The Accumulation of Phosphate in Mucor

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The Biological Method of Phosphorus Recovery: Fungus

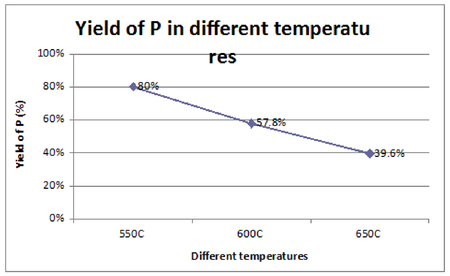
1. Phosphorus is an important element in agricuture but it also causes the eutrophication in water if the amount is excess. So far, the chemical method of Phosophorus removal is considered to be too complicated and expensive. A convenient biological way is going to be introduced in this project.
2. For many kinds of fungi, they have the ability to store Poly-phosphate in their cells if the amount of phosphorus taken in from external is excess. The accumulation of Poly-phosphorus can occupies 5%-7% of the dry cell mass; that is a considerable amount in phosphorus recovery.

The Development of Phosphorous Anslysis Method

·$MgCl_2$·$7H_2O$: Maximum the burning yield

1. Burning is a quick and convenient way to break all the cells in an organism so that the phosphate compound can be released completely. 550C is a commonly used temperature.

2. However, a drawback of burning is that it may cause the volatilization of some phosphate compounds. Result shows that burning at 550C also causes the volatilization; the yield of P is only 80% of the initial amount before burning, and as the burning temperature goes higher, the less yield we will get.



3. The addition of auxiliary liming agant into the dry sample before burning is a good way to prevent P element from being volatilized. $MgCl_2$·$7H_2O$ is used in the following experiments and it has shown an effectively effect to ensure the high yield of P element. This does a great help to the mass balance of P, which is also the kep point of this project.

Idea of The New Method: Mass&Concentration Balance

1. The fungus used in following experiments is mucor.
2. The phosphorus source for Mucor in this project is KH_2PO_4. In mucor cells, phorphorus may exist in organic phosphate forms. Due to the high temperature of burning, organic phosphates are tansferred into inorganic phoshate forms that are soluble in 7M HNO3. The final HNO_3&Biomass solution is to be tested.


Mucor (Wikipedia)

3. Mass Balance of P: P initial in medium = P after in supernatant + P after in Mucor

If the volumes of solutions in two sides are same, we have:

P initial concentration in medium = P after concentration in supernatant + P after concentration in Mucor (mg/L)

We make the initial volume of medium in each flask 100ml. when we are dissolving the phosphate from biomass, we need to make all samples to be 100ml solutions.

the table below shows the concentration balance of P in different tenperatures with the addition of $MgCl_2$·$7H_2O$; the replication for each temperature is 3(n=3).

P unit mg/L	P initial	P supernatant	P Biomass	Total	Error (Abs)
450C	515	336	154	490	4.8%
500C	515	335	159	494	4.1%
550C	515	348	149	497	3.5%
600C	535	319	166	485	9.3%
650C	535	365	137	502	6.2%

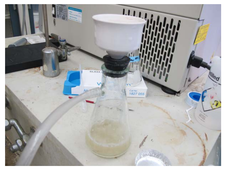
It is clear that the concentration balance equation is applicable; $MgCl_2$·$7H_2O$ also works as a very good auxiliary liming agant and it minimize the error. Based on the result, 550C burning is accepted in the following experiments. The $MgCl_2$·$7H_2O$ added is 220mg/L, 5ml for each sample.

Cell Cultivation and Phosphorus Removal

General Procedure: Grouping, Extracting and testing

1. Mucor, which is a common fungus in nature, is tested.
2. Culture the mucor in two groups, high concentrtaion of P (500mg/L) and Low concentrtaion of P (only about 2mg/L). We will measure the amount of P in 2, 4, 6, 8 days. The replication for all the following experiments is 3(n=3).

