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Characterization of Oxygen Mass Transfer Rate (kLa) Using a Statistical Design of Experiment (DoE) for Bioreactor Scale-Up

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Abstract

Bioreactor production development and optimization are typically performed at a small scale to reduce their cost and time requirement. Bioreactor production is scaled-up often based on keeping volumetric mass transfer coefficient (kLa) constant. To facilitate a successful transfer of an optimized small-scale cell culture bioreactor production to lab, pilot, and industrial scale, the kLa profiles for these bioreactors should be determined. Although a number of correlations and predictive models for kLa are available, they do not have universal applicability.

The purpose of this study was to use a statistical Design of Experiments (DoE) approach to characterize kLa in 3L, 7L, and 50L bioreactors, and hence to use this information for bioreactor scale-up. Dissolved oxygen sensors were used to measure dissolved oxygen concentrations in the bioreactors. kLa was determined by using the dynamic re-oxygenation method. Response surface design of experiment was used to characterize kLa in the bioreactors. Agitation, air flow rate, and volume were included in the experimental design as input parameters, and kLa was used as the output parameter. Results of these studies is discussed in this presentation. The correlations determined here will be used as a tool for bioreactor production development, scale up and tech transfer.

Experimental Procedures and Data analysis

1. kLa was determined with water in different scales of bioreactor using the dynamic oxygen absorption method:

$$\ln(C^* - C_L) = -k_L a.t$$

2. Clark-type polarographic electrode was used for sensing the dissolved oxygen concentrations in liquid samples.
3. The 16-18-run Response Surface design were applied to kLa for identifying the effects of **volume**, **agitation,** and **air flow rate** on the kLa.
4. The design includes 3 replicates of the central point to determine the experimental error.

Experimental Designs for k=3 input Parameters

- **Input parameters :** Volume (L), agitation (rpm), and air flow rate (LPM)
- **Output parameter:** kLa (1/hr)
- **Response Surface Design:** The RSD had a total of 16-18 runs including 2-4 center points.

