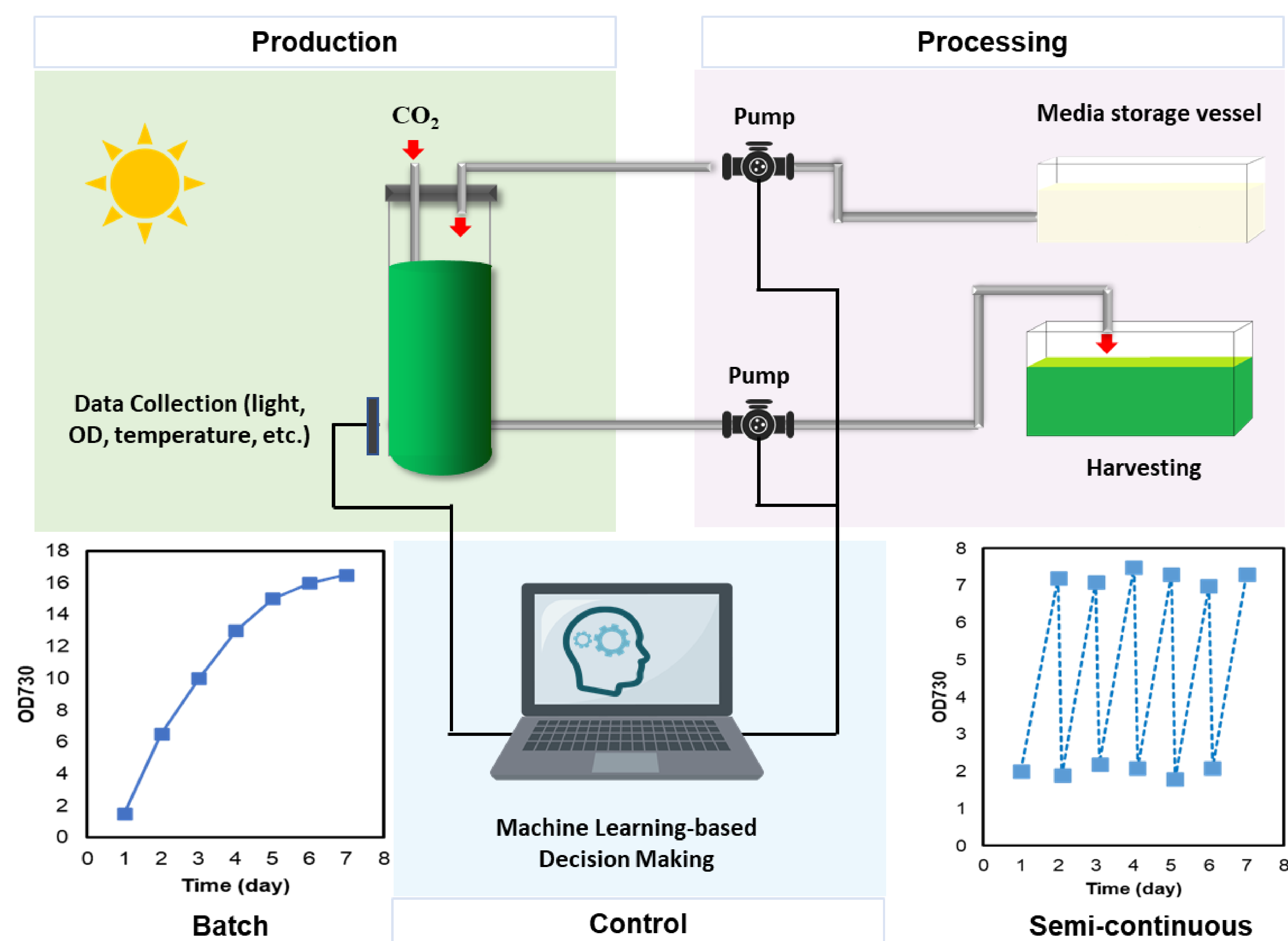


Simplified Harvesting Achieved by Cyanobacterial Cell Surface Engineering Economically Enables Machine Learning-informed Semi-continuous Cultivation for Sustainable Biofuel Production

