GFP fluorescence, and GFP fluorescence emission rate (qF) were calculated over the exponential growth phase. * Indicates significant difference ($n = 4$; $p < 0.05$), compared to the WT strain under the same conditions.

partially explain the growth rate reduction and accumulated oxygen transfer increase.

Recombinant protein expression at low temperatures can increase the production of correctly folded proteins in *E. coli* (San-Miguel et al. 2013). The use of expression systems engineered to induce protein expression in response to low temperature, is attractive to attain high expression levels (Lin et al. 2024). Therefore, a set of cultures at 20°C in the mineral medium was carried out, which included the *rpoH* mutant. As explained above, increasing the IPTG concentration from 0.2 to 0.3 mM did not increase the GFP fluorescence in cultures at 37°C. Therefore, for the cultures at 20°C, only 0.1 and 0.2 mM IPTG were used. The OTR values in cultures at 20°C were very low, compared to cultures at 37°C (Figures 2a and 3b). This may be due to the low oxygen consumption rate of the cells, as the growth at 20°C was slow (some cultures lasted up to 50 h, Figure 3a). The strains exhibited prolonged *lag* phases, lasting between 10 and 20 h (Figure 3a). The GFP fluorescence increased parallel to cell growth. In cultures of the *rpoS* mutant, both the GFP fluorescence and biomass signals strongly increased immediately after the OTR decrease, indicating glucose exhaustion (Figure 3b). This could be related to the downregulation of stationary phase genes in this mutant, which in turn may release cellular resources for further GFP expression. The specific growth rate was severely reduced in the *rpoD*, *rpoS,* and *rpoH* mutants, compared to the WT (Figure 3b). With exception of the *rpoN* mutant, all the other strains attained higher biomass than the WT (Figure 3b). The maximal GFP fluorescence was similar in cultures of the WT and *rpoN* and *fliA* mutant strains, while for the *fecI* mutant, it was 20% lower than for the WT under the two induction levels (Figure 3b). In contrast, GFP expression was remarkably higher in the *rpoD*, *rpoH,* and *rpoS* mutant strains than in the WT (Figure 3b).

Particularly, the GFP fluorescence in cultures of the *rpoS* mutant was up to 19-fold higher than in cultures of the WT strain. Changing the IPTG concentration from 0.1 to 0.2 mM increased GFP fluorescence by 23% in cultures of the *rpoS* mutant strain, whereas no relevant changes were observed for the other strains. The *rpoS* mutant exhibited the highest specific GFP fluorescence yield and fluorescence emission rate, which were up to nine and fourfold greater, respectively, than those of the WT strain. At 20°C, *E. coli* cells experience a cold shock, which lowers cell growth and biomass yields, as can be seen comparing the results of Figure 2 with those of Figure 3. The sigma factor σ^S is essential for the response to cold shock, as it is involved in this stress response and activates genes that code for membrane proteins to strengthen them (White-Ziegler et al. 2008). A deletion of the *rpoS* gene leads to a lower growth of the cells (Figure 3b), which are more sensitive to the cold shock due to the lack of membrane support. However, at 20°C, the highest GFP fluorescence emission rate (q_F) can be observed for this mutant. The suppressed cold stress response appears to provide additional metabolic space for GFP expression. Therefore, the *rpoS* deletion seems to be an attractive option for improving recombinant protein expression at low temperatures in *E. coli*.

The specific GFP fluorescence was threefold higher in the *rpoD* mutant than in the WT strain (Figure 3b). It has been reported that mutations in the sequence of *rpoD* reduces its capacity to bind to the RNA polymerase core, creating conditions similar to reduced levels of *rpoD* expression (Jishage et al. 2002). A mutation in the sequence of *rpoD* led to a fourfold higher expression of a eukaryotic integral membrane protein at 20°C in batch mode. The authors also reported higher biomass formation in fed-batch cultures in shake flask (Tomatis et al. 2019). In agreement with such information, the lower expression of *rpoD* in the mutant strain used in the present study, also resulted in a substantial increase of recombinant protein expression and, therefore, is an interesting target to increase the production of difficult-to-express proteins.

