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Title: A Method for Adapting *Euglena gracilis* to Cassava-Based Substrates for Heterotrophic Cultivation

Authors and Affiliations:

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

If **Yes**, can you record movies/images using your own microscope camera?

No

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye.**

OMAX Sartorius compound light microscope

PRACTICUM224-1S 0033150256

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

Scope shots: 4.2.1, 4.3.1, 4.5.1, 4.9.1, 5.11.1.

Videographer: Please film above mentioned shots using a scope kit.

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **No**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 46

Number of Shots: 57

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~**REQUIRED:** Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Adrian Guaman Vargas:** Our research focuses on adapting *Euglena gracilis* to cassava waste and studying growth, cyanide reduction, and protein-rich biomass production.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Adrian Guaman Vargas:** This protocol can be applied in sustainable cassava processing, agricultural waste valorization, feed production, and low-cost biotechnology systems.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Adrian Guaman Vargas:** This protocol allows researchers to measure *Euglena* growth, biomass production, cyanide reduction, and nutritional potential in cassava-based environments.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Adrian Guaman Vargas:** The critical challenge in this protocol is maintaining stable temperature, consistent culture conditions, and balanced microbial communities for *Euglena* growth.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Adrian Guaman Vargas:** Future studies can optimize scale-up, improve protein yield, and test industrial applications using diverse cassava waste streams on a large scale.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

All procedures were performed according to institutional biosafety and chemical safety requirements for handling cyanogenic plant materials

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Preparation and Fermentation of Cassava-Based Media

Demonstrator: Zsofia Hatvani

- 2.1. To begin, label sterile 500 milliliter vented-cap flasks with the substrate type as garri [1]. Mark the target concentration on the flask as 10, 20, 30, 40, or 50 grams per liter [2]. Label the flask with the replicate identification and planned sterilization condition as autoclaved or non-autoclaved [3].
 - 2.1.1. WIDE: Talent labeling the 500 milliliter vented-cap flasks as garri.
***Videographer:** Please obtain CU shot as well for this shot* Videographer's NOTE: Shows labeling, take 1 wide shot, take 2 close up
 - 2.1.2. Close-up shot of the talent labeling the target concentrations on the flask.
Videographer's NOTE: 2.1.2 and 2.1.3 are close-up shots of the labels already applied
 - 2.1.3. Close-up shot of the talent labeling the replicate identification and sterilization condition on the flask.
- 2.2. Place a weigh boat on the analytical balance [1]. After pressing **TARE** or **Zero** to set to 0.000 grams, weigh the garri to match the final working volume [2].
 - 2.2.1. Talent placing a weigh boat on the analytical balance.
 - 2.2.2. Close-up shot of the analytical balance display showing different target weights.
- 2.3. Aseptically add the weighed garri into a sterile 500 milliliter flask containing 300 milliliters of deionized or distilled water [1]. Reapply the vented cap to each flask [2].
 - 2.3.1. Talent aseptically transferring the weighed garri into a sterile 500 milliliter flask containing water.
 - 2.3.2. Talent securing the vented cap onto the flask.
- 2.4. Mix each suspension for 3 minutes until the mixture is homogenized [1]. If magnetic stirring is used, place the flask on a stir plate and set the speed to 200 g to form a shallow vortex without splashing [2]. Allow the suspension to hydrate undisturbed for 10 minutes at 25 degrees Celsius [3].
 - 2.4.1. Talent holding and gently mixing the flask containing the garri suspension.
 - 2.4.2. Close-up shot of the flask on the stir plate showing a shallow vortex forming in the suspension.
 - 2.4.3. Talent placing the prepared flask undisturbed at 25°C.

- 2.5. Tighten the vent cap immediately prior to fermentation and swirl the flask by hand 5 times to gently homogenize the mixture and resuspend the settled material [1].
 - 2.5.1. Talent tightening the vent cap and swirling the flask by hand.
- 2.6. To prepare additional cassava-product media, label the flasks and weigh the substrate as previously demonstrated to achieve 30 grams per liter based on the working volume [1].
 - 2.6.1. Talent adding the substrate onto the weigh boat placed on the analytical balance.
- 2.7. Aseptically add the weighed substrate into a sterile flask containing 300 milliliters of deionized or distilled water [1]. Reapply the vented cap to each flask [2].
 - 2.7.1. Talent aseptically transferring the weighed substrate into a sterile flask containing water. Videographer's NOTE: 2.7.1 is combined with 2.7.2
 - 2.7.2. Talent securing the vented cap onto the flask.
- 2.8. Hydrate and mix the suspension as previously demonstrated [1].
 - 2.8.1. Close-up shot of the hydrated suspension showing evenly dispersed solids without dry clumps.

