Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Material

Exposure of *Escherichia coli* to cadmium telluride quantum dots, silver or cupric oxide nanoparticles during aerobic respiratory *versus* anaerobic fermentative growth on D-(+)-glucose

Joanne Vassallo, Rich Boden and Richard D. Handy

Supplementary Information. Complete and partial (mixed-acid fermentation) oxidation of D-(+)-glucose in *Escherichia coli* K-12 MG1655.

The change in Gibbs energy (ΔG°) for the complete oxidation of glucose is presented in Eq. (1):

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad (1)$$
$$\Delta G° = -2{,}456 \text{ kJ (mol glucose respired)}^{-1}$$

The change in Gibbs energy for the partial (mixed-acid fermentation) of glucose is described in Eq.(2):

$$C_6H_{12}O_6 \rightarrow 0.003 \text{ 2,3-butanediol} + 0.002 \text{ acetoin} + 0.505 \text{ ethanol} + 0.003 \text{ glycerol} + 0.072 \text{ butyric acid} + 0.387 \text{ acetic acid} + 0.860 \text{ formic acid} + 0.148 \text{ succinic acid} + 0.700 \text{ lactic acid} + 0.018 \ CO_2 + 0.003 \ H_2 \quad (2)$$
$$\Delta G° = -135 \text{ kJ (mol glucose fermented)}^{-1}$$

This was determined *de novo* from the standard Gibbs energy of formation (ΔG_f) values.[1-3] Mixed acid fermentation product ratios are for *E. coli* K-12 MG1655 fermenting glucose at starting pH > 7.0.[4]

Using the methodology of Kelly[5] for approximating the maxima for ATP synthesis, based purely on thermodynamics and not on biological ATP yields, possible on the basis of the above Gibbs energy changes and taking that for the formation of ATP as determined *de novo* from ΔG_f values obtained,[1, 6, 7] resulting in Eq. (3):

$$MgATP^{2-} + H_2O \rightarrow MgADP^{-} + H_2PO_4^{2-} \quad (3)$$
$$\Delta G° = -29.43 \text{ kJ (mol } MgATP^{2-} \text{ hydrolysed)}^{-1}$$

$MgATP^{2-}$ is the dominant form of ATP in the living cell (> 80 %).[8]

