Work Package 5 Task 3.2

The Production of Fish Feed enriched with poly-unsaturated fatty acids

Corinda Erasmus

Annali Jacobs

Gerda Lombard

Petrus van Zyl

Judy Reddy

Ntombikayise Nkomo

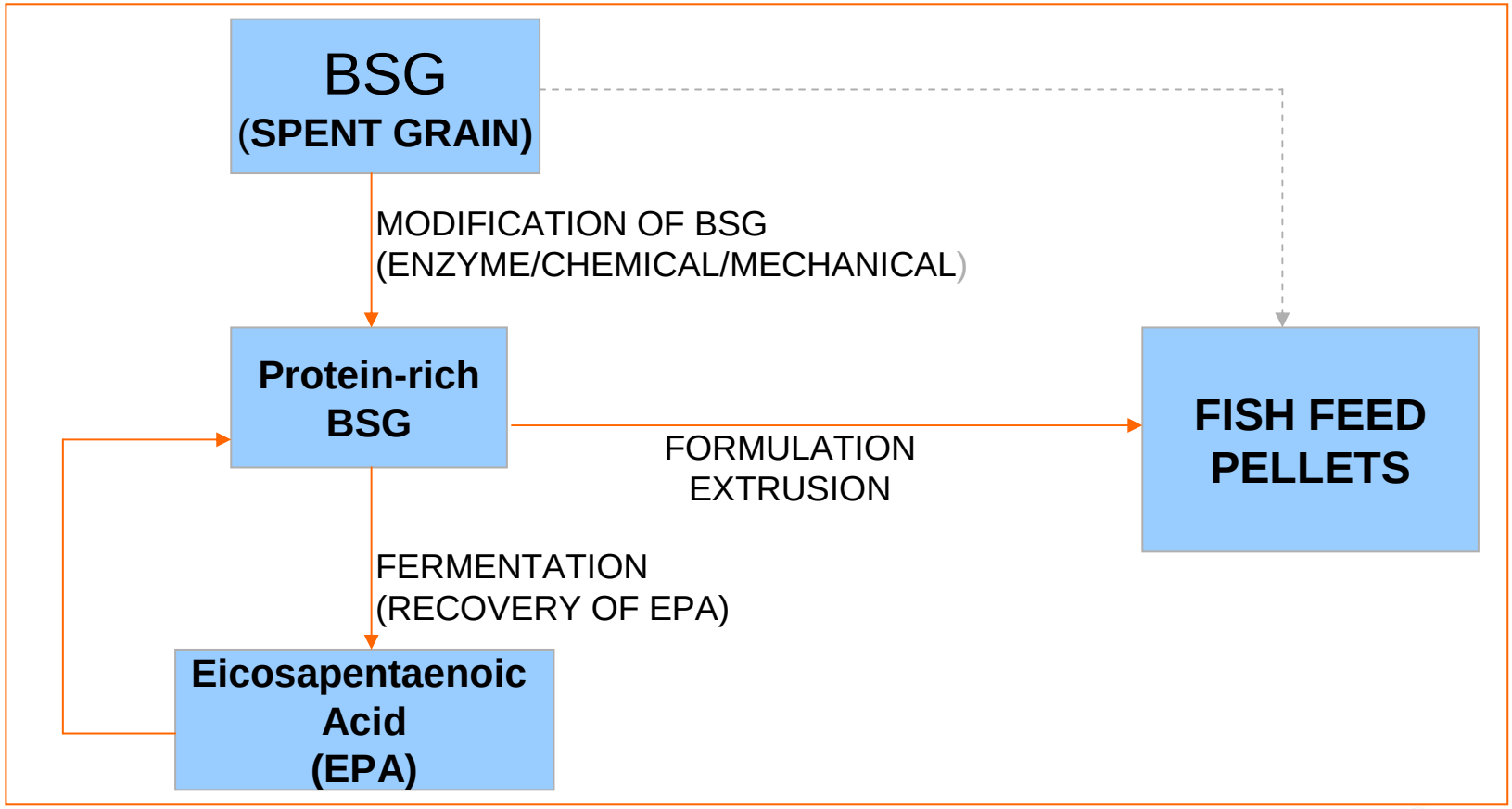
Elizabeth Timme

Partner 11

Activities Planned for Year 2

- Development of fermentation process for EPA production
- Scale-up of fermentation
- Formulation of feed with fermented BSG
- Extrusion of fermented BSG
- Fish feeding trials with fermented BSG

