Elucidating the Importance of Numeracy Skills for Undergraduate Students in Life Sciences Using the Oxygen Requirement in Yeast as an Example

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Current education in biology is devoid of mathematics in many countries, probably because many relevant biological processes are explained from a qualitative point of view rather than addressing the quantitative aspects of these phenomena. Here, we employ a case study from the yeast physiology to illustrate the importance of numeracy skills for a deeper understanding of relevant biological problems. Yeast anaerobic growth on sugars is a widespread process as it is the basis for beer, bread, and winemaking and it is much akin to lactic acid fermentation in muscle cells in response to an increased energy demand. To study the physiology of yeasts under controlled conditions and being able to compare the results quantitatively, one ought to perform measurements and calculations involving concentrations of oxygen, biomass, and organic compounds. To set-up an "anaerobic" culture of *Saccharomyces cerevisiae* in a defined medium, one needs to calculate how much oxygen must enter the cultivation system, to meet the requirements for ergosterol and oleic acid biosyntheses, both of which require oxygen. Using basic physicochemical principles and simple mathematical skills, students will be able to compute the oxygen requirement for yeast growth under such "anaerobic" conditions.

Introduction

Microbial physiology is a prospering area in the field of industrial biotechnology (Raghevendran, Nielsen, and Olsson 2005) and attracts students from science as well as engineering backgrounds. It is a quantitative subject with emphasis on yields, titres and rates (Gombert and van Maris 2015) and students with a biology background might face some challenges understanding the concepts and numbers involved. Most undergraduate students in life sciences do not get exposed to mathematical training in their studies and often memorise facts without much exposure to data analysis and interpretation (Brewer and Smith 2011; J. Koenig and Pike 2014). The textbooks employed in the curriculum compounds the issue with their mile wide, inch deep approach (Duncan, Lubman, and Hoskins 2011). Although a redesigned undergraduate biology course with a new textbook that integrates mathematics and biology offers a breath of fresh air (Barsoum et al. 2013), books on microbiology deal with rates in a lacklustre manner. The latest edition of Brock's microbiology, the most commonly employed textbook in the undergraduate microbiology course includes just the calculation of growth rate in the chapter on microbial metabolism (Madigan et al. 2015). Several reports (Bergevin 2010; Feser, Vasaly, and Herrera 2013; Marsteller 2010), books (Milo and Phillips 2014; Phillips, Kondev, and Theriot 2016), examples (Alberty 1991; Flamholz et al. 2014; Milo 2013; Planinšič and Vollmer 2008; Sender, Fuchs, and Milo 2016; Shastri and Morgan 2004; Torres 1998) and online tools (Adamec 2017; J. L. Koenig 2017; Shah et al. 2013; "Math Bench" 2016; "QUBES - Home" 2016; "Science in the Classroom" 2017) have been published showcasing the importance of quantitative approach in biological studies. These efforts make a strong case that undergraduate teaching in life sciences should place more emphasis on team learning, mathematical knowledge and interdisciplinary collaboration.

A paradigm is vital for a shift in the mindset of educationists and departmental heads in creating courses that have more relevance in the 21st century (in the areas of sustainability & innovation). To provide a context-based application of mathematical knowledge to address relevant biological questions, we chose the "anaerobic" cultivation of the yeast *Saccharomyces cerevisiae* in a defined medium as an example to understand a well-studied fermentation metabolism from a quantitative perspective. The goals of this article are:

- To expose students to a familiar problem that requires some quantitative skills

- To make connections between mathematics and biology

- To bring concepts from physical chemistry to address a question in microbial physiology

- To be familiar with numbers in yeast physiology using back of the envelope calculations