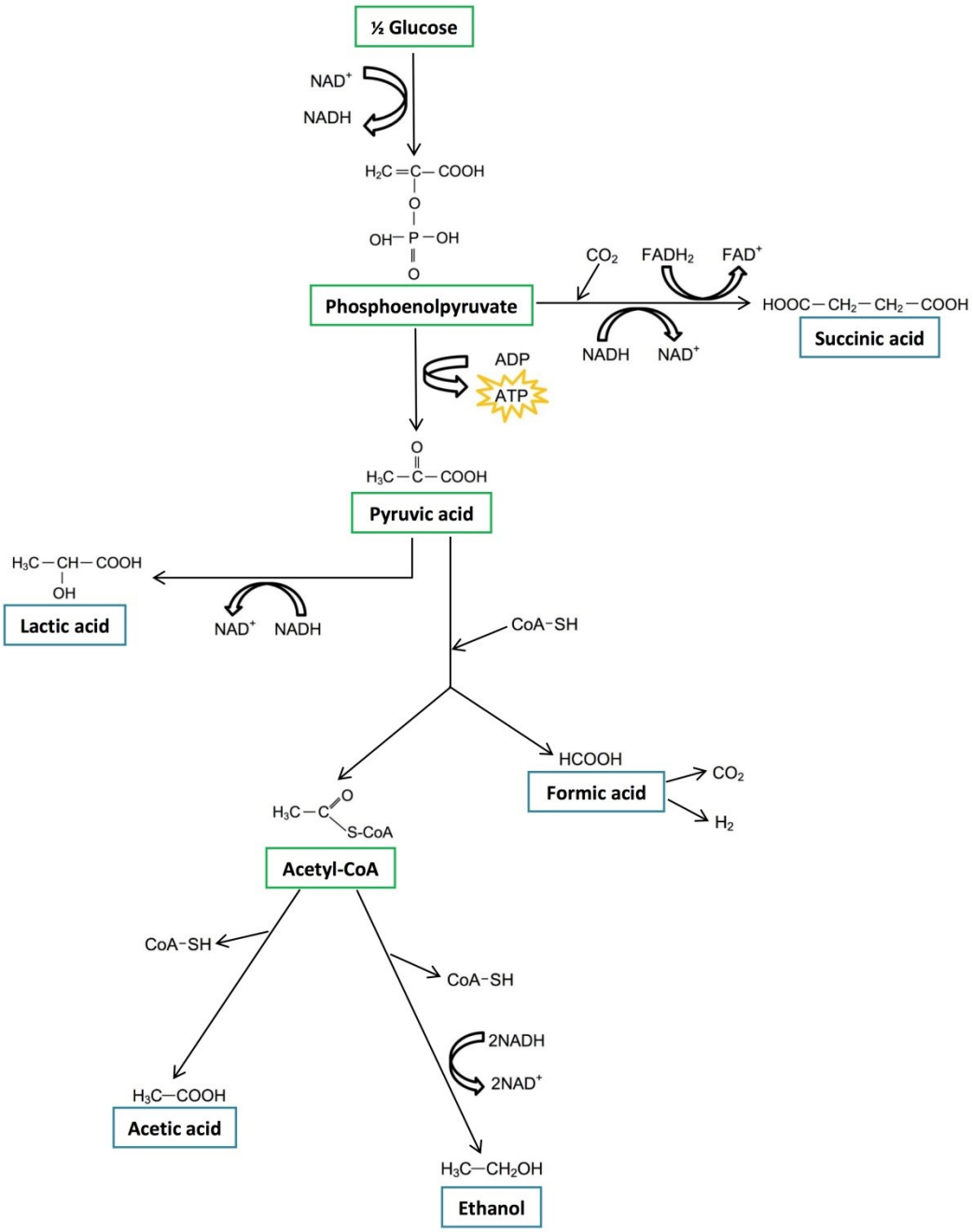
From the above data, glucose oxidation can yield a thermodynamic maximum of 68.1 ATP/glucose (i.e., -2456 kJ/29.43 kJ mol^{-1}) and fermentation 3.8 ATP/glucose (i.e., -135 kJ/29.43 kJ mol^{-1}), which is an enormous difference. Given the biosynthetic cost of assimilation of glucose into biomass ($C_{12}H_{24}O_6N_3$ = 306 g) is 31 mol ATP *per* mol biomass,[9] biomass yields during fermentation are much lower.

The maintenance coefficient (m_S) is a measure of the cost of the existence of a cell,[10] in terms of how much substrate must be oxidised to provide the energy needed for survival. This