GFP expression was also evaluated in the widely used LB medium, at 37°C and with 0.1 and 0.2 mM IPTG. The results are shown in Figure 4. No growth of the *rpoH* mutant was observed, and consequently, this strain is excluded from Figure 4. The OTR time profile suggests the exhaustion of several nutrients during cell growth, which agrees with the complex composition of this culture medium (Figure 4a). Like in previous experiments, the GFP fluorescence increased proportionally to the cell growth (Figure 4a). The growth rate was lower in all mutants and induction levels, except for the *rpoN* mutant with 0.1 and 0.2 mM IPTG, and the *fecI* mutant with 0.1 mM IPTG (Figure 4b). The individual deletion of the sigma factors resulted in lower attained biomass, except in the case of the *rpoN* mutant. The unaffected growth rate and biomass synthesis of the *rpoN* mutant suggest that the genes controlled by this sigma factor are less relevant in the rich LB than in the mineral media, in which the growth rate of the *rpoN* mutant was noticeably lower than that of the WT (Figures 1–3). Furthermore, the biomass attained in cultures of the *rpoN* mutant was very similar to those of the WT, which contrasts with the rest of the mutant strains, that exhibited lower biomass values (Figure 4b). In contrast, only the *rpoD*, *rpoS,* and *fliA* mutants reached maximal GFP fluorescence values greater than the WT. During the exponential growth phase, the specific GFP fluorescence of the *fecI* and *fliA* mutants was approx. 20% lower than the corresponding value for the WT. The *rpoN* mutant achieved a specific GFP fluorescence value 50% higher than that of the WT. Remarkably, the specific GFP fluorescence of mutant strains *rpoS* and *rpoD* were approx. Three and ninefold greater than the achieved by the WT (Figure 4b). Consequently, the specific GFP fluorescence emission rate of the mutant strain *rpoD* was approx. Sevenfold higher compared to the WT (Figure 4b). In general, the accumulated oxygen transferred in cultures of the mutant strains was slightly but significantly ($p < 0.05$) lower than that of the WT (Figure 4b). The lowest value was observed in cultures of the mutant strain *rpoD*, which was approx. 20% lower than that of cultures of the WT. The higher GFP expression and lower oxygen consumption render the *rpoD* mutant an excellent option for protein expression in a rich medium. A reason for the superior performance of this strain could be related to a lower proteomic burden in the rich LB medium, due to lesser expression of the proteins controlled by the transcriptional factor RpoD. For instance, McKenna and coworkers created a library of *rpoD* gene mutations and found that the most truncated mutant led to strongly increased expression of a full-length antibody at 30°C in a rich medium, although the exact reasons for this were not clarified (McKenna et al. 2019). Further studies are required to fine-tune the expression level of *rpoD* as a strategy to optimize recombinant protein expression in rich media.

