Customer Needs Statement

Autonomous Benchtop Bioreactor for the New UF Biofoundry

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Overview

Industry 4.0 advances manufacturing by interlinking emerging technologies including robotics, biotechnology, and the internet of things through machine-to-machine communication. Coined by Klaus Schwab[1], Industry 4.0 portends a new era in fabrication enabled by smart machines that manufacture products without human intervention. Raw materials go in, and finished products come out on-demand and with no user involvement.

The University of Florida Synthetic Biology Consortium is creating an Industry 4.0 UF Biofoundry. To initiate this Biofoundry, the Consortium seeks an autonomous bioreactor with coupled liquid handling capacity that can manufacture and interrogate biological samples in larger volumes at lower cost than current commercial alternatives. Successful designs must integrate at least five existing capabilities into one autonomous unit: (1) well plate / conical tube fluid handling; (2) sample shakers; (3) cell incubators; (4) optical metabolic sensors and diagnostics; and (5) operation support features such as environmental control and waste disposal.

Following an Industry 4.0 approach, separately engineered raw materials like bacteria or yeast will go into the device, and with no human intervention, these will grow to produce substantial amounts of well-monitored microbial culture. Ultimate goals for the biofoundry include producing products like biofuels, biopolymers, pharmaceuticals, and even edible biomass.

As mechanical engineers, you will design only the smart device (leaving programming the biological cells and generating products their cultures synthesize to the Consortium). Commercial microplate readers can monitor cultures, but the Consortium requires several improvements, including: (1) a wider working volume range [e.g., from 20μL to 20mL]; (2) a wider culture condition control [e.g., atmospheric gas control, temperature control, and shaking]; and (3) full automation [e.g., automated transfer of liquid from one well in a plate to another, from one conical tube to another, or between plates and conical tubes]. The proposed system will have three key functions:

1. *Cell Culturing:* the device must culture microbes in compartments or vessels that are fully enclosed, easily interchangeable, and allow for media exchange.
2. *Liquid Handling:* the device must move cultures suspended in liquid between compartments or vessels, provide nutrients in media to cultures, dispose of waste, and sterilize itself.
3. *Culture Monitoring:* the device must continuously measure its internal temperature and pressure as well as culture optical density (OD) and fluorescent intensity (FI). The device must

[1] K Schwab, The Fourth Industrial Revolution, Currency, Jan. 2017.

report cell culture condition and metabolic data to the user and for culture condition closed loop control.

Known challenges to overcome include: (1) eliminating contamination if inter-well exchange introduces foreign materials; and (2) satisfactory liquid handling while eliminating exposure to the local atmosphere/environment.

General Desired Features

1. Operational lifetime of at least 10 years
2. Prototype production cost does not exceed $10,000
3. Moveable by one person after disassembly
4. Fits on a research benchtop, taking into consideration:
    a. Clearance through doors (when assembled)
    b. Benchtop loadbearing capacity
    c. Benchtop footprint
5. Runs from a single standard 120 VAC wall outlet
6. Has an easily accessible interior for cleaning
7. Includes an easily actuated emergency shut-off that safely that stops all functions
8. Has an intuitive user interface
9. Has a visual indicator that is easily seen by the user and nearby personnel, and that shows:
    a. System on/off
    b. Process mode, elapsed process time, and remaining process time
    c. If an error occurred
10. Is programmable: control parameters can be changed by the user, and more complex processes can be added
11. Only nonporous materials contact cell cultures
12. Only nonreactive materials contact lab chemicals
13. Appropriate for operation in a BSL-2 space
14. Has an exterior surface that is not too hot to comfortably touch
15. Is capable of sequestering and neutralizing its own liquid and solid waste

Microbiological Requirements

16. Cultures microbes in fully enclosed compartments or vessels that are interchangeable
17. Maintains environmental conditions independently for each well plate or tube
18. Is capable of safely injecting, measuring, and regulating the composition of the following gases in each compartment holding a well plate or tube: N_2, O_2, CO_2, CH_4, H_2
19. Is capable of incubation periods long enough to permit 1,000 *E. coli* culture generations (~two weeks)
20. Maintains cultures in a well plate or tube at a constant temperature within a range from 4°C to 70°C

21. Maintains internal setpoint temperature with time and spatial variation less than ±2.5°C
22. Uniformly heats/cools wells/tubes within the desired temperature range
23. Mitigates condensation on cooled well plate and tube surfaces
24. Reaches setpoint culture temperature in less than 15 minutes
25. Accommodates existing culture well plates of the following sizes: 6, 24, 48, 96, deep 96, 384
26. Accommodates existing conical tubes of the following sizes: 15mL & 50mL
27. Includes a photobioreactor mode to illuminate photosynthesis-capable cell cultures (e.g., cyanobacteria) with white light
28. Shakes well plates and tubes in Linear, Orbital, and Double Orbital patterns
29. Has shaking patterns that are independent for each well plate or tube

Measurements Required

30. Measures optical density (OD) in all individual wells and conical tubes[2]
31. Measures fluorescent intensity (FI) in all individual wells and conical tubes
32. For OD/FI measurement, sustains adequate light intensity to make measurements at wavelengths not lethal to cells[3]
33. Processes a 384 well plate through OD/FI measurements in less than 6.5 minutes

Liquid Handling

34. Is capable of automated liquid handling with fluid addition/subtraction from each well or tube
35. Dispenses fluid without creating aerosols
36. Achieves dispense rates from 225 uL/s to 300 uL/s
37. Deposits a minimum/maximum aliquot fluid volume from 5–20,000 µL
38. Achieves dispensing volume accuracy of ±0.1 µL
39. Achieves dispensing volume precision ±0.01 µL
40. No cross contamination between individual wells/tubes during liquid handling

[2] https://bitesizebio.com/41100/is-your-bacterial-culture-still-growing/
[3] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4611486/