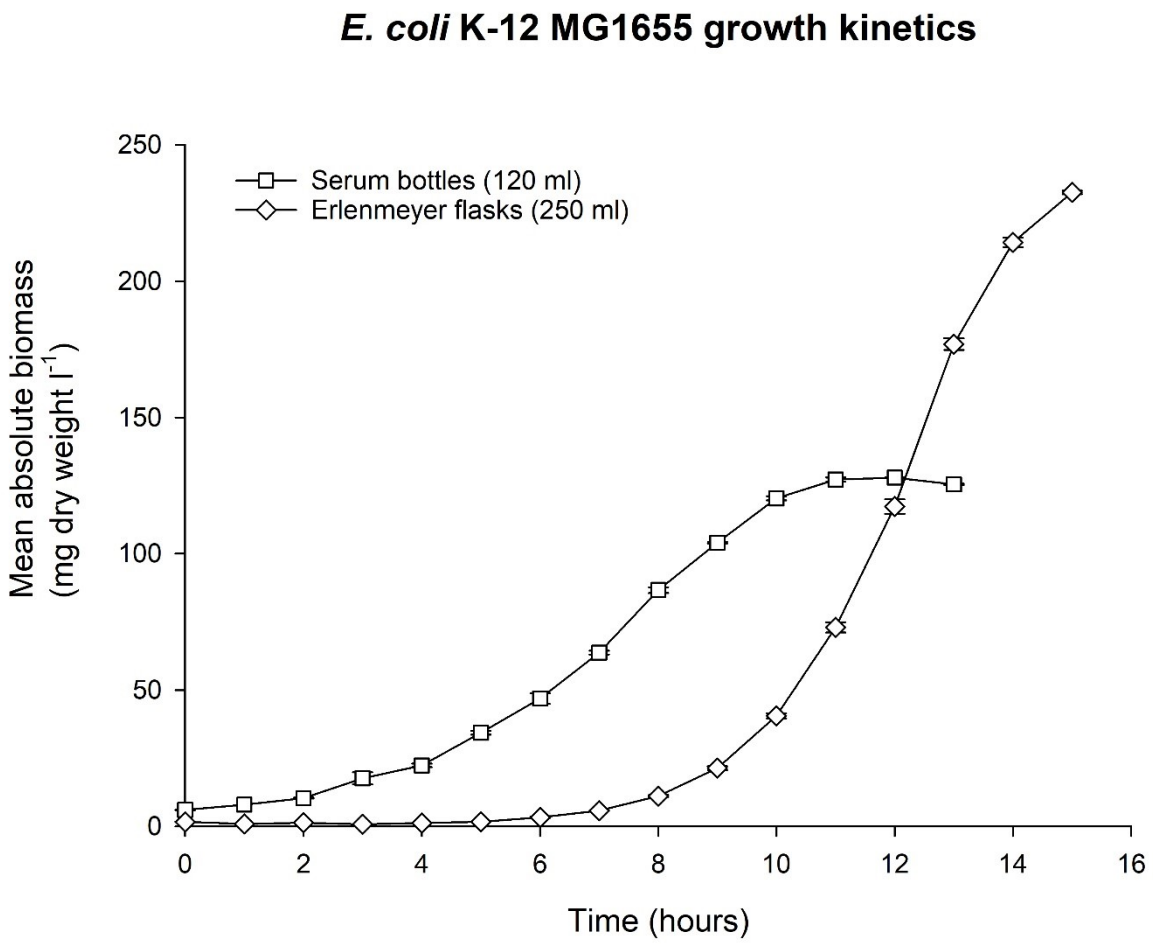
does not include costs of cell division, turnover of cell material, maintaining concentration gradients, motility, and repairing damage. It is expressed in the amount of substrate required to maintain 1 g of biomass for 1 h. It has been studied in various forms from the 1940s and is well-established in microbial physiology as a measure of the 'cost of living'.

As m_S can only be determined from continuous-flow cultures grown in the chemostat,[10] we cannot express our data herein in terms of it, but it is a useful parameter for consideration of the effect of nanomaterials on *E. coli* K-12 MG1655 grown under each condition. Typical m_S values for organisms growing aerobically on glucose are in the region of 0.05 - 0.1 mmol glucose (g dry biomass)$^{-1}$ h^{-1}, whereas during fermentation they can rise to as much as 2 - 4 mmol glucose (g dry biomass)$^{-1}$ h^{-1}.[11-13]