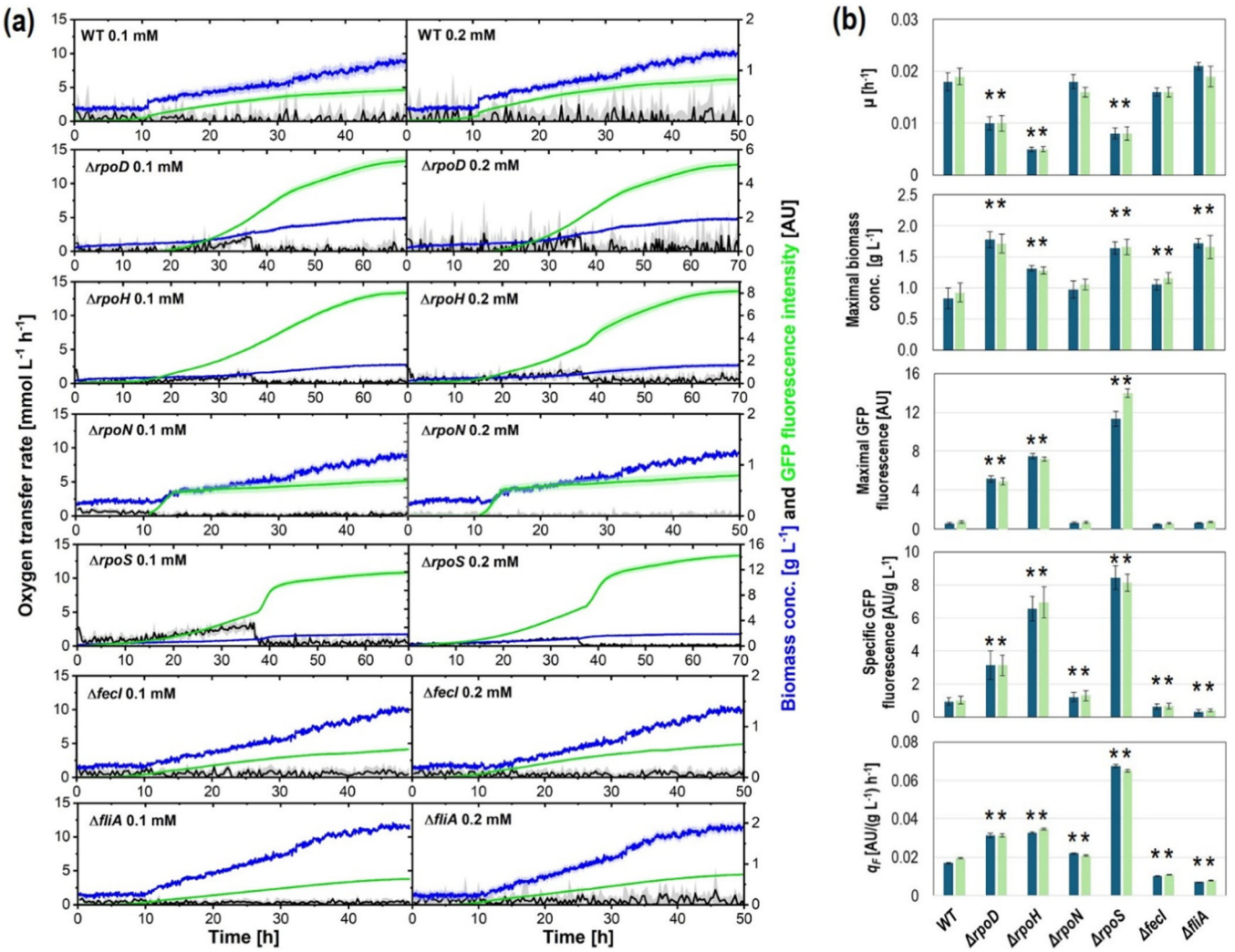

FIGURE 3 | (a) Culture profile of the strains bearing the plasmid pV21 in mineral medium at 20°C. GFP expression was induced with 0.1 or 0.2 mM IPTG from inoculation. Biomass concentration was calculated from the online monitored scattered light intensity. The oxygen transfer rate and GFP fluorescence were also monitored. Shaded bands indicate the standard deviation between replicates. (b) Main culture parameters using 0.1 (blue bars), or 0.2 (green bars) mM IPTG. Specific growth rate (μ), GFP fluorescence, and GFP fluorescence emission rate (qF) were calculated over the exponential growth phase. * Indicates significant difference ($n = 4$; $p < 0.05$), compared to the WT strain under the same conditions.

Taken together, the results show that deletion of sigma factors is a useful tool to improve the production of recombinant protein in *E. coli*, since they clearly affect the expression levels, growth rates, and oxygen consumption. As this study shows, the best target for deletion will depend on the culture conditions like temperature and media, and should be decided depending on the application. Namely, recombinant protein expression at low temperatures is benefited by *rpoS*, *rpoH,* and *rpoD* knockouts. At 37°C, *rpoD* deletion showed the best results in LB, while in a mineral medium, the *fliA* was a superior producer.