- To be able to guestimate in biology and eliminate potential outliers

- To feel comfortable with rates

A practical problem

Although it is habitual to talk about the *anaerobic* fermentation of sugars by the yeast *S. cerevisiae*, strictly speaking, this process is not completely devoid of consumption of molecular oxygen (O_2).
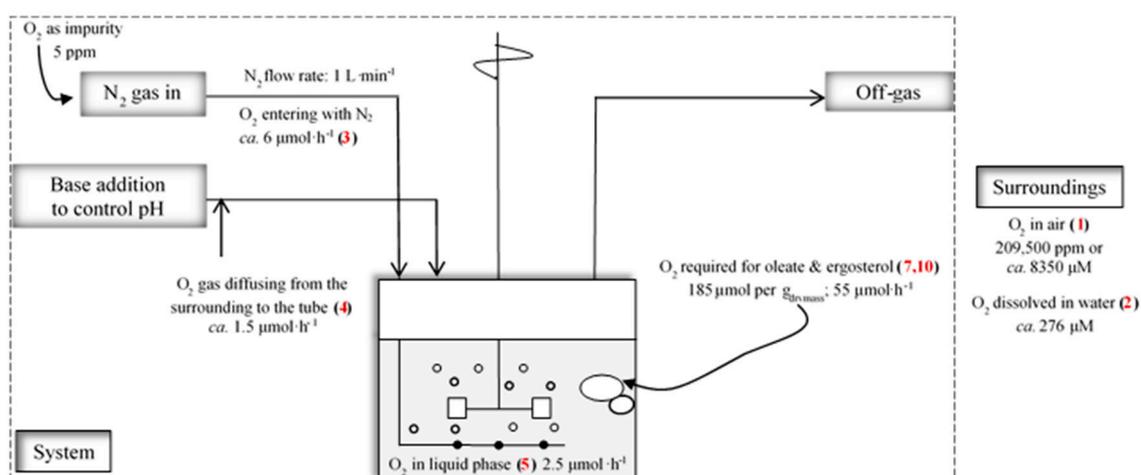

Figure *1* describes the set-up used to carry out the anaerobic fermentation. Various questions might arise in the minds of the students related to the diffusion of O_2 into laboratory cultivation systems, to the solubility of O_2 in a liquid medium, to the mass transfer from gas-to-liquid-to-yeast, and the influence of all these factors on the yeast physiology. These questions can be formulated as follows:

1. What is the concentration of O_2 in the atmosphere, in $mol \cdot L^{-1}$? ~ 8,300 μM

2. How much can O_2 be dissolved in water? ~ 280 μM

3. How much O_2 enters a bioreactor (or a shake flask) when ultrapure N_2 (commercially available as a gas compressed to high pressures in cylinders) with 5 ppm of O_2 is sparged at a rate of $0.5 \ L \cdot min^{-1}$ per unit liquid volume in the bioreactor? $\sim 6 \ \frac{\mu mol \ O_2}{h}$

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4. How much O_2 diffuses through the tubing material from the surroundings ($\sim 1.5\ \frac{\mu mol\ O_2}{h}$)? What precautions are needed to minimise O_2 entry into the system?

5. How much of these two sources of O_2 (present as an impurity with the incoming N_2 gas and through diffusion from the surroundings) dissolves in the liquid medium? $\sim 2.4\ \frac{\mu mol\ O_2}{h}$

6. Are there essential compounds in yeast that require O_2 for their biosyntheses? Yes: oleate and ergosterol

7. How much O_2 is needed to biosynthesise these two compounds: ergosterol and oleate? ~ 184 $\mu mol\ O_2$ per g dry cell mass

8. Given an initial sugar concentration, how much microbial biomass, on a dry basis, could be obtained by cultivating yeast under such "anaerobic" conditions? ~ 0.1 g dry cell mass per g of glucose

9. How much ergosterol and oleate are present in this dry biomass? 2 mg and 35 mg respectively per g of dry cell mass

10. For a given maximum specific growth rate ($\mu_{max} = 0.3\ h^{-1}$), what is the minimum amount of O_2 needed, in $\mu mol \cdot h^{-1}$, to synthesise these two compounds? $\sim 55\ \frac{\mu mol\ O_2}{h}$

11. Finally, would a culture sparged with 5 ppm O_2 under the conditions described here grow anaerobically without the exogenous supply of these growth factors or would additional O_2 be needed to meet the demand? Demand is $\sim 55\ \frac{\mu mol\ O_2}{h}$ while the supply is only $\sim 2.5\ \frac{\mu mol\ O_2}{h}$. Thus, cells will require O_2 or the growth factors must be supplied exogenously for proper growth.