3. To extract phosphorus from cells, filtering is the first step. Filtering the mucor in medium to get dry samples to ensure no P element from the medium would affect the result.
4. Dry the samples in 100C to get dry biomass.
5. Burn dry biomass at 550C with $MgCl_2$·$7H_2O$ as a Auxiliary Liming Agent in crucible for 1h.
6. Using 7M HNO3 to dissolve the ash left, and add distilled water into the solution until 100ml.


Filtering


Samples to be dried

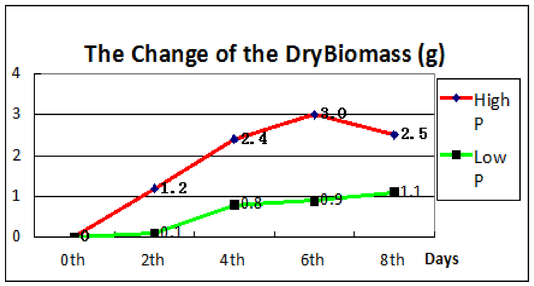
Reagents

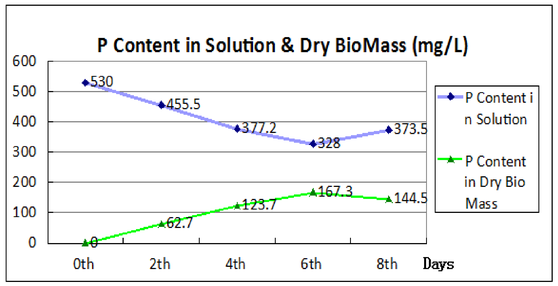
7. P concentration of the initial medium, P concentration of the supernatant after cultured and P concentration of the biomass solution are the three groups to be tested. TNT845 is a high range phosphorus testing reagent that can give the concentration of inorganic P between 2-20mg/L.
8. The overall P concentration in this project is very high; so dilution should be done make the sample soutions fit the range of reagents.
9. After a series of reactions, use spectrometer to get the concentrations. (unit: mg/L)
• **10.** The initial concentration of P is about 530mg/L for high P group; 2.3mg/L for low P group.

Results and Analysis

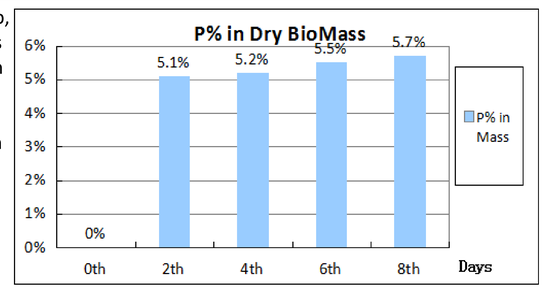
1. The left graph shows below indicates the change of the dry biomass vs days.
2. The upper right graph indicates the tendency of the change of P concentration in medium and biomass vs days.
3. The low right graph indicates the tendency of change of P content in biomass vs days. The calculation equation is:

P% = (P content in mucor) / (Dry biomass)





→ No phosphate is accumulated in low P group. For the low P group, the growth of mucor is limited; only 1/3 of the maximum biomass is measured compared with the high P group. A simple calculation can be done for the P needed to grow for mucor until a certain weight. In this case, P needed for mucor to grow in high P group is M=(3g/1.1g)(2.3mg/L)=6.3gmg/L, which is nearly 30 times less than the Phosphate accumulated in cells in 6th day.
→ It is obvious that mucor is able to accumulate phosphate inside the cell if there is enough phosphorus source; The mass percentage is near 6%, which is a considerable amount.
→ The growth period is about 1 week, after that apoptosis occurs among cells; Most of the Phosphorus has been absorbed by Mucor cells in first 2 days.



Summary and Conclusion

1. The phosphate accumulated in the Mucor can occupy 6% of the cell dry mass; it means that mucor can be use as an efficient carrier of the phosphorus removal process.
2. It is still nuknown whether the P element stored in cell is in the form of poly-phosphate; but it is true that the phosphate stored in cell can be dissolved in 7M HNO3 solution into an inorganic phosphate compound.
3. More strains of fungus can be tested to see which one shows a better ability of storing Phosphorus.

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