2 | Materials and Methods

2.1 | Strains and Plasmid

The K-12 derivative BW25113 *Escherichia coli* was used as wild type (WT). The mutans strains with individual interruptions of the genes *rpoD*, *rpoN*, *rpoS*, *fecI,* and *fliA*, were obtained from the Keio Collection (Baba et al. 2006). The gene *rpoH* was interrupted using the method described by Datsenko and Wanner (2000), except that the mutant strain was incubated at 20°C. The strains were transformed with the high copy number plasmid pV21, which contains the gene of the GFP under transcriptional control of the *lac* promoter, and a gene conferring spectinomycin resistance (Velazquez et al. 2022). Transformed and untransformed strains were plated in LB-agar in Petri dishes and grown at 37°C for 14–18 h, except for the *rpoH* mutant, which was grown at 20°C for 48 h. One colony per strain was selected and grown in Terrific Broth (TB) medium at 20°C (for the *rpoH* mutant) or 37°C (for all the other strains) until reaching an optical density at 600 nm (OD_{600}) of approximately 6–8. Then, 0.9 mL of such culture broth was mixed with a sterile solution of glycerol (80% v/v) and immediately frozen at −80°C. The genotypes of the mutant strains were confirmed by PCR amplification of the involved gene using the oligomers described in the Supplementary File. To further confirm the PCR product of the Δ*rpoD* strain, 6 colonies were sampled and subjected to PCR amplification.