FLOW DIAGRAM OF THE PRODUCTION OF EPA-ENRICHED FISH FEED

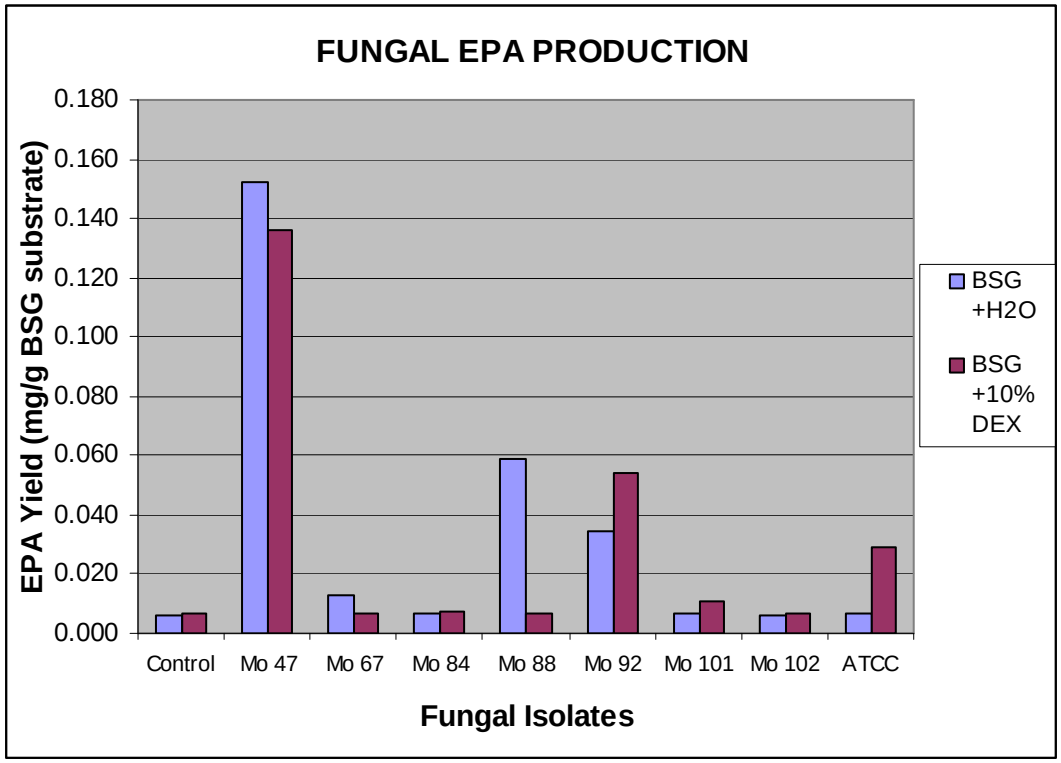


Development of fermentation process for EPA production

- develop a synthetic fermentation medium with higher levels of nutrients based on a literature study
- 8% dextrose, yeast extract and KNO_3 and 1% linseed oil
- biomass production up from <10 g/L (with the non-optimal medium) to >30 g/L
- Biomass production improved from 9,5 g/L to 39,3 g/L for Mo 130
- EPA production up from <10 mg/L for most isolates to approximately 100 mg/L
- EPA production for Mo 130 up from 77,6 mg/L to 155,8 mg/L
- EPA production mentioned in literature range from 48 mg/L (Cheng *et al*, 1999) to 1 880 mg/L (Shimuzu *et al*, 1989).