3. Fermentation and Sterilization of Cassava Media

Demonstrator: Zsofia Hatvani

- 3.1. Confirm that flasks are fitted with a sterile gas-permeable vented cap to permit gas exchange while minimizing environmental contamination [1].
 - 3.1.1. Close-up shot of the talent checking that each flask is fitted with a sterile gas-permeable vented cap.
- 3.2. Place the flasks upright at 25 degrees Celsius in a designated area away from direct sunlight and high-traffic airflow and incubate the flasks without agitation for a minimum of 72 hours [1].
 - 3.2.1. Talent arranging the flasks upright in a designated storage area away from direct sunlight and leaving it undisturbed.
- 3.3. Record the fermentation start date and time for each flask and condition [1].
 - 3.3.1. Talent recording the fermentation start date and time for each flask and condition in a laboratory log sheet.
- 3.4. Confirm that each 500 milliliter flask contains no more than 300 milliliters of liquid following fermentation to prevent boil-over during sterilization [1].

- 3.4.1. Close-up shot of the liquid level inside the flask showing the volume below two-thirds of the total flask capacity.
- 3.5. Once the vented cap is loosened to a finger-tight position, apply autoclave indicator tape to the flask [1].
 - 3.5.1. Talent applying autoclave indicator tape to the flask.
- 3.6. After loading the flasks into an autoclave-safe tray, place the tray into the autoclave and select the **P13 Liquid** (*P-thirteen*) cycle [1].
 - 3.6.1. Close up shot of talent selecting the **P13 Liquid** cycle in the autoclave display screen.
- 3.7. Set the sterilization parameters to 121 degrees Celsius for 30 minutes for liquid sterilization [1]. Start the autoclave cycle [2].
 - 3.7.1. Close up shot of talent setting the sterilization parameters to 121 degrees Celsius for 30 minutes in the autoclave display.
 - 3.7.2. Show the talent pressing the control to start the autoclave cycle.
- 3.8. After the cycle completes, allow the autoclave chamber pressure to return to atmospheric pressure and open the autoclave door slowly [1]. Using heat-resistant gloves, remove the flasks and allow them to cool to room temperature [2].
 - 3.8.1. Talent slowly opening the autoclave door. Videographer's NOTE: Take 1 wide shot, take 2 close up
 - 3.8.2. Talent wearing heat-resistant gloves while removing the flasks and placing them on the bench to cool.
- 3.9. Store the cooled flasks overnight at room temperature prior to inoculation [1].
 - 3.9.1. Talent placing the cooled flasks in the designated storage area at room temperature.
- 3.10. Use the fermented cassava media directly without autoclaving to preserve native microbial communities [1].
 - 3.10.1. Close-up shot of the non-autoclaved fermented cassava media inside the flask.
- 3.11. Inoculate the non-autoclaved media immediately after the 72-hour fermentation period to minimize unintended environmental contamination [1].
 - 3.11.1. Talent handling the non-autoclaved media immediately following the fermentation period.
- 3.12. Maintain consistent handling across replicates by using the same type 500 milliliter vented-cap flasks, the same working volume, and the same fermentation duration for all substrate conditions [1].

- 3.12.1. Shot of multiple replicate flasks arranged together showing identical flask types and working volumes. Videographer's NOTE: Take 1 wide shot, take 2 close up