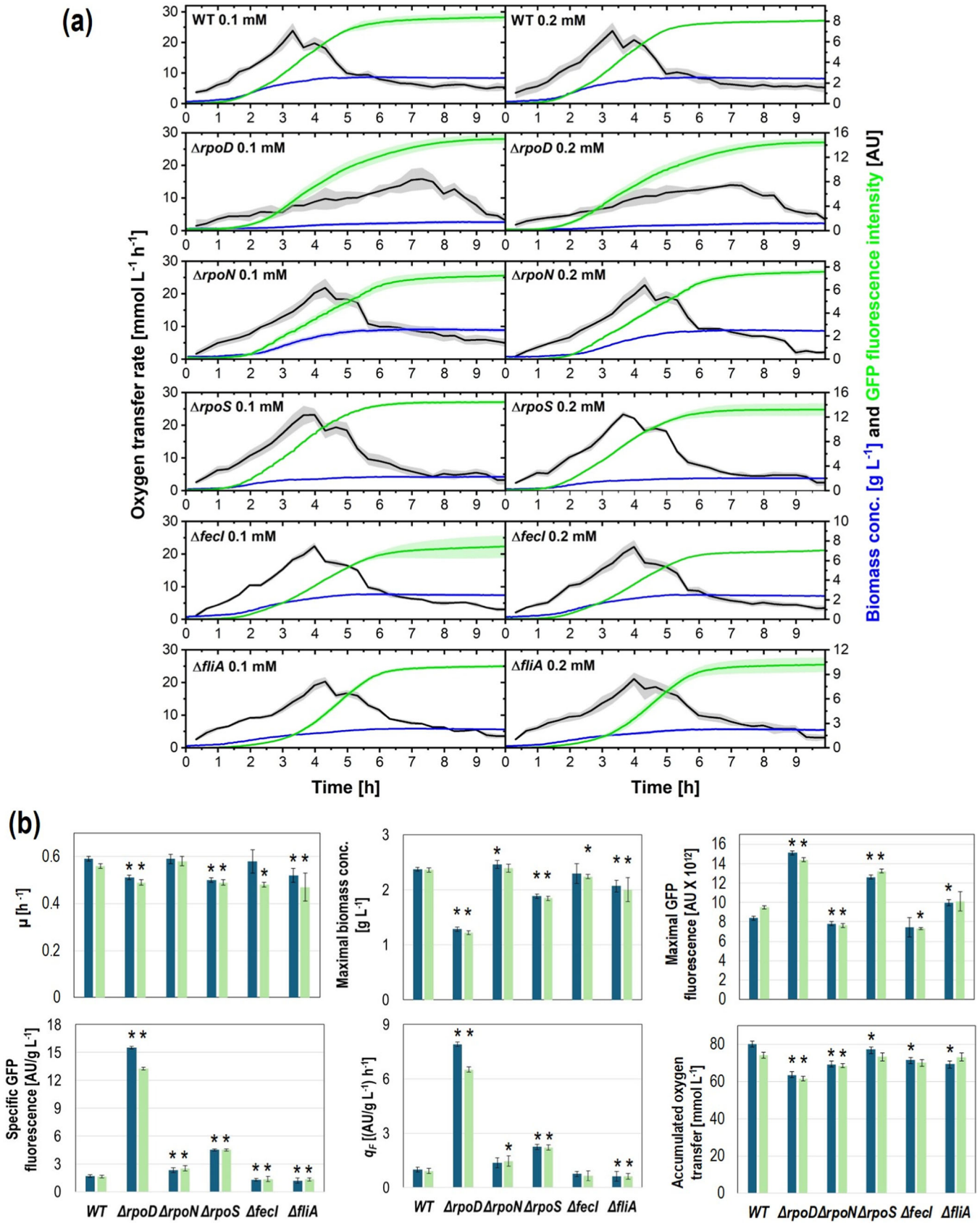

FIGURE 4 | (a) Culture profile of the strains bearing the plasmid pV21 in LB medium at 37°C. GFP expression was induced with 0.1 or 0.2 mM IPTG from inoculation. Biomass concentration was calculated from the online monitored scattered light intensity. The oxygen transfer rate and GFP fluorescence were also monitored. Shaded bands indicate the standard deviation between replicates. (b) Main culture parameters using 0.1 (blue bars), or 0.2 (green bars) mM IPTG. Specific growth rate (μ), GFP fluorescence, and GFP fluorescence emission rate (qF) were calculated over the exponential growth phase. * Indicates significant difference ($n = 4$; $p < 0.05$), compared to the WT strain under the same conditions.

2.2 | Media Composition

All chemicals applied for media preparation were of analytical grade and purchased from Carl Roth GmbH, if not stated otherwise. The composition of LB was (in g L^{-1}): yeast extract, 5; tryptone, 10; NaCl, 5. The composition of TB was (in g L^{-1}): yeast extract, 24; tryptone, 20; glycerol, 4. The mineral medium used was modified from a previous report (Wilms et al. 2001). The mineral medium composition was (in g L^{-1}): $(NH_4)_2SO_4$, 6.5; NH_4Cl, 0.5; K_2HPO_4, 3.0; Na_2SO_4, 2.0; $MgSO_4 \cdot 7H_2O$, 0.5; thiamine hydrochloride 0.01; MOPS, 41.85; plus 1 mL of trace elements solution per L of medium. Spectinomycin was purchased from Sigma Aldrich. The initial pH was adjusted to 7.4 by adding 0.2 M NaOH. The trace elements solution was prepared and autoclaved separately. The composition of the trace element solution was (in g L^{-1}): $ZnSO_4 \cdot 7H_2O$, 0.54; $CuSO_4 \cdot 5H_2O$, 0.48; $MnSO_4 \cdot H_2O$, 0.30; $CoCl_2 \cdot 6H_2O$, 0.54; $FeCl_3 \cdot 6H_2O$, 41.76; $CaCl_2 \cdot 2H_2O$, 1.98; $Na_2EDTA \cdot 2H_2O$, 33.4. Glucose was added from a separately autoclaved solution at a concentration of 500 g L^{-1}, to final concentrations of 4 g L^{-1} for cultures of the untransformed strains, or 3.5 g L^{-1} for cultures of the plasmid-bearing strains. For all cultures of plasmid-bearing cells, the media were supplemented with 0.1 g L^{-1} spectinomycin. GFP expression was induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) added before inoculation at final concentrations of 0.1, 0.2, or 0.3 mM.