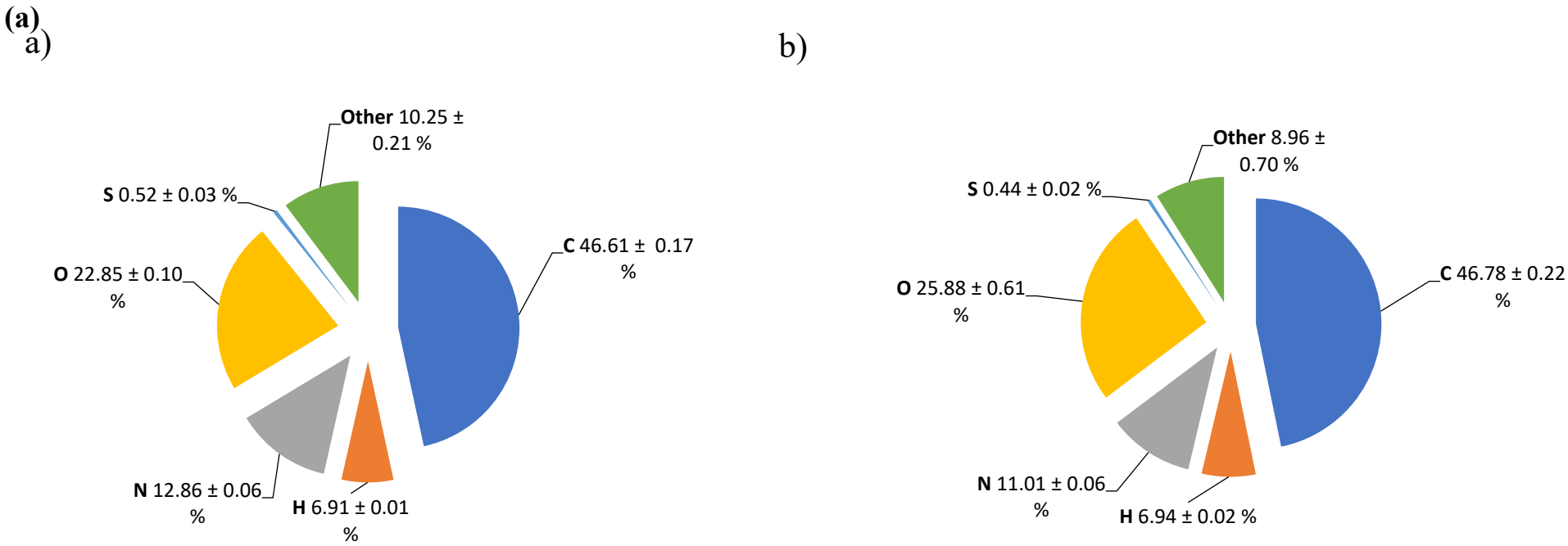
Supplemental Fig. S1 *Escherichia coli* K-12 MG1655 mixed-acid fermentation pathway as adapted from Stanier et al.[14] and Clark.[15] For figure clarity, the protons (H^+) are presented on the acidic products acetate, formate, succinate, lactate and pyruvate (fermentation products).


½ Glucose
NAD+
NADH
H2C=C–COOH
O
OH–P–OH
O
Phosphoenolpyruvate
CO2
FADH2
FAD+
HOOC–CH2–CH2–COOH
Succinic acid
NADH
NAD+
ADP
ATP
O
H3C–C–COOH
Pyruvic acid
H3C–CH–COOH
OH
Lactic acid
NAD+
NADH
CoA–SH
HCOOH
Formic acid
CO2
H2
H3C–C
O
S-CoA
Acetyl-CoA
CoA–SH
CoA–SH
2NADH
2NAD+
H3C–COOH
Acetic acid
H3C–CH2OH
Ethanol

Supplemental Fig. S2 Growth curve analysis for *E. coli* K-12 MG1655 incubated under oxic conditions in 250 ml Erlenmeyer flasks (n = 3) and anoxic conditions in 120 ml sealed serum bottles (n = 3), with measured mean ± S.E.M absolute biomass (mg dry weight l^{-1}) on the *y-axis*, against time in hours on the *x-axis*.



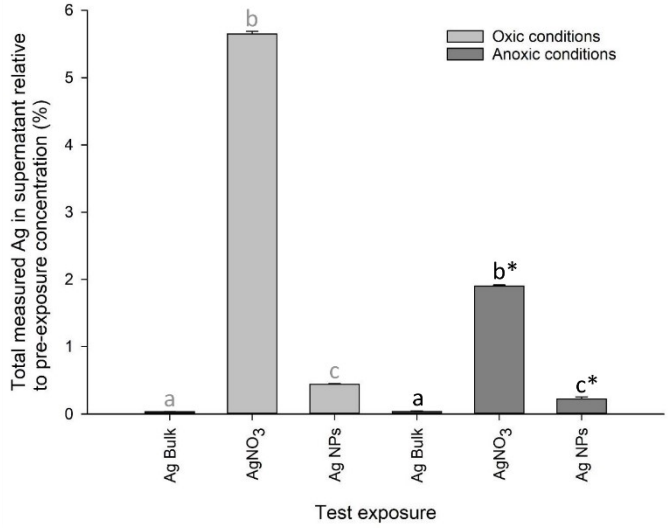
Supplemental Fig. S3 Micro-elemental composition of *E. coli* K-12 MG1655 incubated under: a) oxic conditions, b) anoxic conditions (n = 3); data are presented as mean ± S.E.M, for carbon (C), hydrogen (H), oxygen (O), nitrogen (N), sulfur (S) and other elemental percentage composition (%), as analysed from 3 replicate dried pellets of bacterial biomass, and with two technical replicates analysed for each elemental assay.



