**Centrifugation Separation Strategies**

**Theory:**

Density-based centrifugation:

The mixture to be separated consists of components of different densities. This mixture is put in one of the standard gradients and then centrifuged.

A standard gradient is a liquid of varying density that is used for density-based centrifugation. It has the following properties:

* Its maximum density should be greater than the highest density in the mixture.
* It should be chemically inert towards the components of the mixture.
* It should not be electrically charged.

The centrifugation then takes place. After centrifugation, different substances get accumulated at the portion in the gradient where the density of the gradient is equal to the density of the substance. This way, all the components of the mixture are separated in different layers.

**Program Model:**

Input:

1. Densities of the components of the mixture
2. The volume of each component in the mixture
3. The component being looked for and the expected purity
4. The height of the centrifugation tube
5. The minimum height to which any component can be blown out using pipette (We call this blow limit henceforth)

Output:

1. The sequence of centrifugation.

Each element in the sequence of centrifugation will consist of:

* 1. the radius of the tube to be used
  2. the component separated and its total percentage separation so far
  3. the time of rotation
  4. the angular velocity
  5. the current composition of the mixture solution after separation

Constants:

* Purity Limit

Separated Proportion of a component is the ratio of the volume of the component separated so far to the total volume of the component given in the original input.

When the separated proportion >= Purity Limit, we assume the component is completely separated.

Purity Limit = 99%

* Gradient

The gradient used is Cesium Chloride (CsCl). This is a commonly used gradient in separation of materials of biological interest like virus, bacteria, yeast, DNA etc. by centrifugation. Its density is greater than most of the substances encountered in centrifugation as we shall see later in the examples below.

* Gradient Proportion

This is the ratio of the volume of the gradient that the system adds to the mixture to the total volume of the mixture given. This gradient is added only in the first step.

Gradient Proportion = 10%

* The time and speed of rotation for every centrifugation step will depend on the maximum of the rotation effort required for each component to separate.

This is given by the formula:

**t = [(18u)/(DP2 \* (ρ - ρP)\* w2)]**

where,

t = time of rotation

u = fluid viscosity

DP = particle diameter,

which is calculated using the density of the particle

ρP = particle density

ρ = gradient density

Thus, we can see t\*w2 (= rotation effort) is constant for a given component.

Once, we find the maximum rotation effort in the given mixture, we distribute the efforts equally among its two factors time and square of speed

i.e. t\*w2 (= rotation effort)

w2 = sqrt(rotation effort)

w = sqrt(w2)

w = rounded up to the nearest value in the list of available angular speeds

t = (rotation effort)/ w2

The list of available angular speeds: **500 rpm, 1000rp, 5000 rpm, 10000 rpm.**

**Algorithm:**

Input:

1. The number of elements in the mixture
2. Name, volume, density, particle diameter of each of the element in the mixture
3. Name of the element to be separated and purity expected.
4. Maximum height of the centrifugation tube
5. Blow limit

Output:

Centrifugation strategy which consists of an ordered list of centrifugation steps needed for the desired separation to take place.

Each centrifugation step consists of:

1. Radius of the centrifugation tube
2. Element separated in this step and its total separation percentage
3. Time needed for centrifugation
4. Speed of centrifugation needed out of allowed set of tuneable speeds
5. A message

if the physical constraints of the centrifugation tube doesn’t allow the separation of the top most element beyond its current separation

Algorithm for the centrifugation strategy:

1. If we have already separated the material which was supposed to be separated and its purity is greater than the expected purity, then STOP.
2. If the input elements do not contain the element to be separated, then STOP.
3. Find the radius of centrifugation tube needed for centrifugation step using the volume and the height present in the input.
4. Get the mixture element with minimum density. It is the topmost element after centrifugation.
5. Find the percentage separation of topmost element that we are separating in this step as follows:
   1. Separable height of topmost element = proportional height – blow limit
   2. If (Separable height of topmost element < 0.01) then, conclude this material cannot be separated further and add a message in the result accordingly. Go to Step 7.
   3. Volume of topmost element separated in this step = Pi \* radius \* radius \* (Separable height of topmost element)
   4. Original volume of the topmost element = Current volume of the topmost element /(1 – its percentage separated so far/100)
   5. Percentage of topmost element separated in this step = Volume of topmost element separated in this step/(Original volume of the topmost element) \* 100
   6. Total Percentage of topmost element separated = Percentage of topmost element separated in earlier steps + Percentage of topmost element separated in this step
6. Calculate maximum time and speed needed for centrifugation step as follows:
   1. efforts = MAX((particle diameter)^2 \* (density of gradient - density of element)/(18\*viscosity of the gradient))
   2. speed = efforts(1/4) and rounded up to the nearest value in the list of speeds
   3. time = efforts/speed2
7. Add this step to the centrifugation steps.
8. If we have separated more than 99% of topmost element we consider this element completely separated and do not consider it fort the rest of centrifugations.
9. Repeat steps 1 to 8 till we reach the STOP condition.

