Point-of-use production of bio-ethylene oxide for medical sterilisation using a chemical looping approach

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Highlights

- Small-scale production of ethylene oxide (EO) with a concentration of 0.6vol%.
- *Bacillus subtilis* spores effectively inactivated with EO produced *via* chemical looping epoxidation.
- Point-of-use production of bio-EO demonstrated suitable for sterilisation

1. Introduction

Ethylene oxide (EO) is one of the most effective sterilising agents in healthcare, with roughly half of all medical devices (MD) in the U.S. being sterilised with EO [1]. Not only is EO bactericidal, sporicidal, and virucidal but also its efficacy remains unbeatable in mild and non-destructive conditions (<63°C, 40-80% relative humidity). Thus, EO is often the only acceptable sterilisation method for many delicate and complex MDs, such as endoscopes, pacemakers, and surgical meshes [1]. The hazard associated with EO makes handling, transportation, and storage both difficult and expensive. Consequently, sterilisation with EO might not be readily available to healthcare facilities in low-resource and rural settings where the reuse of MD is common practice. In such situations, reuse of MD without effective sterilisation often leads to the spread of healthcare-associated infections.

Industrially, EO is produced *via* the selective oxidation of ethylene, referred to as epoxidation, in which fossil-fuel-derived ethylene and gaseous oxygen are passed over a bed of $Ag/\alpha\text{-}Al_2O_3$ [2] – one of the highest CO_2-emitting processes in the chemical sector [3]. To prevent the formation of flammable mixtures, the process operates with low single-pass conversion and large volumes of ballast gas, necessitating significant downstream processing to obtain high-purity EO. Consequently, the incumbent method for EO production can only feasibly operate at a large scale.

We recently demonstrated a novel approach for small-scale and on-demand production of bio-EO from bio-ethanol [4] making use of a chemical looping epoxidation (CLE) approach. Instead of gaseous oxygen, the CLE approach provides oxygen to the epoxidation reaction from a mixed-metal oxide (strontium ferrite), termed an oxygen carrier. Using oxygen carriers eliminates the flammability hazards and downstream processing associated with EO production. Thus, the novel epoxidation method is ideal for the point-of-use production of EO, with an especially promising application of sterilisation of MDs.

Here we demonstrate such an application, utilising the CLE-produced EO to inactivate the vegetative and spore forms of *Bacillus subtilis* – one of the most resilient microbes and a standard organism used to assess sterilisation methods [5].

2. Methods

Strontium ferrite ($SrFeO_{3-\delta}$) was prepared by ball milling stoichiometric amounts of $SrCO_3$ and Fe_2O_3, followed by calcination at 1000°C for 12h. The resulting perovskite was impregnated with $AgNO_3$ solution, followed by calcination at 650°C for 5h, with a target loading of 15wt% Ag ($Ag/SrFeO_{3-\delta}$).

The production of EO was performed in a stainless-steel tube loaded with 4.00 g SiC (inert material) above 2.00 g $Ag/SrFeO_{3-\delta}$. The reactor tube was placed within a tubular furnace, operated at 295°C. The feed gas was introduced at the top of the reactor at 200 mL/min. The gases were altered in chemical looping (CL) cycles of: 1) 2 min purge in N_2; 2) 1.5 min reduction with 5vol% C_2H_4/ N_2; 3) 2 min purge in N_2; and 4) 15 min oxidation in 21±0.5vol% O_2/N_2 (all gases BOC). The composition of the gas at the outlet of the reactor was measured with an online Fourier Transform Infrared spectrometer (MKS), or gas chromatograph (Agilent, 7890A).

A 2 µL sample of *B. subtilis* spores (strain PY79) diluted to an optical density of OD_{600}=1 in water was deposited onto a glass cover slip, and placed within a desiccator to dry overnight. The spores were loaded into a 250 mL sample bottle connected to the outlet of the CLE reactor. The spores were exposed to the outlet gas (up to 6,000 ppm EO) for various contact times (4-24 h) and temperatures (20-50°C). Following exposure to EO, the bacterial samples were placed onto nutrient agar, kept in an incubation oven at 37°C, and imaged every 30 min with phase-contrast microscopy to monitor germination and outgrowth. Two samples were made using two slips with bacterial spores. The control experiment was performed by immediately introducing the spores to nutrient agar followed by imaging, with no exposure to EO.

3. Results and discussion

Ethylene oxide inactivates microbes *via* the alkylation of cellular constituents. Depending on the extent to which various cellular components are damaged, spores can be capable of germination, but are metabolically inactive, lacking the ability to grow and reproduce [6]. Thus, the reduction in outgrowth is a better indicator of inactivation. In the CLE experiments, a concentration of ~0.6vol% EO was produced and delivered to the sample bottle containing the *B. subtilis* spores (**Fig. 1a**). The fraction of spores that germinated and outgrew following 50 h of exposure to EO are compared to a control (**Fig. 1b** and **c**). The exposure to EO resulted in a 53.7±5.4% reduction in germination, and a 98.5±1.2% (~2-log) reduction in outgrowth. Whilst industrial sterilisation is commonly carried out with ~8vol% EO, regulating bodies, such as the FDA, have encouraged the use of lower the concentrations of EO, to both limit the environmental impact of the sterilising gas that is exhausted and to reduce the lengthy aeration times for removing residual EO [7]. In that context, our results indicate that low concentration sterilisation using bio-EO from CLE is a feasible and sustainable approach that would be safer to operate and reduce the risks of residual EO.

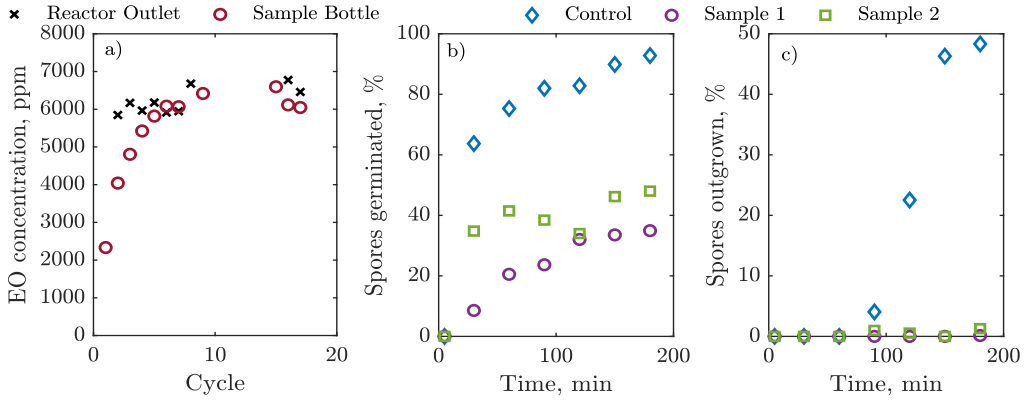

Figure 1. a) Concentration of EO within the sample bottle and at the outlet of the reactor tube, measured by GC. **b)** Fraction of *B. subtilis* spores that germinated, and **c)** outgrew over 180 min of monitoring.

4. Conclusions

Sterilisation of highly resilient *B. subtilis* spores was carried out with 0.6vol% EO produced by chemical looping epoxidation and a ~2-log reduction in viable microbes in comparison to the control was achieved. Our novel method for producing bio-EO holds promise for more sustainable, safer and accessible sterilisation, especially for healthcare facilities in low-resource and rural settings.

References

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Keywords
Ethylene oxide, sterilisation, sustainable development, epoxidation