Suggested learning outcomes for the above questions

Learning outcomes for questions 1 & 2: Students will be able to calculate mole fraction, convert *ppm* O_2 to *mol·L⁻¹* using the picket fence method. They will be able to apply Dalton's law, Henry's Law (see Box, in the supplementary material, for definitions,) and Ideal gas law. *Equations 1-4* in the supplementary material.

Thought problems: Is the solubility of O_2 dependent on temperature, on dissolved solids (compare freshwater vs sea water) and, on elevation (sea level vs places at high altitude)?

Learning outcomes for questions 3 & 4: Students will be able to perform unit conversion from *vvm* to *mol. h^{-1}*. They will be able to explain the mechanism involved in the transfer of O_2 from the gaseous phase to the liquid phase. They will be able to calculate the rate of transfer of O_2 from the gaseous phase to the liquid phase. *Equations 5-7; 11-12* in the supplementary material.

Thought problems: Is the transfer of O_2 dependent on bubble size of the incoming N_2 gas, the agitational speed of the impeller, the flow rate of N_2, the volume of liquid and any other parameters that you can think of that can influence the dissolution of O_2 in the liquid phase?

Learning Outcomes for question 5: Students will be able to apply Fick's law of diffusion to calculate the O_2 entering the system. They will be able to explain their choice of tubing material based on their permeability coefficient for O_2. *Equations 8-10* in the supplementary material.

Thought problems: What is the typical permeability coefficient of a biological membrane? What is the primary mode of movement of molecules (metabolites, proteins, RNA) inside the cell? How much time would it take for a metabolite to leave the cell by diffusion (Milo and Phillips 2014)?

Learning outcomes for question 6: Students will be able to use the scientific publications database to acquire a list of all the reactions from the latest genome-scale model of yeast with O_2 as a reactant. They will be able to manipulate the electronic spreadsheet to calculate the percentage of metabolic reactions.

Thought problems: How many metabolites are listed in the genome-scale model (Österlund et al. 2013)? What are the assumptions made in the model? What can you get from the model? What use does a yeast metabolic model serve? How would you improve the model?

Learning outcomes for questions 7, 8 & 9: From the genome-scale model, and from published literature, students will be able to identify the compounds that are indispensable for the growth of yeast in the absence of O_2. They will be able to distinguish between glucose non-repressed and glucose repressed growth and calculate the maximum dry biomass produced under anaerobic conditions from an initial glucose concentration of 10 g·L^{-1}. They will also be able to calculate the amount of ergosterol and oleic acid needed in the formation of one gram of yeast dry mass. *Equations 13-14* in the supplementary material.

Thought problems: Must ergosterol and oleic acid be supplied exogenously when a complex medium such as molasses or yeast extract, peptone (YP) is used? How are these molecules transported inside the cell? What role do these molecules play and why are they important? Would growth cease if these two compounds are not added? Based on the mass of a single yeast cell, calculate the number of yeast cells that are present in 1 g dry cell mass per L?

Learning outcomes for questions 10 & 11: Students will be able to explain what a maximum specific growth rate is and how it can be used to calculate the metabolic O_2 demand per hour. *Equations 15-16* in the supplementary material.

Thought problems: How is the maximum specific growth rate related to the doubling time? Would the metabolic O_2 demand be different between a prokaryote and a eukaryote, between different genera of yeasts, between aerobic and anaerobic conditions? From a biochemical point of view, is there a way to engineer cells with an increased affinity for O_2? What is the typical K_m of the enzyme involved in the ergosterol and oleic acid biosyntheses?

The various values that can be calculated related to O_2 are shown in red in figure 1 (see Supplementary information for detailed calculations). Students will notice that the O_2 demand of 55 $\mu mol \cdot h^{-1}$ is clearly not met by its supply of 2.5 $\mu mol \cdot h^{-1}$. Hence, to cultivate *S. cerevisiae* under ergosterol-free and oleic acid-free anaerobic conditions, an exogenous supply of O_2 is necessary. This is the reason why ergosterol and oleic acid are supplemented while performing anaerobic cultivations with *S. cerevisiae* in a defined medium.