4. Establishment of *Euglena gracilis* starter Cultures

Demonstrator: Zsofia Hatvani

- 4.1. Inoculate *Euglena gracilis* into fresh heterotrophic nutrient medium [1]. Incubate the cultures in complete darkness at 28 degrees Celsius with continuous orbital shaking at 120 g until the cultures reach mid-logarithmic growth [2].
- 4.1.1. Talent inoculating *E. gracilis* into fresh heterotrophic nutrient medium.
- 4.1.2. Talent placing the culture flasks onto an orbital shaker in a dark incubation area.
- 4.2. Using a compound light microscope in brightfield mode, examine the cultures at 100× (hundred-x) to 400× (four-hundred-x) total magnification and confirm active motility, elongated cell morphology, and low levels of encystment [1].
- 4.2.1. SCOPE: Show actively motile elongated *E. gracilis* cells with minimal encystment and low debris. Videographer's NOTE: Scope shot shows the spinning motion of particles
- 4.3. Exclude cultures exhibiting excessive cellular debris, abnormal morphology, or elevated encystment prior to adaptation experiments [1].
- 4.3.1. SCOPE: Show culture fields containing excessive debris, irregular cell morphology, or encysted cells being identified for exclusion.
- 4.4. After gently homogenizing the starter cultures by tightening the vent cap, swirl the flasks by hand for 15 seconds to ensure uniform cell distribution without inducing shear stress [1].
- 4.4.1. Talent gently swirling the flask by hand.
- 4.5. Using a Neubauer hemocytometer, determine the cell density [1].
- 4.5.1. SCOPE: Show *E. gracilis* cells distributed within the Neubauer hemocytometer grid.
- 4.6. Once the culture is mixed by swirling the flask for 15 seconds, pipette 1 milliliter of culture into a sterile 1.5 milliliter microcentrifuge tube [1].
- 4.6.1. Talent transferring 1.0 milliliter of culture into a sterile 1.5 milliliter microcentrifuge tube using a pipette.

- 4.7. Clean the hemocytometer and coverslip with 70% ethanol and allow the hemocytometer and coverslip to air-dry [1].
 - 4.7.1. Talent cleaning the hemocytometer and coverslip with 70% ethanol and keeping it aside for air-drying.
- 4.8. Pipette 10 microliters of culture onto the two grids on the hemocytometer and place the coverslip onto the wet sample [1].
 - 4.8.1. Close-up shot of the talent pipetting culture onto the two grids of the hemocytometer and placing the coverslip onto the loaded hemocytometer.
- 4.9. Using brightfield microscopy with a 10× (ten-x) objective, focus on the grid and count the cells within a defined set of squares on the large grid by applying the standard boundary rules [1].
 - 4.9.1. SCOPE: Show cells distributed within the hemocytometer squares during counting.
- 4.10. Repeat the cell counts on the second chamber and calculate the mean cells per milliliter and cell density [1-TXT].
 - 4.10.1. Talent doing the calculation on a whiteboard.

$$\text{TXT: cells/mL} = \frac{\text{mean cells per square} \times 10^4 \times \text{dilution factor}}{1}$$
- 4.11. Adjust the inoculation volumes to achieve a final inoculum of 5% volume by volume across all experimental media [1].
 - 4.11.1. Close-up shot of the talent using a pipette to measure and transfer the inoculation volume into the experimental media flask.
- 4.12. Prepare separate inocula for each treatment condition to maintain consistent initial cell densities between experimental groups [1].
 - 4.12.1. Shot of multiple labeled inoculum flasks prepared separately for different treatment conditions.

5. Adaptation and Monitoring of *E. gracilis* Cultures

Demonstrator: Adrian Guaman Vargas

- 5.1. Add the prepared *E. gracilis* inoculum to autoclaved and non-autoclaved cassava media prepared at final garri concentrations of 10, 20, and 30 grams per liter [1].
 - 5.1.1. Talent inoculating labeled autoclaved and non-autoclaved cassava media flasks with the prepared *E. gracilis* inoculum.
- 5.2. Use equal inoculation volumes across all treatment conditions to standardize the initial cell density [1].