2.3 | Microbioreactor Cultures

The strains were characterized in microbioreactor cultures in lysogeny broth (LB) or in the mineral medium supplemented with 4 g L^{-1} glucose. For cultures in LB, a preculture was performed by transferring 0.05 mL of the cryopreserved cells to 8 mL of LB medium contained in a 250 mL Erlenmeyer flask. For cultures in the mineral medium, 0.05 mL of the cryopreserved cells were transferred to 8 mL of TB medium contained in a 250 mL Erlenmeyer flask. The cells were grown at 20 (for the *rpoH* mutant) or 37°C (for all other strains), and 350 rpm in an orbital shaker of 50 mm shaking diameter for 6 h. For the main cultures in mineral medium, 0.05 mL from the first preculture in TB medium were transferred to 8 mL of mineral medium contained in a 250 mL Erlenmeyer flask and cultured at 20 (for the *rpoH* mutant) or 37°C (for all other strains), and 350 rpm in an orbital shaker of 50 mm shaking diameter until mid-exponential growth phase (approx. 14–16 h). The obtained cells were washed with 0.9% sterile NaCl solution and used to inoculate the main cultures. Microbioreactor cultures were carried out in 48-round transparent bottom wells microtiter plates (MTP) (Beckman Coulter) with a liquid volume of 0.75 mL per well, an initial OD_{600} of 0.2 units, at 20 or 37°C, 1000 rpm and 3 mm shaking diameter (Climo-Shaker ISF1-X, Kuhner). The shaken monitoring system, named μRAMOS-BioLector combination, is constructed in-house, and enables measurement of the OTR in every individual well of the MTP based on the oxygen partial pressure and oxygen-dependent emission of fluorescence sensors (Flitsch et al. 2016), and synchronized online measurement of fluorescence and scattered light (Ladner et al. 2016). The MTPs were covered with a gas-permeable polyolefin sealing foil (HJ-Bioanalytik GmbH), to reduce evaporation and prevent contaminations. Cell growth was monitored by measuring the scattered light (ScL) intensity at a wavelength of 620 nm. Biomass concentration was calculated from the ScL readings using an experimental correlation for *E. coli* (Kensy et al. 2009). The wavelengths for excitation and fluorescence emission of GFP were 480 and 507 nm, while for NADH were 340 and 460 nm, respectively.

2.4 | *rpoD* Gene Transcription Level

Triplicate cultures of the WT and *rpoD* mutant strains were carried out in the mineral medium at 37°C as explained above. Samples were taken during the exponential growth of the cells. RNA extraction, purification, RT-qPCR conditions, and relative transcription level calculation are described in detail elsewhere (Aguilar et al. 2012).

2.5 | Data Analysis

The data from fluorescence and scattered light (ScL) intensities are represented as the corresponding reading minus the lowest reading (usually obtained during the first 20 min of culture). The specific fluorescence (SF) was calculated as the mean of the fluorescence divided by the ScL readings in every time point during the period involved. Specific yields were calculated by the proper mass balances over the time periods involved. The specific fluorescence emission rate was calculated by multiplying the specific growth rate by the specific GFP fluorescence intensity. Accumulated oxygen transfer was calculated by integration of the area under the curve in the plot of OTR versus time using the Integration gadget of the Origin software (OriginLab Corporation). For comparison between two groups means, unpaired Student's *t*-tests were performed.

Author Contributions

A. R. Lara, G. Gosset, and **J. Büchs:** conceptualization. **L. Pohlen, E. García, N. Flores, L. M. Martínez, A. R. Lara,** and **G. Gosset:** experimental design, planning, execution, data acquisition, analysis. **A. R. Lara, G. Gosset,** and **J. Büchs:** funding acquisition. **A. R. Lara, G. Gosset, J. Büchs, L. Pohlen, E. García, N. Flores,** and **L. M. Martínez:** writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.