Conclusions

We hope that our 'tips' article will improve the quantitative skills by providing a richer context (home-brewed beer and conducting anaerobic experiments without an anaerobic chamber, for instance) for students to engage in a stimulating discussion. As the results of the calculations are available in the accompanying material, both students and teachers can be involved in a fish-bowl exercise so that the intended object of learning can be lived (discerned) through connections at multiple levels (gas, liquid, yeast metabolism and rates) (Marton 2014). By invoking simple equations, students might feel more enthused and be less fearful in an unfamiliar situation.

This work demonstrates the significance of basic knowledge of chemistry and mathematics in addressing an important question in microbial physiology. Therefore, it constitutes a clear and beautiful example of the necessity of improving the level of numeracy skills and mathematics in the curricula of the Life Sciences courses. With the explosion of data in biological research, the future undergraduates must be well prepared to embrace this challenge. To this end, universities and funding agencies must encourage cross-fertilisation of disciplines, as 21st-century science teaching and research can no longer be conducted in isolation.

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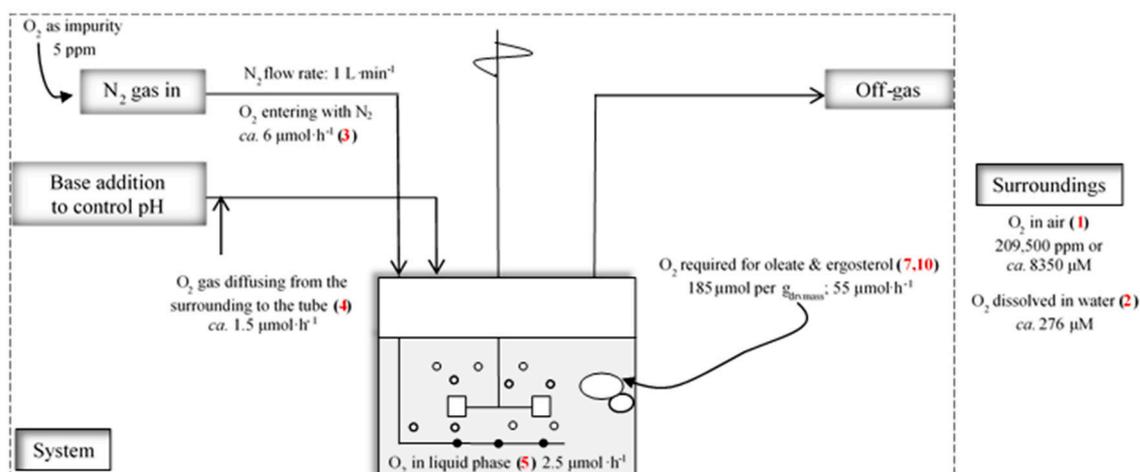
Figure 1. Set up of a typical laboratory anaerobic bioreactor. The bioreactor is usually sparged with N_2 gas containing < *5 ppm* O_2 throughout the course of the fermentation, to prevent O_2 from entering the system and to maintain an anoxic atmosphere. Tubing used for pumping the sugar solution and base constitute potential sources of entry for O_2. O_2 in numbers are indicated along with their question numbers mentioned in the text. O_2 concentration in the air (1) is an order of magnitude higher than in pure water, at 25 °C (2). O_2 as an impurity in N_2, enters the bioreactor (3). Some O_2 diffuse into the system due to imperfect sealing as well as through the tubing that dispenses base/acid to the reactor (4); the diffusion is calculated based on a tubing length of 30 cm while in real life, this value may be higher. All the gaseous phase O_2 is not readily available for use by yeast. It has to be dissolved in the liquid for the yeast to access and only a third of it actually dissolves in the liquid phase and this constitutes the supply (5). Once O_2 is in the liquid phase, it has to enter inside the yeast to synthesise the two compounds: ergosterol and oleic acid, whose biosynthesis require O_2 and this constitutes the the demand (7,10). As we are concerned with a flow system where yeast is multiplying, all the variables involve time. The change of a variable with time constitutes the rate and we have the following variables in our system: flow rate of N_2, O_2 difusion rate from the surrroundings, the rate at which the gaseous O_2 is transferred into the medium, the rate at which yeast multiplies and consumes O_2 for the biosynthesis of the two growth factors: oleate and ergosterol.