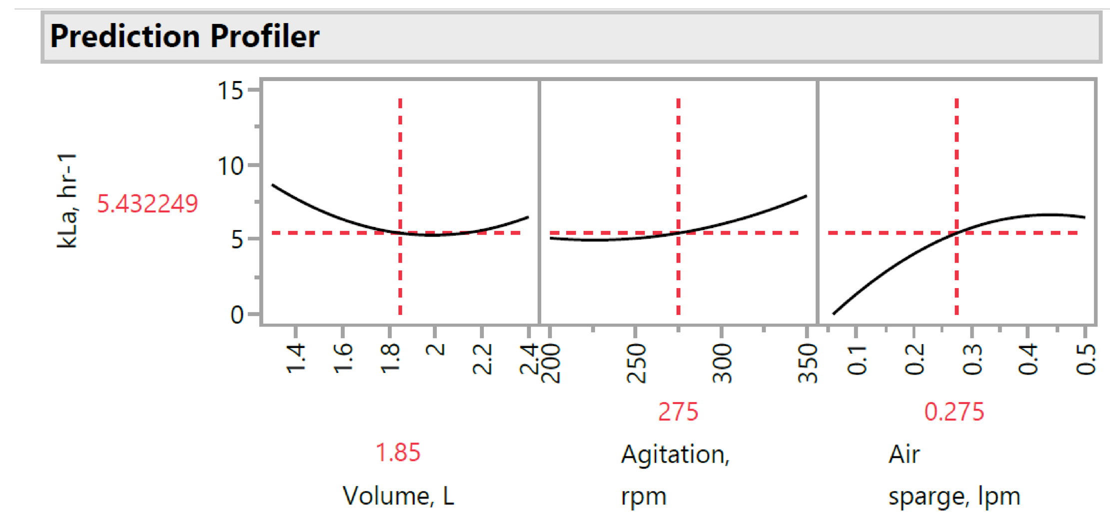
Experimental Design Results

1. 3L Response Surface Design

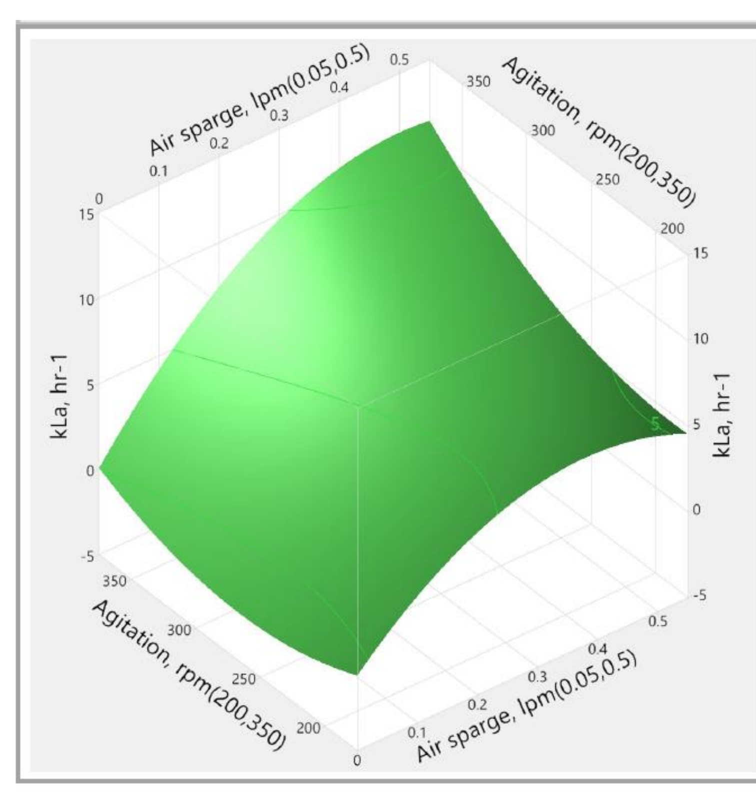
Sorted Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Air sparge, lpm(0.05,0.5)	3.3854118	0.237282	14.27	<.0001*
Agitation, rpm(200,350)	1.4160882	0.237282	5.97	0.0003*
Volume, L(1.3,2.4)	-1.084176	0.237282	-4.57	0.0018*
Agitation, rpm*Air sparge, lpm	0.947975	0.244584	3.88	0.0047*
Volume, L*Air sparge, lpm	-0.46485	0.244584	-1.90	0.0939
Air sparge, lpm*Air sparge, lpm	-2.335796	1.601744	-1.46	0.1829
Volume, L*Volume, L	2.1654044	1.601744	1.35	0.2134
Agitation, rpm*Agitation, rpm	1.0836044	1.601744	0.68	0.5178
Volume, L*Agitation, rpm	0.1561	0.244584	0.64	0.5412

Prediction Profiler



kLa Surface Profiler for 3L



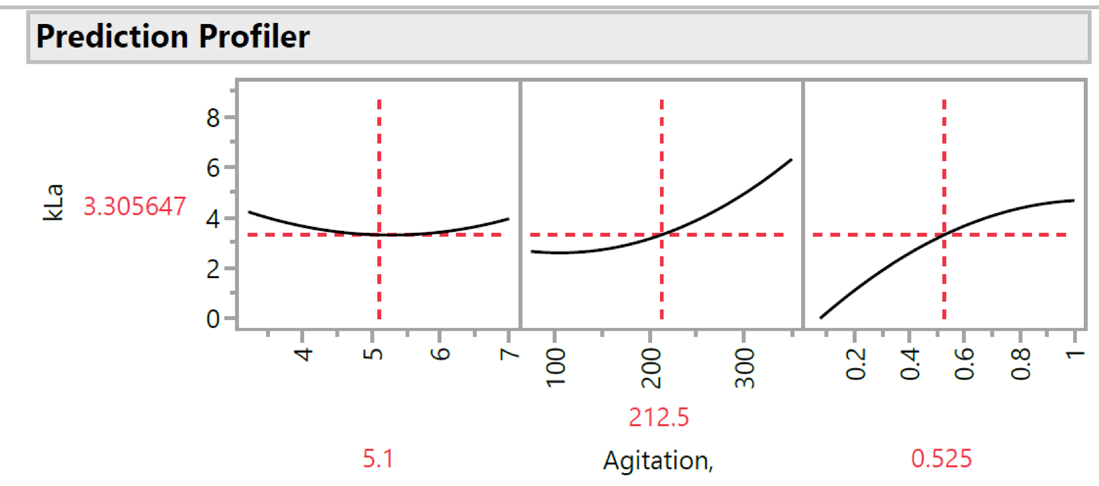
Summary: Air rate, agitation, volume, and agitation and air rate interaction are statistically significant.