**Sample Run:**

Sample Inputs and Outputs to the Program:

Input 1:

1. Maximum height of the test tube used 500 mm
2. Blow limit 2mm
3. Component Table:

|  |  |  |  |
| --- | --- | --- | --- |
| Component | Volume(in mL) | Density (in g/mL) | Diameter of molecule(in mm) |
| Mitochondria | 20 | 1.15 | 0.0005 |
| Bacteria | 30 | 1.09 | 0.002 |

Mixture element being looked for:

Bacteria with 99% purity

Output 1:

Iteration 1

Centrifugation Strategy..

Radius of the centrifugation tube: 5.91727

Mixture element Bacteria, 99.26668 separated

Duration of rotation: 101 sec

Speed of rotation: 500 rpm

Now, the mixture contains:

Mitochondria : 20.0

Bacteria : 0.2199974

CsCl : 5.0

Input 2:

1. Maximum height of the test tube used 1000 mm
2. Blow limit 2mm
3. Component Table:

|  |  |  |  |
| --- | --- | --- | --- |
| Component | Volume(in mL) | Density (in g/mL) | Diameter of molecule(in mm) |
| Mitochondria | 20 | 1.15 | 0.0005 |
| Bacteria | 30 | 1.09 | 0.002 |
| Yeast | 20 | 1.1 | 0.01 |

Mixture element being looked for:

Yeast with 99.53% purity

Output 2:

Iteration 1:

Centrifugation Strategy..

Radius of the centrifugation tube: 4.9507437

Mixture element Bacteria, 99.48667 separated

Duration of rotation: 101 sec

Speed of rotation: 500 rpm

Now, the mixture contains:

Mitochondria : 20.0

Bacteria : 0.15399742

Yeast : 20.0

CsCl : 7.0

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Iteration 2:

Radius of the centrifugation tube: 3.867889

Mixture element Yeast, 99.53 separated

Duration of rotation: 101 sec

Speed of rotation: 500 rpm

Now, the mixture contains:

Mitochondria : 20.0

Yeast : 0.09399986

CsCl : 7.0

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Input 3:

1. Maximum height of the test tube used 900 mm
2. Blow limit 2mm
3. Component Table:

|  |  |  |  |
| --- | --- | --- | --- |
| Component | Volume(in mL) | Density (in g/mL) | Diameter of molecule(in mm) |
| Mitochondria | 20 | 1.15 | 0.0005 |
| DNA | 10 | 1.7 | 0.000002 |
| Bacteria | 30 | 1.09 | 0.002 |
| Virus | 15 | 1.4 | 0.0004 |
| Yeast | 20 | 1.1 | 0.01 |

Mixture element being looked for:

DNA with 97.5% purity

Output 3:

Iteration 1:

Centrifugation Strategy..

Radius of the centrifugation tube: 6.079417

Mixture element Bacteria, 99.22592 separated

Duration of rotation: 491266 sec

Speed of rotation: 2000 rpm

Now, the mixture contains:

Mitochondria : 20.0

DNA : 10.0

Bacteria : 0.23222351

Virus : 15.0

Yeast : 20.0

CsCl : 9.5

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Iteration 2:

Radius of the centrifugation tube: 5.1331263

Mixture element Yeast, 99.172226 separated

Duration of rotation: 491266 sec

Speed of rotation: 2000 rpm

Now, the mixture contains:

Mitochondria : 20.0

DNA : 10.0

Virus : 15.0

Yeast : 0.16555595

CsCl : 9.5

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Iteration 3:

Radius of the centrifugation tube: 4.3903794

Mixture element Mitochondria, 99.39445 separated

Duration of rotation: 491266 sec

Speed of rotation: 2000 rpm

Now, the mixture contains:

Mitochondria : 0.121110916

DNA : 10.0

Virus : 15.0

CsCl : 9.5

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Iteration 4:

Radius of the centrifugation tube: 3.4931188

Mixture element Virus, 99.488884 separated

Duration of rotation: 491266 sec

Speed of rotation: 2000 rpm

Now, the mixture contains:

DNA : 10.0

Virus : 0.076667786

CsCl : 9.5

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Iteration 5:

Radius of the centrifugation tube: 2.6261597

Mixture element DNA, 99.56668 separated

Duration of rotation: 491266 sec

Speed of rotation: 2000 rpm

Now, the mixture contains:

DNA : 0.0433321

CsCl : 9.5

**Analysis of the result:**

1. For every sample input, the component with lowest density is centrifuged first.
2. With every centrifugation, the radius of the centrifuging tube reduces, in order to increase its height and attain more purity.
3. The denser the components, the greater are the duration of rotation for centrifugation.
4. The number of steps of centrifugation depends on the position of the target component in the ascending order of the components’ densities and its proportion.

**Further plan:**

Optimization of the rotation efforts

Currently, we are mathematically optimizing the time and speed by distributing the rotation effort equally between the two.

The plan is to make the system intelligent enough to consider practical limitations on extremely high time and speed values and adjust the time, speed values accordingly.

Selection of gradient

Currently, the system uses only one standard gradient i.e. Cesium Chloride. The plan is to feed the system with information of more gradients and to make the system intelligent enough to select the one with the minimum density and viscosity to reduce the rotation efforts.