Rapid prototyping of a single-use bioreactor: conceptional design studies to final product

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Abstract

Driven by market adoption and evolution, Thermo Fisher Scientific has recently focused on expanding its single-use SmartVessel benchtop bioreactor family (originally commercialized by Finesse Solutions). Based on fluid dynamic investigations that were conducted during early design studies, prototypes of a new single-use bioreactor were built by using different rapid prototyping techniques, including stereolithography (SLA), direct deposition modelling (DPM) and urethane casting (UC). Finding materials suitable for use in a cGMP environment, in combination with an appropriate manufacturing method, is challenging. Despite the large variety of available plastic materials, the polymers must meet special requirements, including the absence of animal-component derived material, Bisphenol A, latex, phthalates, and they must also be free of cytotoxic and carcinogenic components. Ideally the materials that meet the aforementioned criteria are also gamma irradiation compatible to levels in excess of 40 kGy.

This poster is based on a case study, and provides some insights into the bioreactor development process, from conceptional designs to a final product. In addition, the final design's engineering characteristics, with respect to animal cell culture applications, are presented for the first time.

Computational modelling for bioreactor development

Computational Fluid Dynamics (CFD) is a powerful tool to use in the early phases of bioreactor development. It provides insights into the fluid flow, mixing and shear, without a need for physical prototypes [1]. Due to the low aeration rates used in typical cell cultures, single-phase models are often sufficient for the prediction of steady-state flow patterns, which are usually well-predicted in modern CFD codes. The commercially available Fluent CFD package (ANSYS, v17.0) was used for this case study. It provides the different turbulence models required to solve the RANS approach. The agitator rotation was described by using the Multiple-Reference-Frame method (MRF).

Additive manufacturing used for prototyping

Over the last four decades, a variety of rapid prototyping techniques have been developed to enable rapid, cost-effective prototype manufacturing, based on 3D CAD models [2]. The most important for the current project were:

- Stereolithography (SLA); A photopolymer resin is polymerized and cured in a layer-upon-layer process.
- Fused deposition modeling (FDM); A thermoplastic is melted and deposited through a heated nozzle extruder before it solidifies during cooling.
- Urethane casting (UC); A thermoplastic is injection molded, using a silicone mold that was created based on a master pattern, which is made by subtractive and/or additive manufacturing (e.g. 3D printing).

The prototypes shown in Figure 2 were used for engineering characterization, including the measurement of the (oxygen) mass transfer, the mixing time and the power input. These experiments were realized in accordance with the DECHEMA guidelines reported by ref. [3]. Briefly, the methods can be summarized as:

- Oxygen mass transfer: The PBS model media was oxygen saturated after complete oxygen removal by nitrogen stripping. The k_La value was obtained from the slope of the linearized saturation profile.
- Mixing time: The duration required to achieve 95% homogeneity after rapid addition of 3M KCl solution was determined. The conductivity change was measured with a single-use conductivity probe installed close to the vessel bottom.
- Power input: The torque required during the agitator rotation was measured with a torque transducer (T20WN from HBM, Germany) that was attached to the agitator drive. An air-bearing was used to reduce the friction during rotation.

Table 1. Summary of vessel specifications

Characteristic	Description
Total volume	13.5 L
Max. working volume	10.0 L
Min. working volume	2.0 L
Vessel inner diameter	220–230 mm
Impeller diameter	92 mm
Baffles	Four (4), wall-mounted
Sparger	Ring sparger, 30x1 mm holes Micro sparger, sintered PE
Impellers	Modified Rushton turbine & Segment blade impeller
Sensors	TruFluor pH & DO with integrated temperature measurement

Results

- CFD models can effectively reduce the required number of physical prototypes (30+ geometry candidates vs. three physical models), which results in significant time and cost savings.
- The product development workflow is modified from more traditional approaches by using rapid prototyping techniques. This ultimately leads to lower risk of mold design failures (associated costs of ~0.6 Mio USD in present project).
- Additive manufacturing techniques are capable of producing prototypes with complex geometries.