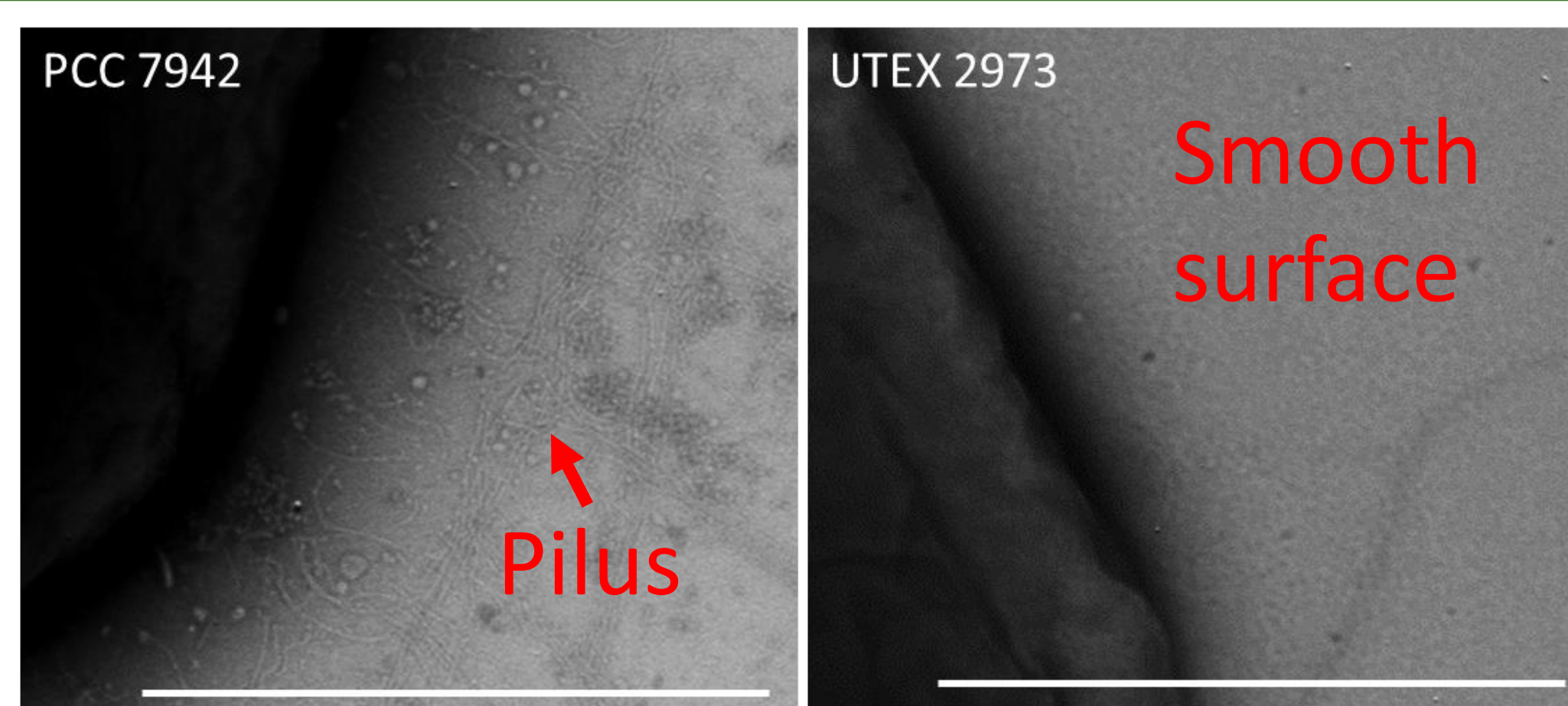
Background

Growth limitation by mutual shading and costly harvesting are two major technical challenges in algal biofuel production. In order to overcome the mutual shading, we developed a machine learning-informed semi-continuous cultivation system (MISC). However, the MISC requires frequent harvesting, so it is economically infeasible unless a low-cost harvesting method is developed.



Material and Methods

Synechococcus elongatus UTEX 2973 (UTEX 2973) was selected as the model strain due to its relatively smooth cell surface (pilN mutation). Limonene, a strong hydrophobic hydrocarbon was chosen to alter cell hydrophobicity.



