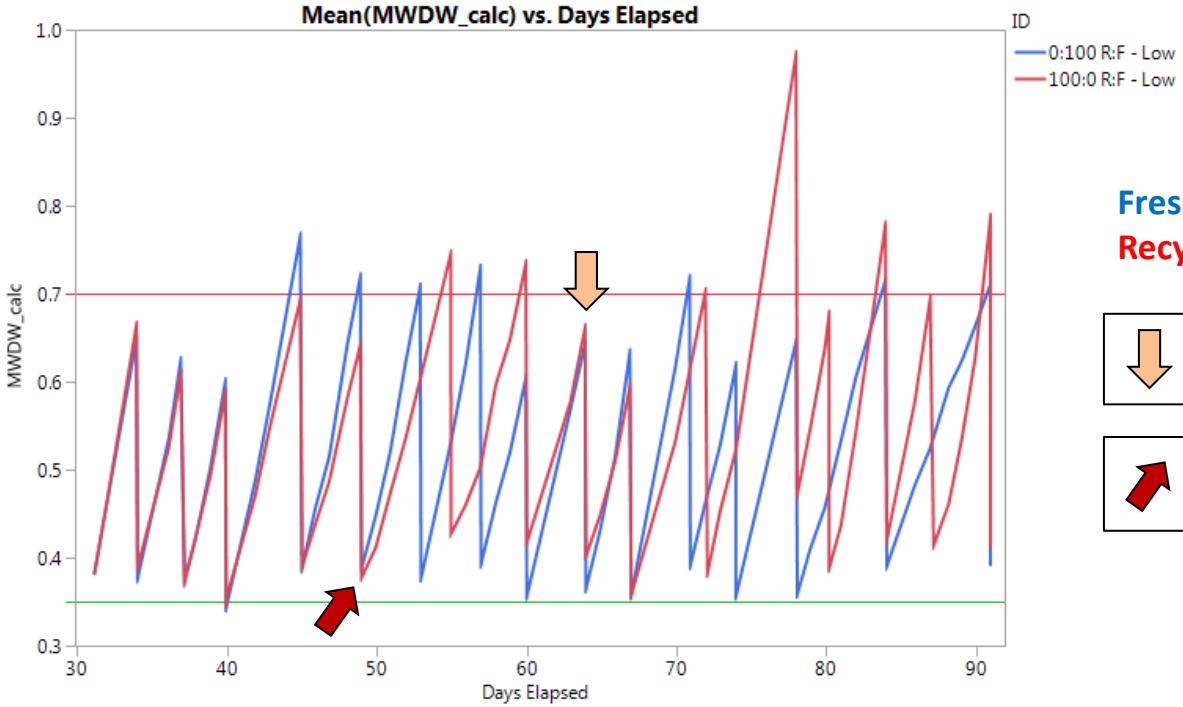
Recycled media flask experiment taken down after 60+ days

SE60445; 16NFC1-101/150:40; continuous light; 25C; 3.5% CO2; 250mL flask cultures in triplicate