Figure 1. Flow chart working steps in case study

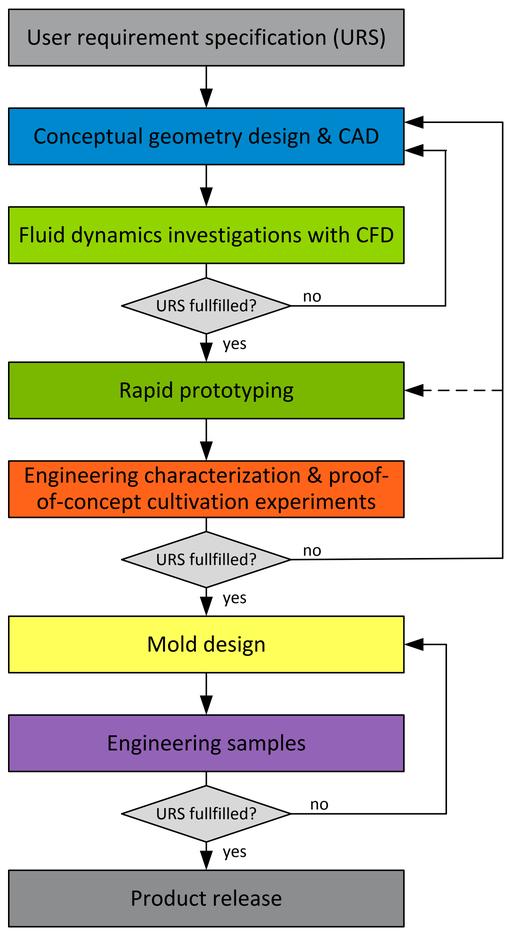
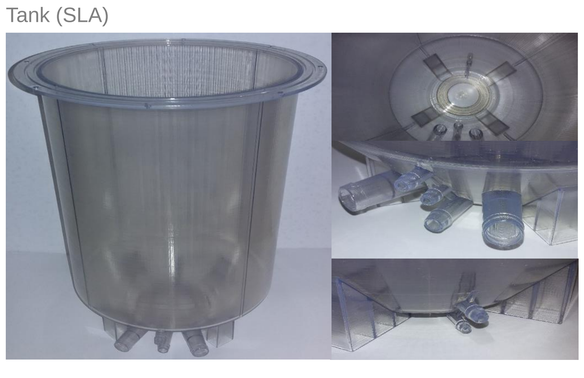
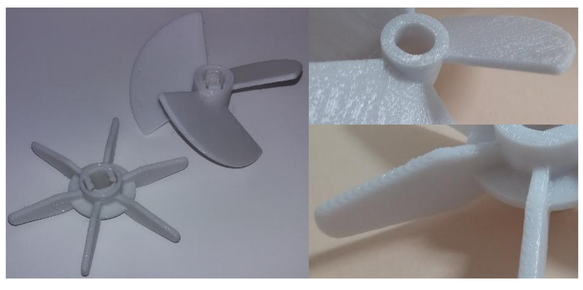


Figure 2. Photos of bioreactor parts that have been manufactured using different rapid prototyping techniques

Tank (SLA)

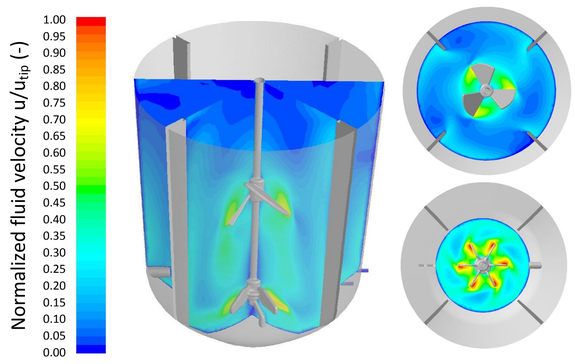


Impeller (FDM)



Head plate and ring sparger (UC)

Figure 3. CFD predicted flow in a baffled bioreactor prototype with multi-stage impellers



- Prototypes were successfully used for engineering characterization in the scope of cell culture applications.
- k_La values were between 5 h^{-1} and 24.2 h^{-1}, depending on sparger design, impeller speed and aeration rate (sufficient for medium to high oxygen demands), see Figure 4.
- Good gas dispersion by the radial flow impeller for homogenous bubble distribution was found (data not shown).
- Mixing times <15 s at moderate agitation indicated good mixing (see Figure 5). No zones of poor mixing were identified.
- Power numbers between 1.2 and 2.5 (see Figure 6) are in comparable range to other bioreactors from the single-use SmartVessel and autoclavable SmartGlass families (data not shown).
- Using a silicone-based heating blanket, temperature control is possible, but temperature distribution needs to be improved (see Figure 7).
- Prototypes were stable for gamma sterilization.