2. 7L Response Surface Design

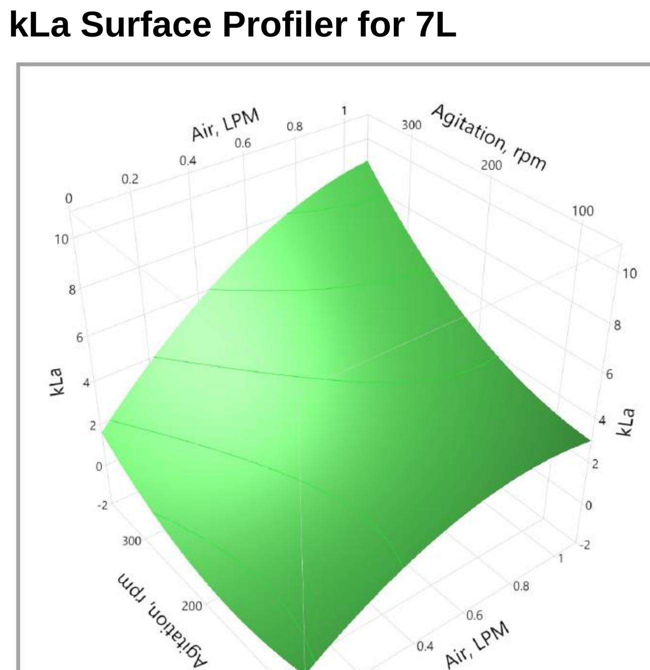
Sorted Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Air, LPM	4.2731042	0.378023	11.30	<.0001*
Agitation, rpm	0.010031	0.001621	6.19	0.0001*
(Agitation, rpm-181.25)*(Air, LPM-0.5745)	0.0127572	0.003977	3.21	0.0094*
(Agitation, rpm-181.25)*(Agitation, rpm-181.25)	6.1979e-5	2.527e-5	2.45	0.0341*
(Air, LPM-0.5745)*(Air, LPM-0.5745)	-4.808919	2.424616	-1.98	0.0754
(Volume, L-5.095)*(Volume, L-5.095)	0.2152952	0.111586	1.93	0.0825
(Volume, L-5.095)*(Agitation, rpm-181.25)	-0.001385	0.000981	-1.41	0.1884
(Volume, L-5.095)*(Air, LPM-0.5745)	0.1862177	0.198277	0.94	0.3698
Volume, L	-0.022291	0.08828	-0.25	0.8058