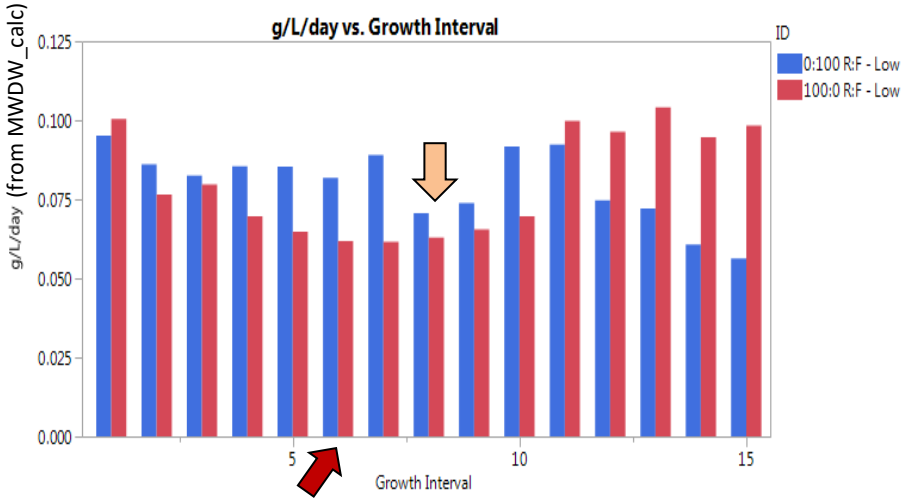


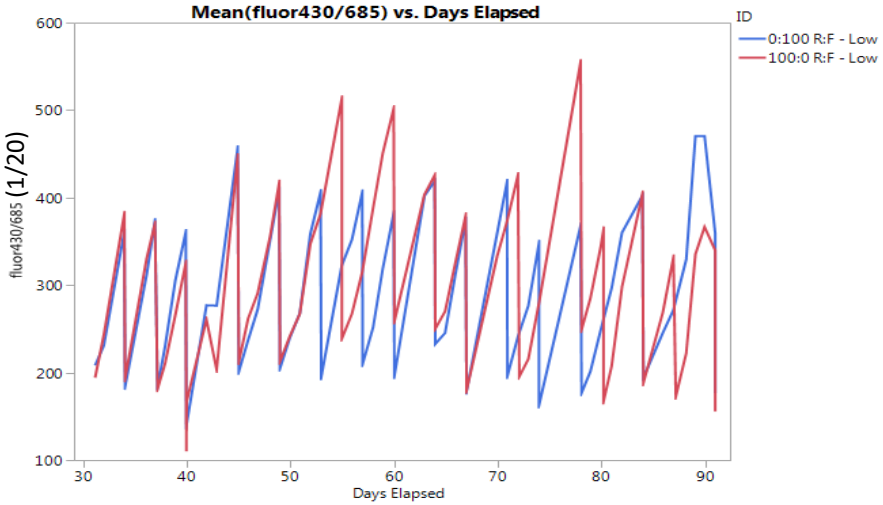
Fresh

Recycled

Supernatant from harvest used in fractionation MGRA

Growth interval 6, where high density flasks diverged

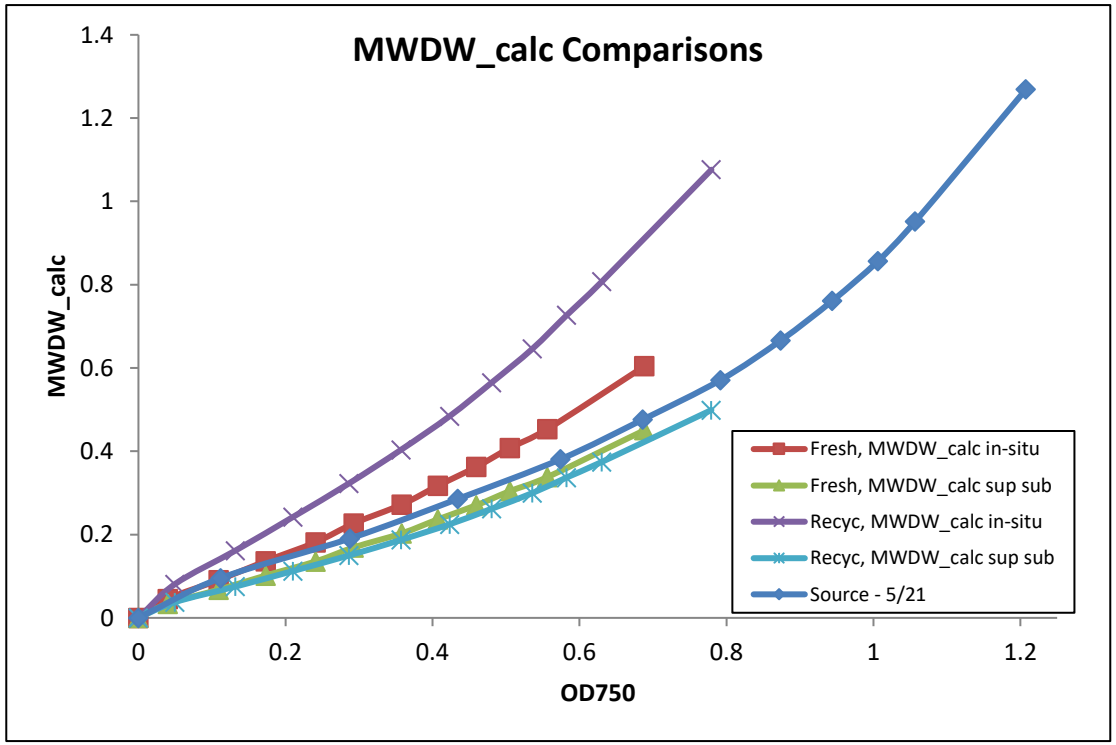




Final cultures and supernatants measured with MWDW, OD