Figure 4. Results of oxygen mass transfer experiments

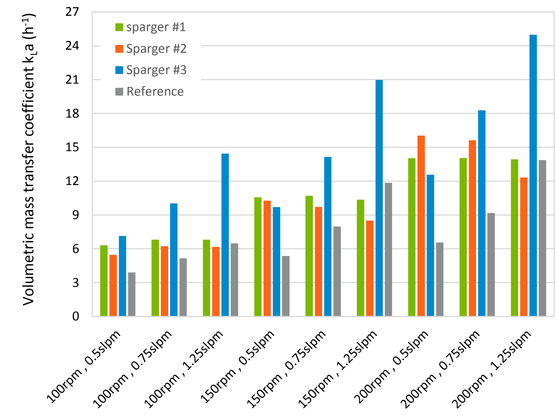


Figure 5. Results of mixing studies

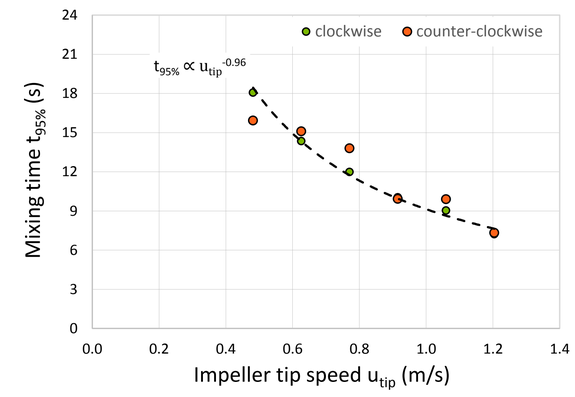


Figure 6. Results of power input measurements

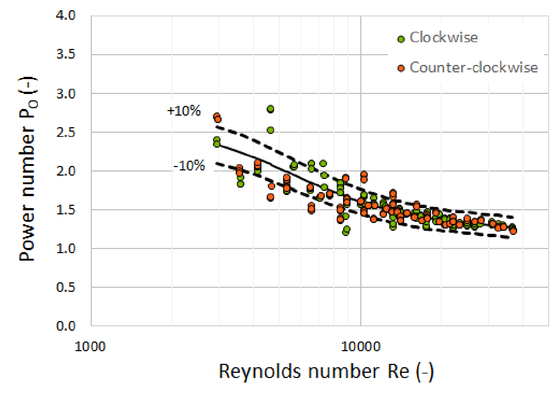


Figure 7. Results of the temperature control with a heating blanket installed around the prototype

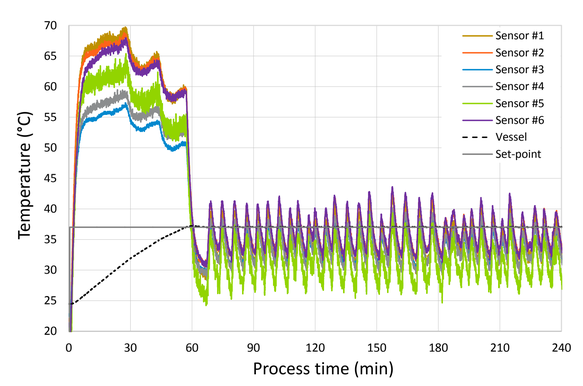


Figure 8. Picture of a final prototype made by injection molding that is placed in a vessel stand for operations

Conclusions

This case study shows the potential for rapid prototyping techniques in bioreactor development, whereas it is not (yet) suitable for mass production.

- Selection from the wide range of available materials must be considered in combination with the manufacturing technique used. The first FDA approved materials meet ISO class VI standards for use in GMP environments.
- There are many suppliers currently providing rapid prototyping services, offering a wide range of techniques and capacities.
- Most materials are stable for gamma sterilization, which is a requirement for sterile cell cultivations.
- The bioreactor prototypes passed the URS evaluation, and the prototype-based injection mold design was finalized (see Figure 8).

Future studies

- Testing of prototypes in animal cell culture applications and optimization of the temperature control are on-going.
- Further testing of long term stability of 3D printing plastics is required.

References

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2. Mohsen Attaran (2017). The rise of 3-D printing: The advantages of additive manufacturing over traditional manufacturing. Business Horizons 60, 677-688.
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