A

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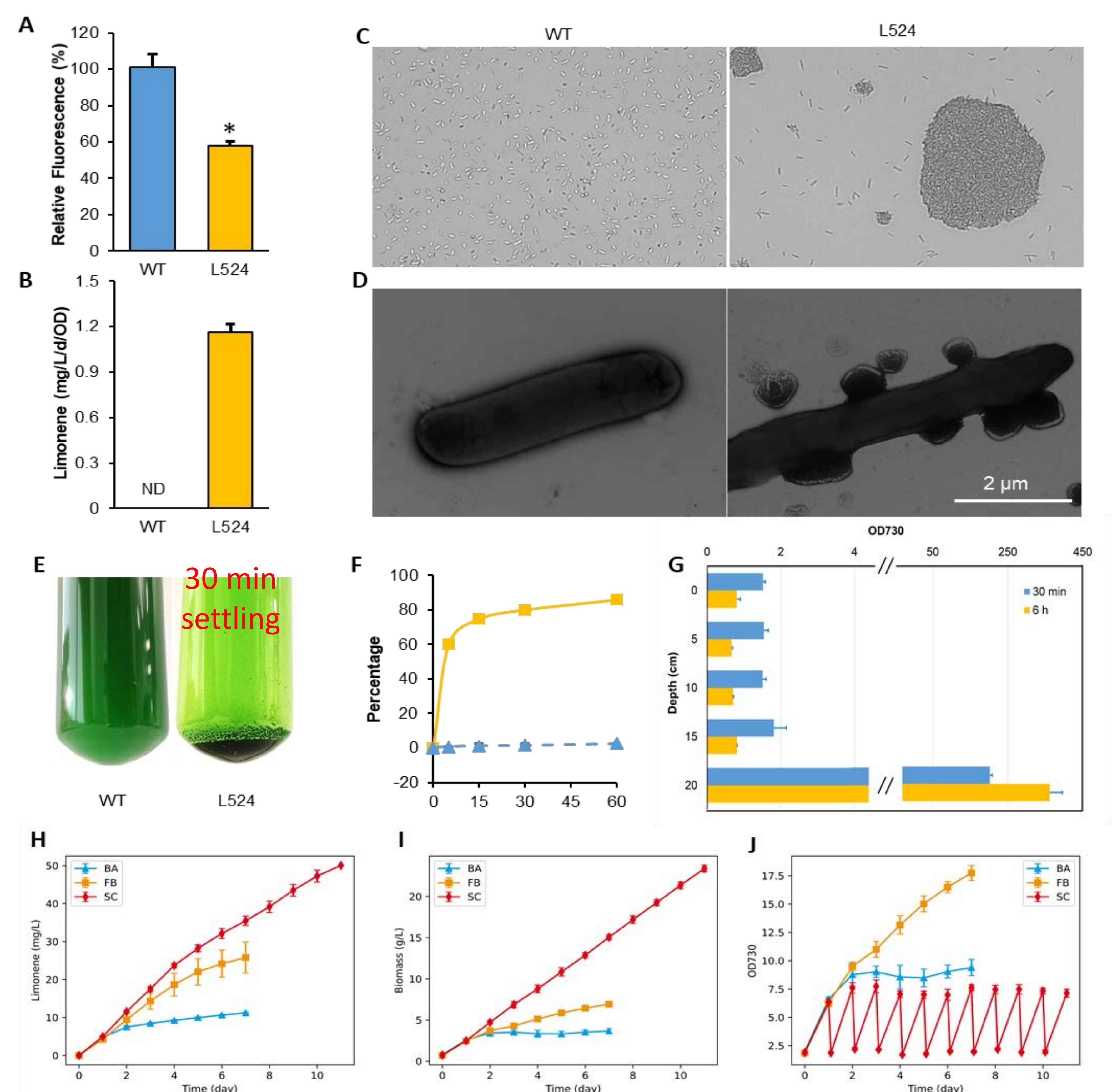
Synechococcus7942 90  CAG GCT GAG ATT GAG CAA ATC ANT ACC GAG ACC ACG TCC TTA ATT CAG
Synechococcus2973  CAG GCT GAG ATT GAG CAA ATC ANT ACC GAG ACC ACG TCC TTA ATT CAG
Synechococcus7942 110  GTC TTC CCG CAG GTC AAA TCC CAG TCT GCT ATC CTC ACC GAC CTG
Synechococcus2973 110  GTC TTC CCG CAG GTC AAA TCC TAG TCT GCT ATC CTC ACC GAC CTG
    
```

B

pLB524 $\xrightarrow{\text{psbA}}$ LS Limonene production

Results

- Limonene production increases cell hydrophobicity;
- Increased cell hydrophobicity drives cell aggregation;
- Cell aggregation enables sedimentation (AES);
- AES is a simple, fast, and low-cost harvesting method with high recovery rate and high solid content in its output.



Conclusion

We developed a simple, fast, and low-cost biomass harvesting method, the AES, by altering cyanobacterial cell surface hydrophobicity with a synthetic biology design. The AES economically enabled the implementation of the MISC, which ultimately addressed the technical barriers in algal cultivation and achieved sustainable biofuel production.