SE60445; 16NFC1-101/150:40; continuous light; 25C; 3.5% CO2; 250mL flask cultures in triplicate

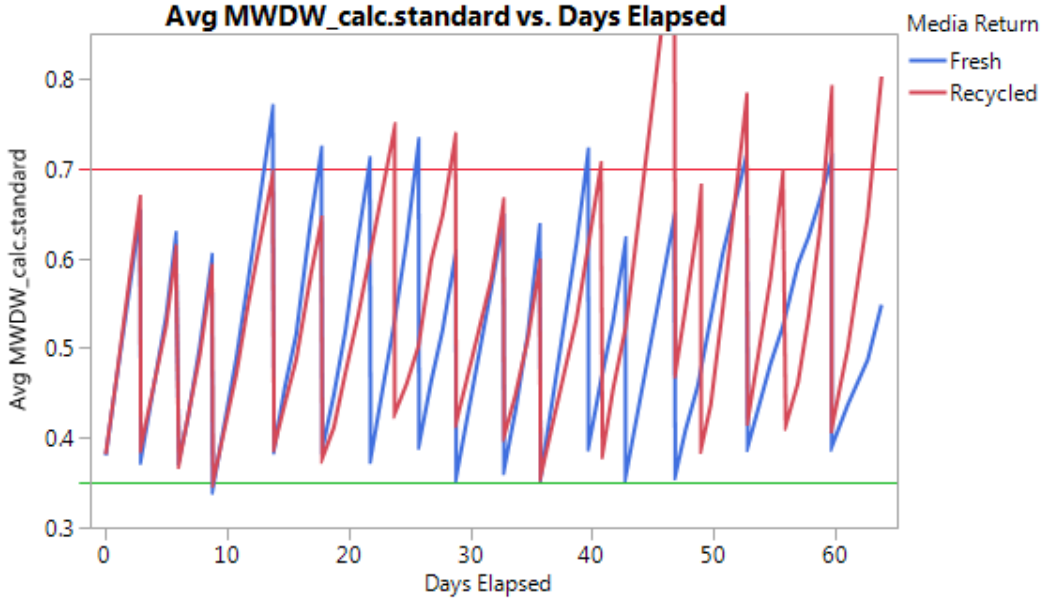
Sample	MWDW (g/L)	MWDW_calc	OD750
Fresh culture	0.605	0.548	0.818
Fresh supernatant	0.154		0.130
Recycled culture	1.076	0.802	1.025
Recycled supernatant	0.577		0.246