Development of fermentation process for EPA production

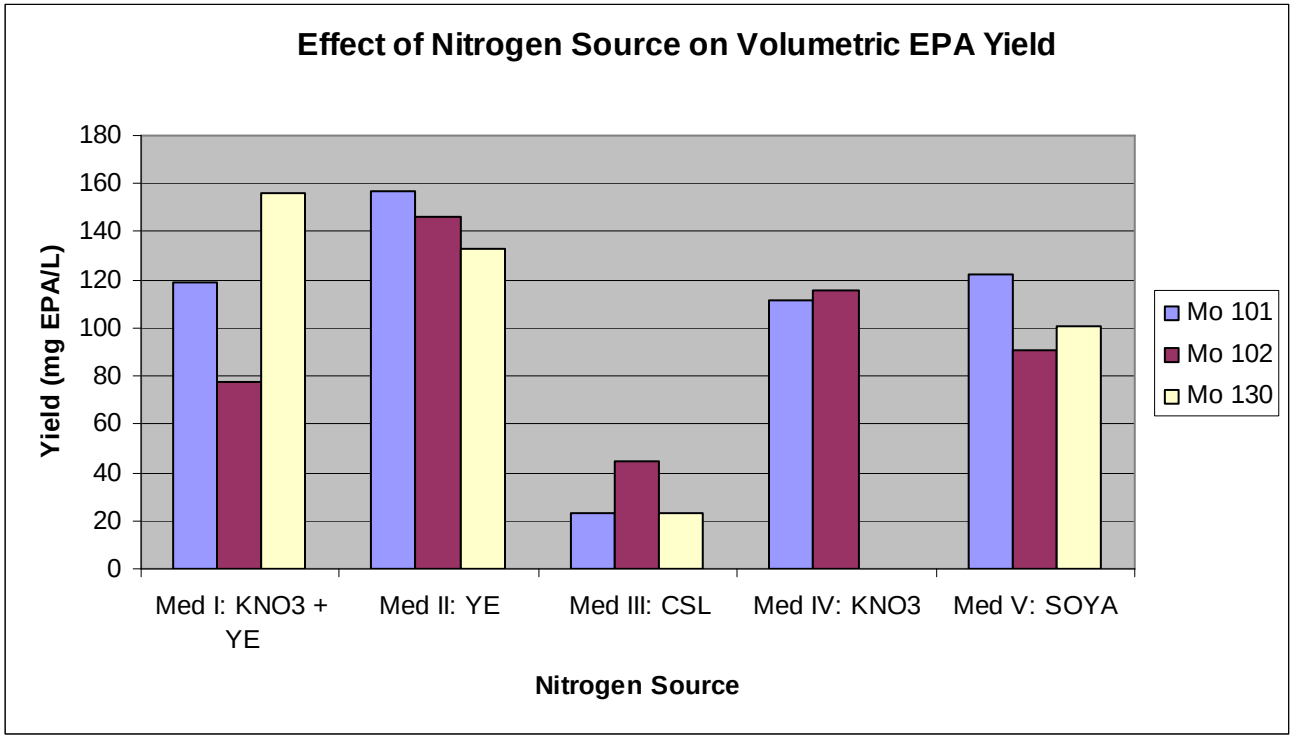
The effect of dextrose concentration on EPA production



Development of fermentation process for EPA production

- Dextrose concentration: no influence in solid state
- Linseed oil concentration: results not available
- Incubation temperature and temperature shift: Longer period at higher temperature promotes growth but lower EPA
- Growth curve: Maximum biomass produced after 8 days for most isolates
- Nitrogen source: Effect on biomass and EPA production

Development of fermentation process for EPA production



Mass production process for fungal biomass

- To study the nutritional effect of fungal biomass on White Stumpnose
- To perform a preliminary assessment of the safety of *Mortierella* biomass in fish diet
- 2,6 kg of wet fungal biomass was produced in shake flask (at ~25 g/L fermentation broth)
- Fish feeding trials still in progress
- ARA (oil produced by *Mortierella spp*) approved for addition to infant formula by:
  - Netherlands - State Journal March 8, 1995 (71)
  - US FDA as GRAS (Notice GRN 000041, May 17, 2001)
  - Commercial infant formula sold in 60 countries incl. France, Portugal, Netherlands, Spain, Turkey and the United Kingdom
- SCO safer than fish oil. Mercury detected in all European women tested – most in fish-eating Spanish women

Development of fermentation process for EPA production: The effect of fungal growth conditions on morphology and EPA production.

Fungal pellets in submerged culture vary in size, texture and shape: from ellipsoidal ball shape to a biconcave “doughnut” shaped pellet with a central cavity (Hamanaka *et al*, 2001). In larger pellets, autolysis of central mycelia occurs due to limitations on nutrient and oxygen diffusion.

Activities Planned for Year 3 (fermentation):

1. Study the effect of medium composition on fungal micro- and macro-morphology (nitrogen sources, carbon concentration, LSO concentration)
2. Study the effect of fermentation conditions on fungal micro- and macro-morphology (dissolved oxygen, temperature)
3. Study of the relationship between pellet morphology and EPA production by image analysis and electron microscopy
4. Optimisation of growth conditions and medium to produce small pellets for EPA production
5. Scale-up to 10L fermenter under optimised conditions
6. Techno-economical comparison of EPA production process in:

   current fermentation medium, ferm. medium + soya oilcake and ferm. medium + BSG protein fraction as nitrogen source.