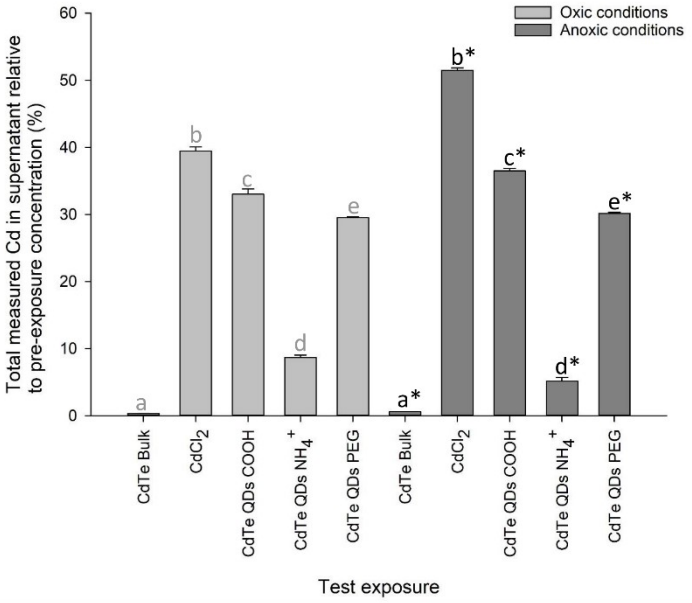
Supplemental Fig. S4 Total measured metal concentration in the supernatant (post-bacterial exposure) relative to the initial pre-exposure calculated total metal concentration, as dosed in the NaCl-EBS medium. Data are presented as percentage mean values ± S.E.M (n = 3); where a) silver, b) copper, c) cadmium and d) tellurium. Different letters indicate significant differences by test material type (ANOVA, $p < 0.05$), in oxic

and anoxic conditions, respectively. Significant differences by test material type (*t*-test, $p < 0.05$) between oxic and anoxic conditions are represented by an asterisk (*).

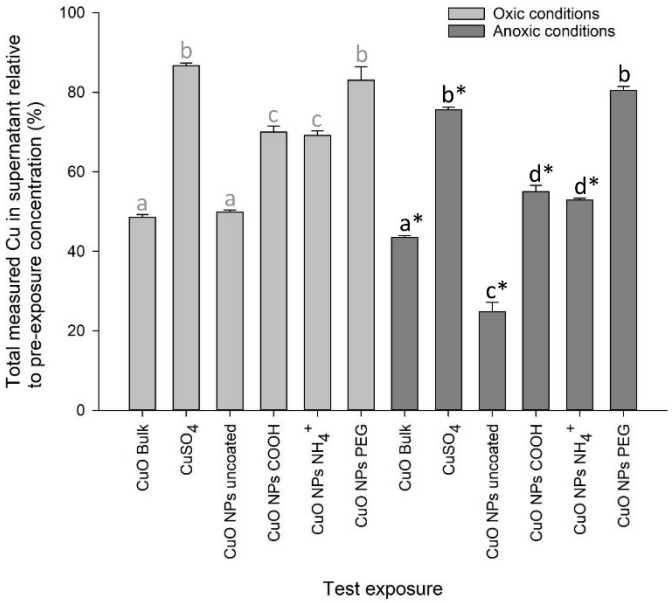
a)


Total measured Ag in supernatant relative to pre-exposure concentration (%)
Oxic conditions
Anoxic conditions
Ag Bulk
AgNO3
Ag NPs
Ag Bulk
AgNO3
Ag NPs
Test exposure