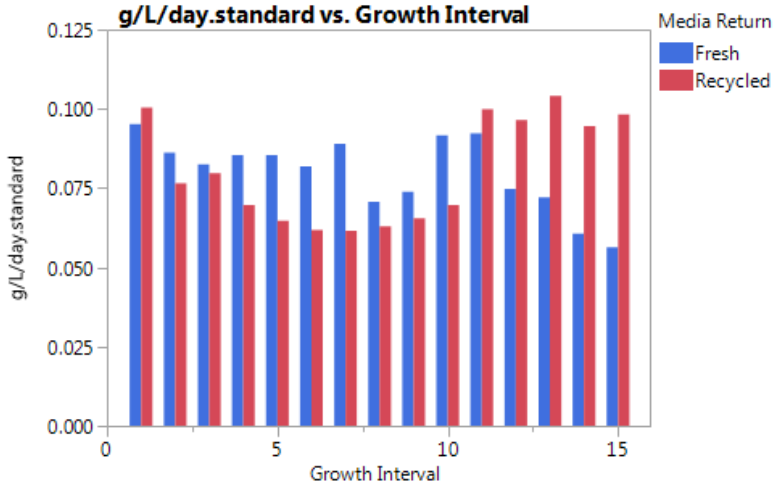


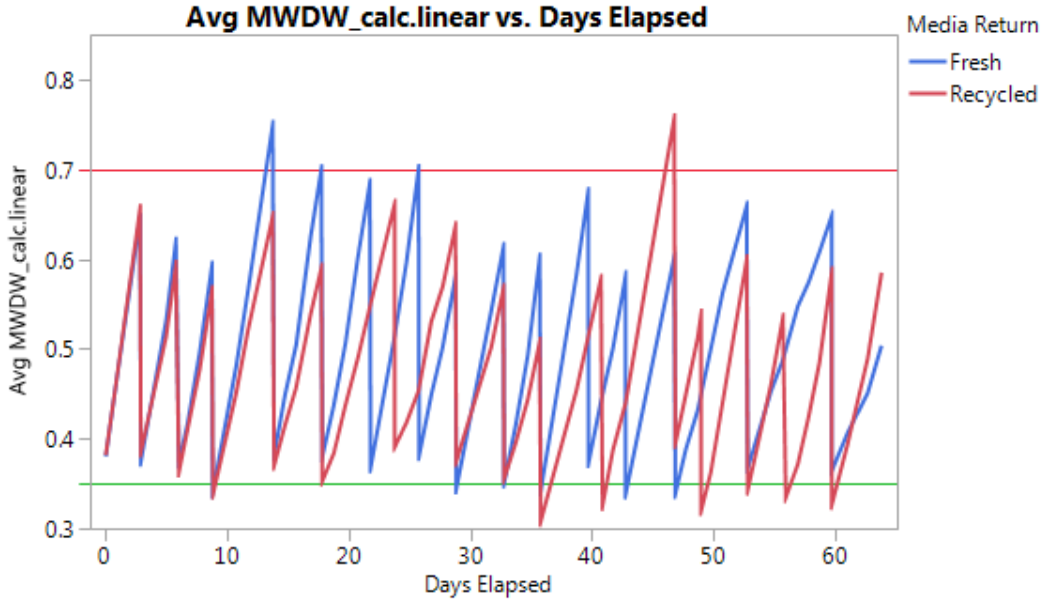
- Supernatants are contributing significantly to MWDW measurements
- Source inoculum MWDW_calc curve resembles “supernatant-subtracted” curves of final cultures

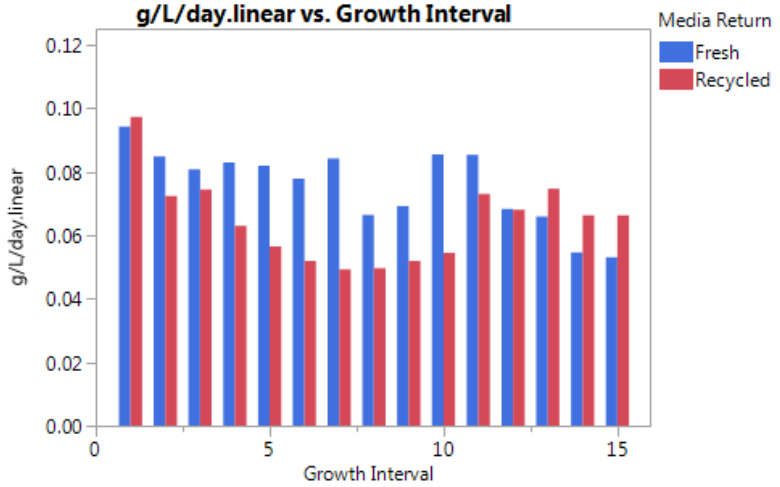
Blanking for calculated supernatant contributions to OD suggests different growth history

SE60445; 16NFC1-101/150:40; continuous light; 25C; 3.5% CO2; 250mL flask cultures in triplicate









“.linear” calculations assume a linearly increasing OD750 contribution from the supernatant over time, back-calculated from final culture reads