NOxIOUS

Filtration of NO_x in the air via denitrifying bacteria

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Background

Air pollution is a global health risk (1)(2). Combustion of fossil fuels releases harmful gases into the air, including nitrogen oxides (NOx) such as NO2. NO2 is an irritant which causes a host of respiratory problems, particularly in vulnerable groups such as asthmatic children, and can in high concentrations lead to lung cancer (3). The concentration of NO2 is often highest near busy roads in urban areas and is often attributed to car engine combustion, however there is a growing body of evidence suggesting that household NO2 can also reach dangerously high levels which can cause respiratory distress (4)(5).

NO2 is generated in homes and professional kitchens primarily by gas stoves. In conjunction with improved insulation technologies and lack of adequate ventilation, this can lead to a build-up of dangerous NOx levels in buildings. Current NOx detectors and filtration equipment/ units on the market available to consumers are large, unsightly and very expensive. The NOxIOUS device is a low-cost biological solution to this growing problem.

Solution and Implementation

Our proposed solution is to create a device that contains bacteria that can breakdown NO_x in the air and produce harmless N_2. *E. coli* was chosen as the chassis due to it being highly characterised with a wide variety of toolkits available for genomic integration. It is also a non-pathogenic class-II strain which is vital for implementation in the home as well as on a wider industrial scale. The synthetic circuits being implemented are from the gram-negative bacteria *R. denitrificans*, some of which are membrane proteins, it is therefore important that the chassis is also gram-negative.

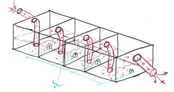
Two different chassis will be used for the denitrification pathway this will bring many benefits to the bacteria. Firstly, it will lighten the metabolic load on the bacteria and reduce the amount of genetic information that needs to integrated into the bacterial chromosome. It will also allow the implementation of a codependent killswitch. A third chassis will then be used for the biosensor application of the device to further lower metabolic load.

Due to long term applications of the device, a plasmid based system is not viable due to its inherent instability as well antibiotic control not being possible. Genomic integration is therefore best, as is it more stable and allows for greater control of copy number.

The device (*a*) itself acts as a physical barrier between the user and the bacteria. Using an ultrafiltrate membrane ensures air and nutrients are able to be pumped in and bacteria are unable to breach containment. The device consists of multiple units (*b*) containing *E. coli* in nutrient-rich LB; the air is bubbled into each subsequently, both reducing the steady-state concentrations of the NO_x by increasing the conversion of the intermediates and facilitating the growth of the *E. coli* by agitating them. The biosensing bacteria are kept in a separate compartment to the denitrification bacteria that is partially transparent.

(*a*) – Solidworks model

(*b*) – sketch of compartments



Regulation and RRI

The proposed device will be implemented in a public space, potentially in the household. As such it is of the utmost importance that the device is compliant with any regulations and is as safe as possible. The project used the Health and Safety Executive's (HSE) Genetically Modified Organisms (Contained Use) Regulations (2014)(6) to guide the design of the project. The regulations stipulate the need for a physical, biological and/or chemical barrier between the GMO and the user – from the outset the device was designed to isolate the GMO from the user via a membrane (physical barrier) and possesses a co-dependence kill switch (biological barrier) that ensures that the bacteria cannot live outside of the device; additionally there is mechanical switch that releases antibacterial chemicals to allow destruction of the population, which also acts as the device's end-of-life procedure. The aforementioned regulations require a thorough risk assessment to be provided to the HSE – the key risks identified were the release of the bacteria or the release of dangerous intermediate molecules; the latter is mitigated by the aforementioned barriers and the former is mitigated by the use of an active air pump to prevent back-diffusion and a multistage system to substantially reduce the steady state outputs of the intermediates. Dual use is a risk that has been accounted for as well – there is no inherent harmful use for denitrifying bacteria, and although N_2O, which has illegal recreational uses, is an intermediate, the intermediates cannot be harvested.

Design and Modelling

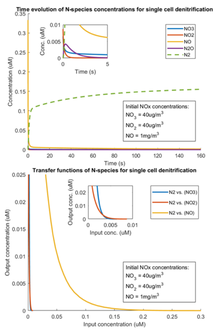
Denitrification Pathway

The denitrification process is based on the denitrification pathway (*c*) occurring in bacteria such as *R. sphaeroides* (7) (8). It includes four nitrogen oxides-reducing enzymes (nap, nirS, norCB, nosZ) under the control of PyeaR promoter. In the absence of nitrogen oxides, the promoter is sequestered by the Nar repressor and NsrR repressor. NO3 and NO2 can sequester the Nar repressor, while NO can sequester the NsrR repressor. This enables RNA polymerase to bind to the PyeaR promoter and initiate the expression of nitrogen-oxides reducing enzymes.(*d*)

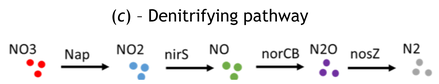
The circuit was assembled on two plasmids, exploiting orthogonality of NO binding to NsrR and NO3 and NO2 binding to Nar repressor. Due to circuit size, single-plasmid assembly was not feasible.