- 5.2.1. Close-up shot of the talent measuring identical inoculation volumes with a pipette for multiple treatment flasks.
- 5.3. After tightening the vented cap, gently swirl the flask in a circular motion for 15 seconds to distribute the cells [1].
 - 5.3.1. Talent gently swirling the flask in a circular motion.
- 5.4. Incubate the cultures in complete darkness at 25 degrees Celsius to initiate heterotrophic growth [1].
 - 5.4.1. Talent placing the inoculated culture flasks into a dark incubation area.
- 5.5. Minimize agitation of the non-autoclaved media to preserve the native microbial community structure and substrate stratification [1].
 - 5.5.1. Close-up shot of the non-autoclaved culture flask remaining undisturbed in the incubation area.
- 5.6. Mix all cultures once daily using gentle manual swirling for 15 seconds to maintain contact between *E. gracilis* cells and suspended cassava material [1].
 - 5.6.1. Talent gently swirling multiple culture flasks by hand.
- 5.7. Using a sterile pipette tip, every 48 hours, aseptically collect 1 milliliter samples from each culture [1].
 - 5.7.1. Talent aseptically collecting culture samples from multiple flasks using sterile pipette tips.
- 5.8. Tighten the vented cap and gently swirl the culture flask for 15 seconds prior to sampling to ensure even distribution of cells and suspended material [1].
 - 5.8.1. Talent tightening the vented cap and gently swirling the flask before sample collection.
- 5.9. After transferring an aliquot of culture into a sterile 1.5 milliliter microcentrifuge tube, dilute the sample with deionized water as needed to improve visualization of individual cells [1].
 - 5.9.1. Talent pipetting the deionized water into a microcentrifuge tube containing the culture sample.
- 5.10. Prepare a wet-mount microscopy slide by pipetting 10 microliters of the diluted sample onto a clean microscope slide and covering the sample with a coverslip [1].
 - 5.10.1. Close-up shot of the talent pipetting diluted sample onto the microscope slide and placing the coverslip onto the wet-mounted sample.

5.11. Using a compound light microscope in brightfield mode, examine the samples at 100× to 400× total magnification and assess cell motility, morphology, and the frequency of encystment as indicators of adaptation status [1].

5.11.1. SCOPE: Show motile *E. gracilis* cells with characteristic morphology and low encystment frequency.

5.12. Every 7 days, aseptically transfer 20% volume by volume of each culture into freshly prepared cassava media with the same garri concentration and sterilization conditions [1].

5.12.1. Talent aseptically transferring culture into freshly prepared cassava media flasks with matching labels.

5.13. Maintain consistent transfer volumes between passages and repeat serial passaging until the cultures exhibit consistent growth, sustained motility, and low encystment frequency in 30 grams per liter cassava media [1].

5.13.1. Shot of multiple culture flasks from successive passages arranged together on the laboratory bench.

5.14. ~~After performing high concentration cassava adaptation and comparative growth analysis of *E. gracilis* cultures, monitor cyanide and harvest biomass for downstream protein analysis~~ Following high-concentration cassava adaptation and comparative growth analysis, use colorimetric strips to assess cyanide levels and observe the developed colour [1]. **NOTE: The VO is modified as per the shot**

5.14.1. ~~Montage shot showing cyanide strip testing of culture supernatants, biomass pellets collected after centrifugation, and preparation of harvested biomass for downstream protein analysis.~~ Talent immersing a colorimetric cyanide test strip into the culture supernatant and showing the colour change in the cyanide strip **Videographer's NOTE: Take 1 shows process, take 2 is close up shot of the cyanide strip color changed**

5.15. After centrifugation, harvest biomass for downstream analysis [1]. **NOTE: VO is added for additional shot.**

Added shot 5.15.1: Shot of biomass pellets collected after centrifugation

Videographer's NOTE: 5.15.1 – added shot of biomass pellets collected after centrifugation

Results

6. Results

- 6.1. Under fully dark cultivation conditions, cultures grown at 30 grams per liter of garri exhibited higher integrated growth [1] than lower concentrations [2].
 - 6.1.1. LAB MEDIA: Figure 1. *Video editor: In the panel labelled 30, emphasize the blue line and dotted blue line labelled as autoclaved and non-autoclaved.*
 - 6.1.2. LAB MEDIA: Figure 1. *Video editor: In the panels labelled 10 and 20 panels, emphasize the blue line and dotted blue line labelled as autoclaved and non-autoclaved.*
- 6.2. Lower garri concentrations of 10 to 20 grams per liter showed detectable but reduced and more variable growth [1].
 - 6.2.1. LAB MEDIA: Figure 1. *Video editor: Emphasize the scattered black dots in the panels labelled 10 and 20.*
- 6.3. Higher concentrations of 40 to 50 grams per liter reached elevated peak cell densities [1] but showed delayed stabilization and greater variability [2].
 - 6.3.1. LAB MEDIA: Figure 2. *Video editor: Emphasize the dashed lines labelled 40 and 50.*
 - 6.3.2. LAB MEDIA: Figure 2. *Video editor: Emphasize the black dots after calendar days 10.*
- 6.4. Trypan Blue exclusion assays indicated that cultures maintained at 30 grams per liter garri retained high viability across sampled time points [1].
 - 6.4.1. LAB MEDIA: Figure 3. *Video editor: In the panel labelled 30, emphasize the blue line labelled as autoclaved between calendar days 3 (the line next to 2.5) and 10 where the line stays close to the top of the graph near 95 to 100% viability.*
- 6.5. Higher garri concentrations showed transient reductions in viability [1], followed by partial recovery with increased variability over time [2].
 - 6.5.1. LAB MEDIA: Figure 4. *Video editor: Emphasize the downward dip between day 0 and day 7 for the lines labelled 40 and 50.*
 - 6.5.2. LAB MEDIA: Figure 4. *Video editor: Emphasize the upward recovery of all 3 lines labelled 30, 40, and 50 from day 7 to day 15.*
- 6.6. Positive outcomes included rounded or semi-encysted cells with reduced motility that remained intact across repeated subculturing [1].