Prediction Profiler



kLa Surface Profiler for 7L



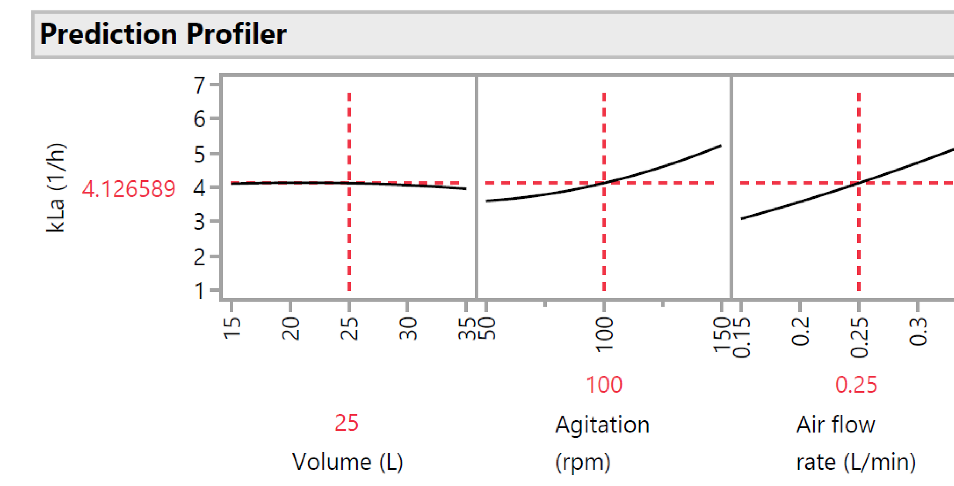
Summary: Air rate, agitation, agitation/air interaction, and agitation square terms are statistically significant.

3. 50L Response Surface Design

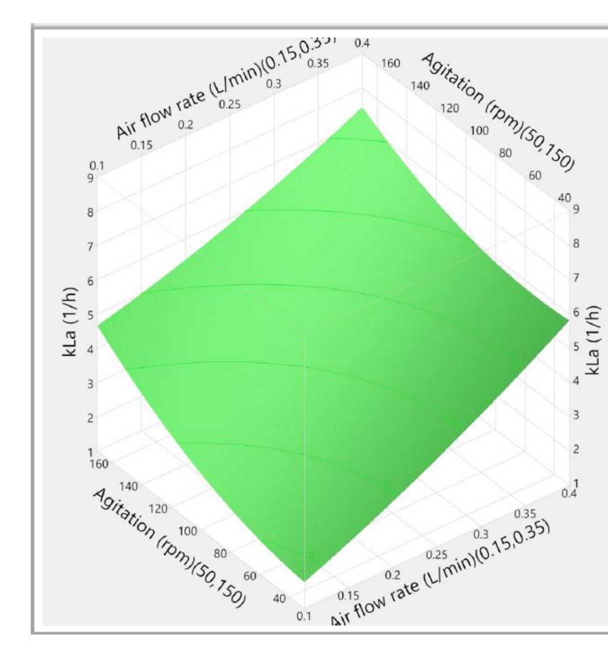
Sorted Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Air flow rate (L/min)(0.15,0.35)	1.1405145	0.246687	4.62	0.0036*
Agitation (rpm)(50,150)	0.8102962	0.246687	3.28	0.0167*
Volume (L)*Agitation (rpm)	0.4525	0.293362	1.54	0.1739
Agitation (rpm)*Agitation (rpm)	0.2924396	0.354119	0.83	0.4405
Agitation (rpm)*Air flow rate (L/min)	-0.15	0.293362	-0.51	0.6274
Volume (L)*Air flow rate (L/min)	-0.085	0.293362	-0.29	0.7818
Volume (L)(15,35)	-0.071372	0.246687	-0.29	0.7821
Air flow rate (L/min)*Air flow rate (L/min)	0.0993025	0.354119	0.28	0.7886
Volume (L)*Volume (L)	-0.090817	0.354119	-0.26	0.8062

Prediction Profiler



kLa Surface Profiler for 50L



Summary: Air rate and agitation are statistically significant, and they have strong positive impact on kLa.

Conclusions

- kLa is an important design parameter and is essential to establish aeration efficiency and to quantify the effects of the operating variables on the delivery of dissolved oxygen.
- A DoE study was preformed to characterize kLa in 3L, 7L, and 50L cell culture bioreactors using response surface design (RSD).
- RSD data analysis showed that agitation and air rate are statistically significant for all the bioreactors.
- They have a strong positive correlation with kLa.
- Created kLa equation for each bioreactor to calculate it for specific operating conditions
- kLa of all three bioreactors are comparable.
- Have better engineering ability to scale up or down and tech transfer a process based on kLa.

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