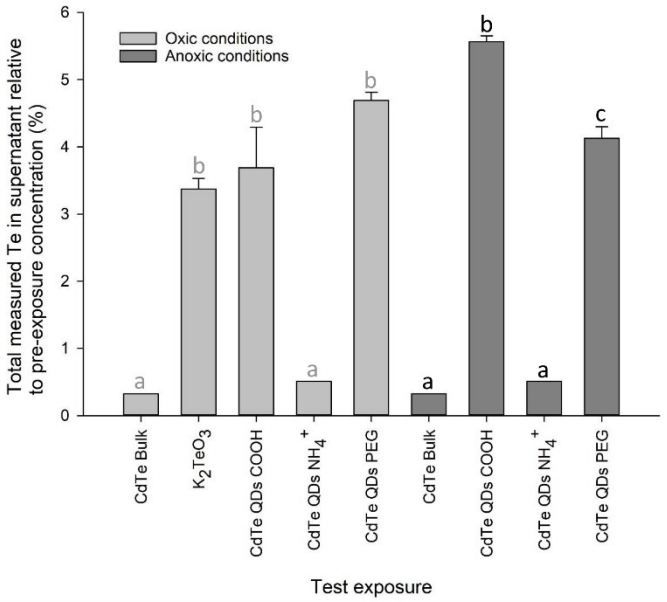
c)


Total measured Cd in supernatant relative to pre-exposure concentration (%)
Oxic conditions
Anoxic conditions
CdTe Bulk
CdCl2
CdTe QDs COOH
CdTe QDs NH4+
CdTe QDs PEG
CdTe Bulk
CdCl2
CdTe QDs COOH
CdTe QDs NH4+
CdTe QDs PEG
Test exposure

b)


Total measured Cu in supernatant relative to pre-exposure concentration (%)
Oxic conditions
Anoxic conditions
CuO Bulk
CuSO4
CuO NPs uncoated
CuO NPs COOH
CuO NPs NH4+
CuO NPs PEG
CuO Bulk
CuSO4
CuO NPs uncoated
CuO NPs COOH
CuO NPs NH4+
CuO NPs PEG
Test exposure

d)


Total measured Te in supernatant relative to pre-exposure concentration (%)
Oxic conditions
Anoxic conditions
CdTe Bulk
K2TeO3
CdTe QDs COOH
CdTe QDs NH4+
CdTe QDs PEG
CdTe Bulk
CdTe QDs COOH
CdTe QDs NH4+
CdTe QDs PEG
Test exposure

References:

1. R. A. Alberty, *Thermodynamics of Biochemical Reactions*, John Wiley & Sons, New Jersey, 2003.
2. Z. Galus, in *Standard Potentials in Aqueous Solutions*, CRC Press, London, 1985.
3. P. N. Ross, in *Standard Potentials in Aqueous Solutions*, CRC Press, London, 1985.
4. A. C. Blackwood, G. A. Ledingham and A. C. Neish, Dissimilation of glucose at controlled pH values by pigmented and non-pigmented strains of *Escherichia coli*, *J Bacteriol*, 1956, **72**, 497-499.
5. D. P. Kelly, in *Companion to Microbiology*, Longman, London, 1979.
6. K. S. V. Santhanam, in *Standard Potentials in Aqueous Solutions*, CRC Press, London, 1985.
7. J. P. Hoare, in *Standard Potentials in Aqueous Solutions*, CRC Press, London, 1985.
8. A. C. Storer and A. Cornish-Bowden, Concentration of $MgATP^{2-}$ and other ions in solution. Calculation of the true concentrations of species present in mixtures of associating ions, *Biochemical Journal*, 1976, **159**, 1-5.
9. C. Anthony, *The Biochemistry of Methylotrophs*, Academic Press London, 1982.
10. R. Boden and L. Hutt, in *Consequences of Microbial Interactions with Hydrocarbons, Oils, and Lipids: Biodegradation and Bioremediation, Handbook of Hydrocarbon and Lipid Microbiology*, Springer Nature, Switzerland, 2018, DOI: 10.1007/978-3-319-44535-9_24-1, pp. 1-42.
11. S. J. Pirt, The maintenance energy of bacteria in growing cultures, *Proceedings of the Royal Society of London*, 1965, **163**, 224-231.
12. S. J. Pirt, *Principles of Microbe and Cell Cultivation*, Blackwell, London, 1975.
13. J. Monod, *Recherches sur la croissance bactériennes*, Masson, Paris, 1942.
14. R. Y. Stanier, Adelberg, E. A., Ingraham, J. L., *General Microbiology*, Macmillan, London, 1977.
15. D. P. Clark, The fermentation pathways of *Escherichia coli*, *FEMS Microbiology Reviews*, 1989, **5**, 223-234.