- 6.6.1. LAB MEDIA: Figure 5. *Video editor: Highlight the rounded cells marked with red circles in the microscopy images*

- 6.7. Adapted *E. gracilis* cultures displayed reproducible growth across multiple substrate types [1] and showed earlier stabilization with reduced late-stage decline [2] relative to wild-type cultures [3].
 - 6.7.1. LAB MEDIA: Figure 6. *Video editor: Show all 4 graph panels labelled PEL, PeG, PT, and PU together*
 - 6.7.2. LAB MEDIA: Figure 6. *Video editor: Emphasize the solid blue curves labelled as GA.*
 - 6.7.3. LAB MEDIA: Figure 6. *Video editor: Emphasize the dotted blue curves labelled as WT.*

- 6.8. Successful implementation of the protocol yielded *E. gracilis* biomass with measurable crude protein content [1], as quantified by external laboratory analysis using the Combustion Dumas method [2]. Protein percentages observed in adapted cultures were comparable to those measured in wild-type cultures [3], with higher total protein output corresponding to increased biomass accumulation [4].
 - 6.8.1. LAB MEDIA: Figure 7. *Video editor: In the bottom-left panel labelled PT, emphasize the black dots and center marker in the group labelled "GA" on the x-axis.*
 - 6.8.2. LAB MEDIA: Figure 7. *Video editor: Emphasize the PT and PEL panels.*
 - 6.8.3. LAB MEDIA: Figure 7. *Video editor: In the top-left panel labelled PeG, highlight the "WT" group and then the "GA" group.*
 - 6.8.4. LAB MEDIA: Figure 7. *Video editor: In the bottom-right panel labelled PEL, emphasize the taller cluster of points above the "GA" label.*

Pronunciation guides:

1. Garri
 Pronunciation link: <https://www.howtopronounce.com/garri>
 IPA: /'gari/
 Phonetic Spelling: gaar·ree

2. Aseptically
 Pronunciation link: <https://www.merriam-webster.com/dictionary/aseptically>
 IPA: /eɪ'septɪkli/
 Phonetic Spelling: ay·sep·tik·lee

