JOVE Jan. 24, 2013

17 Sellers Street

Cambridge, MA 02139

Dear Dr. Kinahan:

Thank you for the opportunity to resubmit the article “'Operation and Maintenance of a Benchtop Bioreactor (JoVE50582)” for consideration to be published in the Journal of Visualized Experiments. The authors have added our responses to the editorial and reviewer comments below.

Editorial comments:  
\* Please re-write your protocol section in imperative tense, as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.) For example, "The vessel and headplate must be cleaned" should be re-written as "Clean the vessel and headplate."

**Author Response: Corrected as recommended**  
\* After being reformatted to comply with JoVE specifications (font size, spacing) your protocol exceeds our 3 page limit. Many short steps could possibly be combined to fit within 3 pages. Please remember to leave a space between each individual numbered step. Alternatively, instead of re-writing the protocol you may select which steps you would like to film and highlight these steps (up to 2.75 pages). Steps that you choose not to film will remain in the written protocol and can be referred to in the video. Lists of equipment, notes or asides that won't be filmed should not be highlighted. Please either shorten OR highlight your protocol.

**Author Response: Corrected as recommended.**  
\* Please update your figure captions. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description.

**Author Response: Corrected as recommended**.   
  
\* Please revise your discussion section. The discussion should cover the following in detail: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

**Author Response: Discussion was rewritten to include the recommended topics.**  
\* JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Please remove all commercial sounding language from your manuscript. For example, the Wave from GE healthcare. If you would like to refer to alternative equipment, please do so more generally.

**Author Response: Company brand names have been removed from the text and replaced with generic descriptions of the other Bioprocessing technologies.**  
Please modify your references section to comply with JoVE instructions for authors. Examples of how to cite various sources can be found in the instructions. Please ensure that each listed reference is cited within the manuscript text.

**Author Comments: Corrected as recommended.**   
  
Reviewer #1 Comments:   
*Summary:*   
In this article, the authors describe the operation and maintenance of a benchtop bioreactor. This article will be very useful for scientists who would like to use this technology. The article clearly lays out the rationale, and the step by step protocol for operation of the instrument. This reviewer found this article to be catering to the needs of both the novice and experts in fermentation technology.  
  
*Major Concerns:*  
Some major comments: There is no description of post operational harvest and processing of the culture and clean up and maintenance of the instrument between runs.

**Author Response: An addition step (#46) was added to describe the method for harvesting and decontaminating the bioreactor vessel contents post run.**The title clearly states the maintenance part but is not dealt with in the article.

**Author Response: Yes, we agree that maintenance was not addressed, therefore, the title should be edited to be “Operation of a Benchtop Bioreactor”**  
The type of fermentor depicted in figure 1 and manufacturer are not spelled out. It would be useful for some who would like to adopt this work. The same is true for many other parts and reagents used in this instrument. It is usual practice to mention the manufacturer and location and in some cases part/ catalog number so that the reader can benefit from that information unless it is the policy of this journal not to furnish such information. Also, the manuscript can benefit if colloquial terms are avoided. Some examples are given below.

**Author Response: The manufacturer and reagent information is included in the tables and not in the narrative as required by JoVE.**  
*Minor Concerns:*  
Abstract:   
1) Line 1: I would change "Fermentation systems have been used" to "Fermentation systems are used"  
2) The viewer will be familiarized with the sterilization process and shown how to inoculate vessel with culture- you do not inoculate the vessel but the medium in the vessel. I suggest changing it to "how to inoculate the growth medium in the vessel"  
3) Basic concepts of operation, sampling and harvesting will also be shown.. will be demonstrated sounds better.  
  
Long abstract: Line 13: Their ability to carefully control..may want to change to "the ability afforded by fermentors to carefully control"   
Line 19: how to inoculate vessel- see comment above  
Line 23: ..is an extension of the simple shake flask. Suggestion: is an extension of the simple shake flask technique for growing cultures.  
Line 34: "consume other chemicals"- suggestion "consume other metabolites"  
Line 44: this sentence is unclear; do you mean to say" feedback control of heating and cooling of this jacket by a sensor inserted into the vessel usually results in temperature control +/- 0.1C around setpoint?  
Line 48: "addition of air" suggestion: "infusion of air"  
Line 51: "not normally available to growth" suggestion: "not normally available for use in shake flask cultures"  
Line 61: "and precise than with a shake flask" Suggestion: "and precise than with a shake flask method"  
Line 67: Would help if brand name, company and location of the company are added in parentheses after the "standard vessel"  
Line 79: "gas addition" suggestion "gas infusion or pumping"  
Line 96: Please add the usual vessel volume and the working volume. For example for a 5 or 10 lit vessel, what is the working volume?  
Line 148: Modify: Autoclave the vessel for 25 to minutes at 121 C, liquid cycle(add the pressure info).   
Line 148-149: the vessel does not come out of the autoclave. Change the sentence to something like:   
Be careful for the vessel will be very hot when removing from the autoclave.  
Line 162: Remove "Put" suggestion "install"  
Line 209: Specify inoculum: bacteria or cell culture and at what concentration: overnight? (CFU/ml) and volume?  
Line 225: change to "Sampling interval is culture dependent"  
Line 231: Refer to figure 1 first somewhere in the text prior to Figure 2. Figure 1 is not mentioned anywhere.  
Line 241: adding a caption or text box above the lines- of what each line represents in the figure would help.   
Line 248: It may also have an important role in development of individualized medicine: in what way?  
Line 259: that mixing occurs due to a rocking motion. "to" is missing

**Author Response: The revised text has incorporated the suggested edits.**

References: Only 4 references are quoted in the body of the manuscript. What about references 5-12. Where are they referenced in the text?

**Author Response: Additional references added and referenced in the text.**  
Figures: Please add a text box above the lines in the graphs to indicate what that line represents, pH or DOC etc..Probably the color legend in the bottom of graph indicates it. But it is not obvious.

**Author Response: Figure legends have been edited to better explain the data presented.**  
Table1: add manufacturer and location of the manufacturer

**Author Response: NA**  
Reviewer #2:   
*Minor Concerns:*  
Discussion might include some insight or direction into how to establish the initial bioreactor conditions, since shake flask growth is not a predictor of successful production in the bioreactor.  
  
**Author Response: Additional information provided as recommended.**

Reviewer #3:   
  
*Minor Concerns:*  
Missing seems to be any discussion regarding possible trouble spots and how to best troubleshoot common problems. Obviously, contamination could be a major issue, and while precautions are listed to avoid contamination, no discussion of how best to detect contamination and resolve it should it become a problem are discussed. Also missing seems to be a discussion on the best means for harvesting the culture.

**Author Response: Additional information provided as recommended.**

Regards

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