Single-cell modelling demonstrates that the circuit is able to convert maximum allowable air limits of the nitrogen oxides (9) to inert nitrogen if the device contains approximately 1010 cells (0.1L - 1.0L), corresponding to the reasonable volume suitable for households. Modelling was done using MATLAB Simbiology module, where chemical reactions were represented by a system of non-linear ODEs with parameters estimated from (10)(11)(12)(13)(14). (*e*,*f*)

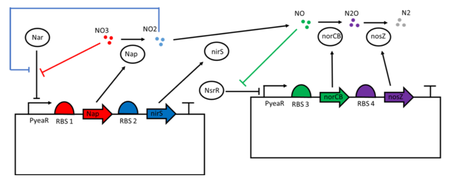
(*e* & *f*) – Concentration time plots + transfer functions



(*c*) – Denitrifying pathway



(*d*) – Biofiltration circuit

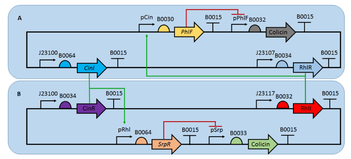


Biological Kill Switch

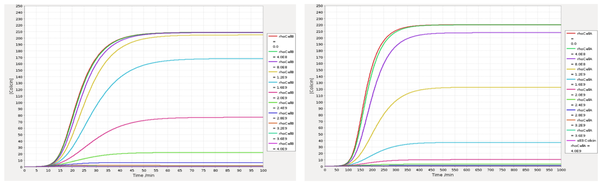
The kill switch for the organisms in this device is based on the idea of mutual co-dependence of two strains of bacteria. Each strain expresses a different signal molecule (C4-HSL in one, and C14-HSL in the other), which it releases into its environment. The survival of the bacteria is dependent on the presence of the signalling molecule from the other strain. This is achieved by using the signalling molecule to facilitate transcription of a repressor molecule, which in turn represses the transcription of a protein that induces cell death - colicin. Therefore, without the presence of the signal molecule, colicin is expressed, and the cell dies.

The kill switch was modelled using CellDesigner (*g*), and parts were chosen from the iGEM registry. C4-HSL and C14-HSL were chosen as signal molecules due to their high degree of orthogonality, and good response characteristics of their associated promoters. The repressors, PhlF and SrpR, were chosen due to their well-characterised behaviours (Glasgow iGEM 2015) and their orthogonality (Stanton et al, 2014. The promoter and RBS parts were chosen from the iGEM registry. The model was used to select parts of specific strengths such that the system is at equilibrium when both bacterial strains have a concentration of $4*10^8$ cells/ml. Colicin is fatal to E. coli cells when in a concentration of 14-200 copies per cell, and so parts were chosen to cause expression below 14 copies at the desired cell concentration, and over 200 at 30% of the desired concentration in both strains to ensure proper functioning of the kill switch. The colicin expression in both strains is highly nonlinear, as desired.(*h*,*i*)

(*g*) – Biological kill switch circuit



(*h*,*i*) – Toxin production over time



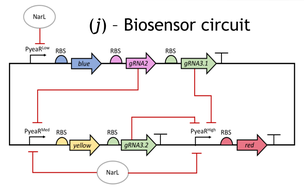
Semi-Quantitative Biosensor

The filtering device would also contain a biosensor strain of *E. coli* bacteria in a separate compartment. They would contain a biosensor circuit which would produce different coloured outputs as a response to increasing concentrations of NOx gases, giving the user an indication of NOx concentration in the air.

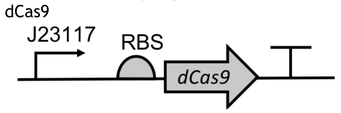
The circuit is made of three parts, where each is controlled by a NOx-responsive promoter that is activated at increasing NOx concentrations. (*j*) This would be achieved by engineered evolution of the PyeaR promoter to have a higher and a lower affinity for the NarL NOx-sensitive repressor. Using such concentration-sensitive promoters will result in expression of blue, yellow, and red chromoprotein at low, intermediate, and high NOx concentrations respectively; using CRISPRi, guide RNAs and constitutively expressed dCas9 (*k*) will silence expression of chromoproteins that would indicate higher NOx concentrations than are present at that time. (*l*,*m*,*n*)

In the model, varying affinities of inhibitor for PyeaR promoters modelled by Hill equations with increasing Km values. For rates of transcription, translation, and degradation, general values for *E. coli* were used.

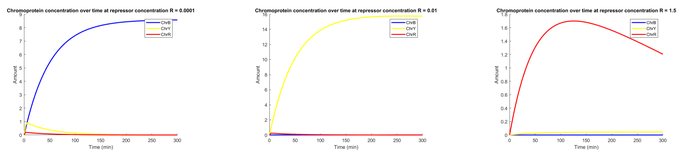
(*j*) – Biosensor circuit



(*k*) – Constitutively expressed dCas9



(*l*,*m* & *n*) – Chromoprotein concentrations at different NO_2 levels



Future Improvements

If the project is taken forward, detailed modelling and lab experiments would be conducted to determine optimal RBS sequences to achieve necessary rates for each biosensor component.

Another potential improvement would be the addition of an antitoxin to the biological kill switch to make the population more robust and less likely to spontaneously die as a result of stochastic processes, such as natural local concentration variance or leaky repressors.

In the future, we hope to up-scale our device for use outside in larger boxes/vats, attached to buildings in cities. We hope the success of our consumer-marketed device will lead to government endorsement and enable widespread implementation of the NOxIOUS solution.

Acknowledgements and References

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