3. Homogenized
 Pronunciation link: <https://www.merriam-webster.com/dictionary/homogenized>

IPA: /hə'mɑ:dʒəˌnaɪzd/

Phonetic Spelling: huh·maa·juh·nyzd

4. Fermentation

Pronunciation link: <https://www.merriam-webster.com/dictionary/fermentation>

IPA: /ˌfɜːməˈteɪʃən/

Phonetic Spelling: fur·muhn·tay·shuhn

5. Autoclave

Pronunciation link: <https://www.merriam-webster.com/dictionary/autoclave>

IPA: /ˈɔːtəˌkleɪv/

Phonetic Spelling: aw·tuh·klayv

6. Sterilization

Pronunciation link: <https://www.merriam-webster.com/dictionary/sterilization>

IPA: /ˌsterələˈzeɪʃən/

Phonetic Spelling: ster·uh·luh·zay·shuhn

7. Incubate

Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>

IPA: /ˈɪŋkjəˌbeɪt/

Phonetic Spelling: ing·kyuh·bayt

8. Euglena

Pronunciation link: <https://www.howtopronounce.com/euglena>

IPA: /juːˈgliːnə/

Phonetic Spelling: yoo·glee·nuh

9. Gracilis

Pronunciation link: <https://www.merriam-webster.com/medical/gracilis>

IPA: /ˈgræsəˌlɪs/

Phonetic Spelling: grass·uh·liss

10. Heterotrophic

Pronunciation link: <https://www.merriam-webster.com/dictionary/heterotrophic>

IPA: /ˌhetərəˈtroʊfɪk/

Phonetic Spelling: het·uh·ruh·troh·fik

11. Logarithmic

Pronunciation link: <https://www.merriam-webster.com/dictionary/logarithmic>

IPA: /ˌlɒːɡəˈrɪðmɪk/

Phonetic Spelling: law·guh·rith·mik

12. Motility

Pronunciation link: <https://www.merriam-webster.com/dictionary/motility>

IPA: /moʊˈtɪləti/

Phonetic Spelling: moh·til·uh·tee

13. Encystment
Pronunciation link: <https://www.howtopronounce.com/encystment>
IPA: /ɛnˈsɪstmənt/
Phonetic Spelling: en·sist·muhnt
14. Neubauer
Pronunciation link: <https://www.howtopronounce.com/neubauer>
IPA: /ˈnoʊˌbaʊər/
Phonetic Spelling: noy·bow·uhr
15. Hemocytometer
Pronunciation link: <https://www.merriam-webster.com/medical/hemocytometer>
IPA: /ˌhiːməsaɪˈtɑːmətər/
Phonetic Spelling: hee·muh·sy·taa·muh·tur
16. Microcentrifuge
Pronunciation link: <https://www.merriam-webster.com/medical/microcentrifuge>
IPA: /ˌmaɪkroʊˈsentrɪfjuːdʒ/
Phonetic Spelling: my·kroh·sen·truh·fyooj
17. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /ˌmaɪkroʊˌliːtərz/
Phonetic Spelling: my·kroh·lee·turz
18. Inoculum
Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculum>
IPA: /ˈɪˌnoːkjələm/
Phonetic Spelling: ih·naa·kyuh·luhm
19. Cyanide
Pronunciation link: <https://www.merriam-webster.com/dictionary/cyanide>
IPA: /ˈsaɪəˌnaɪd/
Phonetic Spelling: sy·uh·nyd
20. Colorimetric
Pronunciation link: <https://www.merriam-webster.com/dictionary/colorimetric>
IPA: /ˌkʌləˈrəˌmetrɪk/
Phonetic Spelling: kul·uh·ruh·met·rik
21. Biomass
Pronunciation link: <https://www.merriam-webster.com/dictionary/biomass>
IPA: /ˈbaɪoʊˌmæs/
Phonetic Spelling: by·oh·mass
22. Trypan
Pronunciation link: <https://www.merriam-webster.com/medical/trypan%20blue>

IPA: /'traɪ.pæn/

Phonetic Spelling: try·pan

23. Viability

Pronunciation link: <https://www.merriam-webster.com/dictionary/viability>

IPA: /ˌvaɪə'bɪləti/

Phonetic Spelling: vy·uh·bil·uh·tee

24. Subculturing

Pronunciation link: <https://www.merriam-webster.com/medical/subculture>

IPA: /'sʌb,kʌltʃərɪŋ/

Phonetic Spelling: sub·kul·chur·ing

25. Combustion

Pronunciation link: <https://www.merriam-webster.com/dictionary/combustion>

IPA: /kəm'bəstʃən/

Phonetic Spelling: kuhm·buhs·chuhn

26. Dumas

Pronunciation link: <https://www.howtopronounce.com/dumas>

IPA: /du:'mɑ:/

Phonetic Spelling: doo·maa

27. Morphology

Pronunciation link: <https://www.merriam-webster.com/dictionary/morphology>

IPA: /mɔ:'rʃɑ:lədʒi/

Phonetic Spelling: mor·faa·luh·jee

28. Orbital

Pronunciation link: <https://www.merriam-webster.com/dictionary/orbital>

IPA: /'ɔ:rbɪtəl/

Phonetic Spelling: or·buh·tl

29. Magnification

Pronunciation link: <https://www.merriam-webster.com/dictionary/magnification>

IPA: /ˌmægnəfə'keɪʃən/

Phonetic Spelling: mag·nuh·fuh·kay·shuhn

30. Centrifugation

Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifugation>

IPA: /ˌsentrəfjə'geɪʃən/

Phonetic Spelling: sen·truh·fyuh·gay·shuhn